Drug Repurposing for Emerging Infectious Diseases and Cancer

Ranbir Chander Sobti
Sunil K. Lal
Ramesh K. Goyal
Editors

Springer

Drug Repurposing for Emerging Infectious Diseases and Cancer

Ranbir Chander Sobti • Sunil K. Lal •
Ramesh K. Goyal
Editors

Drug Repurposing for Emerging Infectious Diseases and Cancer

Springer

Editors
Ranbir Chander Sobti
Department of Biotechnology
Panjab University
Chandigarh, Chandigarh, India

Sunil K. Lal
School of Science
Monash University Malaysia
Bandar Sunway, Selangor, Malaysia

Ramesh K. Goyal
Delhi Pharmaceutical Sciences and Research
New Delhi, India

ISBN 978-981-19-5398-9 ISBN 978-981-19-5399-6 (eBook)
https://doi.org/10.1007/978-981-19-5399-6

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Preface

Medicines have always been discovered and invented with desired and intended purposes and consequences. With deeper understandings, we are able to discover many more facets about these drugs. In mid-1900s when mercaptopurine was introduced, the scientists did encounter its association with a life-threatening condition of bone-marrow toxicity. Similarly in 1950s, another drug succinylcholine, a muscle relaxant, has been associated with respiratory distress.[1] These are both the horrifying conditions of adverse reactions. But these certainly paved the way not only for efficacy of desired drug response but also for the off-target effects of the drugs.

These off-targets do provide a clue about the pharmacodynamic effect of the drugs, i.e., what a particular drug does to the body or what are the biochemical, physiological, and molecular effects of the drug. This actually provided a major clue that if all the biological targets are revealed there is a provision of reconsideration of any drug for alternate purpose. This phenomenon has been termed as the drug repurposing which can be exploited in the treatment of various appalling conditions in a time and cost-effective manner. This reprofiling approach for drugs also provide a way out from traditional method of drug discovery which besides being very expensive ($1.6 billion/drug) are also time consuming (~10–15 years). Moreover, a constant decline in the FDA approval of new drugs especially in the field of oncology necessitated novel approaches to expedite the process of drug discovery.

Drug repurposing is establishment of new medical uses for already known drugs. The repositioning of an active pharmaceutical ingredient that is already available in the market for a new indication is referred to as drug repurposing. Several repositioned drugs, including some very old drugs, have been used throughout the course of medicine historically. This repositioning of drugs was purely through serendipity in those times. In the present scenario, novel methods based on data

[1] Bourne JG, Long action of suxamethonium (succinylcholine) chloride. Br J Anaesth 1953: 25; 116–29.

mining have been developed for identification of new candidates for drug repurposing. The success of drug repositioning in providing benefits in certain diseases brought the global attention on the potential off-target effects of some of the drugs. In view of the fact that these existing drugs have been used previously in humans, their dose regimen with favourable pharmacokinetics and pharmacodynamics properties including any side effects are already in public domain, making these old drugs useful in new drug discovery.

The "repurposing" of existing therapies for alternative disease avoids long regulatory processes, funds, and intellectual property right. It is gaining popularity as an approach to identify new targets of medicines in use. Researchers from Case Western Reserve University School of Medicine and collaborators have received a five-year, $2.8 million grant from the National Institute on Aging to identify FDA-approved medications that could be repurposed to treat Alzheimer's disease. Bedaquiline—new anti-TB drug for treating drug-resistant TB has identified using the principle of repurposing of existing drugs. Celecoxib, a non-steroidal anti-inflammatory drug (NSAID) has demonstrated antibacterial activity by causing the inhibition of DNA and RNA replication, protein synthesis, and cell wall formation, while simultaneously reducing the levels of IL-6, TNF-α, IL-1β, and MCP-1 (monocyte-chemoattractant protein-1) against various pathogens including *S. aureus, Bacillus anthracis, B. subtilis*, and *M. smegmatis*. With the exception of linezolid, celecoxib has also demonstrated synergistic effects with several topical and systemic antimicrobials used against *S. aureus*. Also antidiabetic drug metformin (MET) has been reported to inhibit the intracellular growth of mycobacteria. Quinine, could slow the growth of *Candida* species. Drugs used for obesity treatment (lipase inhibitors) and Cyclipostins and Cyclophostin analogs were reported as promising compounds in the fight against tuberculosis. It targets several serine or cysteine enzymes involved in *M.tb* lipid metabolism and/or in cell wall biosynthesis. Lead compounds exhibited nanomolar inhibition of the enzyme, supporting Rv3802c as a novel TB drug target. It has demonstrated encouraging antibacterial activity against *M. tuberculosis* in vitro. Itraconazole, a triazole antifungal drug, has demonstrated anti-angiogenic property that lead to its usage either alone or in combination with other anticancer drugs in various preclinical models including medulloblastoma, non-small cell lung cancer (NSCLC), and basal cell carcinoma Digoxin, a cardiac glycoside isolated from foxglove and used for treating heart failure and arrhythmia, demonstrated glycoside triggered immunogenic demise of the cancer cells Nitroxoline, an antibiotic, recently demonstrated the anticancer activity in prostate cancer. Rituximab, used to treat rheumatoid arthritis, demonstrated anticancer activity against several cancers.

Drugs used for obesity treatment (lipase inhibitors) and Cyclipostins and Cyclophostin analogs have been reported as promising compounds in the fight against tuberculosis. It targeted several serine or cysteine enzymes involved in *M. tb* lipid metabolism and/or in cell wall biosynthesis. Itraconazole, a triazole antifungal drug has demonstrated anti-angiogenic property that lead to its usage either alone or in combination with other anticancer drugs in various preclinical models including medulloblastoma, non-small cell lung cancer (NSCLC), and basal cell

carcinoma. The major challenge faced by the institutes working on drug repositioning is the relatively weak intellectual property protection on these products, which can lead to reduction in return on their investment further discouraging these companies from developing these drugs.

The major challenge is how to reveal all the biological targets and avoid the adverse reactions. Various approaches for drug repurposing, covering all the aspects are being pursued in drug repurposing direction and are as follows.

Ligand-Based approaches:

(i) Library containing the FDA-approved drugs is tested by High-Throughput screening and statistically evaluated.
(ii) Virtual library of FDA-approved drugs is tested by Reverse Docking to various targets.

Target-Based Approaches:

(i) Targets are identified and screened virtually docking to library of ligands.

In both these approaches, the computational biology is being used as the prime technologies followed by validation under in vitro or in vivo system. In case of oncology repurposing some of the noted examples are isoprenaline, a non-selective β-adrenergic agonist exhibiting cytotoxic potential and fludarabine (purine analogue) for chronic lymphocytic leukaemia. More recently, the use of chloroquin, an antimalarial for the intervention of covid-19 virus reaffirmed the need to look for this aspect for the futuristic therapeutic interventions.

These repurposed therapies offer great advantage as their FDA approvals, adverse drug reportings (ADR), and therapeutic safe doses (TSD) are already determined. Hence in the current scenario, in-depth study of ligand and targets offer great new possibilities for repurposed drugs.

In the backdrop of all these, it is purposed to compile a book entitled *Drug Repurposing for Emerging Infectious Diseases and Cancer.*

Treatments of diseases that are either rare, complex, or novel may not always be available due to high cost of drug development and research. Drug repurposing is an alternative approach to usurp already available drugs or drug candidates with FDA approval which have been initially developed for specific diseases and re-establish their use for other diseases. Modern genomic methods for drug repurposing involve the usage of computational programs and online tools to analyse and ultimately deduce targets with high specificity to be considered as candidates for repositioning. Gene, protein, disease, and drug databases are built from high-throughput experimental, in vitro, in vivo, and clinical data thus providing a reliable basis for drug target acquisition purposes. Key experimental and in silico approaches for modern drug repositioning, namely, signature matching, molecular docking, genome-wide-associated studies, and network-based approaches aided by artificial intelligence will be described in this book, along with research examples that have used these methods.

Drug repositioning for certain diseases such as Alzheimer's disease, cystic fibrosis, and SARS-CoV-2 disease will be discussed in separate sections of this

book. This book will highlight the challenges faced and the future perspectives of Drug Repurposing. Genomic computational approaches for drug repurposing present much potential in identifying drug targets more efficiently and effectively which provides the opportunity to fulfil the gap for the treatment of diseases with little or no cure.

The current pandemic of the coronavirus disease (COVID-19) and past history of fast evolving emerging and re-emerging infectious diseases have put in place an urgent need for new and promising antiviral therapeutics.

Interestingly, there are many cellular pathways and biomolecules that serve as key nodal points for both cancer and infectious disease progression. A good example is the viral RNA-dependent RNA polymerase (RdRp) enzyme that plays a vital role in viral replication of all RNA viruses, including the current SARS-CoV-2, thereby making it a prime and promising candidate for novel antiviral targeting. The human telomerase reverse transcriptase (hTERT), a common catalytic subunit of the telomerase enzyme in many cancers has also been identified with structural and functional similarities to the viral RdRp. Therefore, it becomes important to evaluate and consider anticancer drugs that target hTERT for antiviral RdRp activity, and vice versa. For instance, Floxuridine, an hTERT inhibitor, and VX-222, an HCV RdRp inhibitor now being analysed as a potential antiviral for SARS-CoV-2 and anti-hTERT for cancer, respectively. In this book, we aim at bringing to light this close relationship between emerging infectious diseases and cancer. We plan to punctuate this idea with specific chapters on the great potential of anticancer inhibitors that can be repurposed for infectious diseases. We all agree that cancer is a very studious subject, and there is a vast amount of information on effective drugs against various cancers; however, there is very limited knowledge on the SARS-CoV-2 virus and other recent emerging and re-emerging infectious diseases. Hence, we feel this book will be a great contribution to science and will emerge as a major hot seller in the field.

Newer technologies of OMICs substantiated by computational techniques including artificial intelligence, *etc*. are being tried for rediscovery of functional aspects of disease.

This book is a comprehensive volume on newer technologies as well as their applications in drug discovery with up-to-date information on repurposed and prospective drugs for cancer and infectious diseases with particular reference to Covid-19.

RCS is thankful to the Indian National Science Academy, New Delhi, for providing platform as Senior Scientist and Panjab University, Chandigarh, as Emeritus Professor to continue academic pursuits. He is thankful to his wife Dr Vipin Sobti and daughters Aditi and Aastha and granddaughter Irene but for their support I would not have compiled the book.

Chandigarh, Punjab, India — Ranbir Chander Sobti
Bandar Sunway, Selangor, Malaysia — Sunil K. Lal
New Delhi, India — Ramesh K. Goyal

Contents

Editors and Contributors

About the Editors

Ranbir Chander Sobti former Vice Chancellor, Panjab University, Chandigarh, and Babasaheb Bhimrao Ambedkar University (Central University), Lucknow, and former Education Advisor to the Governor of Bihar, is a scientist, an able administrator and dynamic institution builder.

Starting his career as a Cytogeneticist, he moved on to molecular biology including genomics to understand the susceptibility and disease process of cancer, COPD, AIDS, metabolic syndrome, and kidney diseases. He has also used stem cells and nanoparticles to follow the process of tissue organ development through designed decellularization protocol. Earlier he had worked on the cytogenetics and molecular genetics of various animal groups including humans as well as molecular toxicology of agricultural pollutants using in vitro and in vivo protocols.

He has published more than 350 papers in journals of national and international repute and also published more than 50 books by international publishers.

He is a Fellow of the Third World Academy of Sciences, National Academy of Sciences India, Indian National Science Academy, National Academy of Medical Sciences, National Academy of Agricultural Sciences, Canadian Academy of Cardiovascular Diseases, and few others. He was the General President of Indian Science Congress Association for the 102nd session held at the University of Jammu in 2013. He is the recipient of many prestigious awards like the INSA Young Scientist Medal, UGC Career Award, Punjab Rattan Award, JC Bose Oration and Sriram Oration Awards, and of Life Time Achievement Awards of the Punjab Academy of Sciences, Zoological Society of India, and the Environment Academy of India, besides many other medals and awards of national and international levels. He was awarded Padmashri, the third highest civilian award by the Government of India in 2009 for his contributions to the cause of education. He has chaired/delivered lectures at national and international conferences/symposia/workshops.

Dr. Sobti, an active researcher, is also steadfastly committed to the popularization of science in the community through popular lectures and community engagement programs.

Sunil K. Lal received his PhD from the Georgia Institute of Technology, Atlanta, USA, in Microbial Genetics (1989) following which he joined the faculty at California Institute of Technology, Pasadena (1994). Following this, Prof. Lal joined the ICGEB (International Center for Genetic Engineering and Biotechnology) of the United Nations at New Delhi, India, where he served as an International Research Scientist for 21 years. In 2014 Prof. Lal joined the School of Science, Monash University, Malaysia, where he is currently a Professor of Microbiology and leads an active research group on Systems Virology. Prof. Sunil is internationally well known for his research work in the field of RNA viruses including SARS Coronaviruses, Influenza A virus, and Hepatitis E virus. He has been a visiting scientist to Universiti Malaysia Sarawak, National University of Singapore, Karolinska Institute, and the Centers for Disease Control (CDC) Atlanta. Prof. Sunil has won many prestigious international awards including the Hind Rattan (highest civilian honor from the Govt. of India) and has been the regional representative for the American Society for Microbiology for 9 years besides being an Elected Fellow of the National Academy of Sciences (India). Prof. Sunil is on the editorial board of international journals and is actively involved in peer reviewing some of the top-tier scientific journals. Prof. Sunil has over 200 publications in top international scientific journals and has published four international books on emerging viral diseases.

Ramesh K. Goyal is the Vice Chancellor of Delhi Pharmaceutical Sciences and Research University, New Delhi. He was earlier the Vice Chancellor of the Maharaja Sayajirao University of Baroda; Executive Director (Research & Strategies) at V ClinBio Labs., Sri Ramachandra Medical Center University, Chennai; Director (Pharmacology and Clinical Research) at NMIMS University, Mumbai; Director ISF College of Pharmacy, Moga, Punjab; and Professor at L. M. College of Pharmacy, Ahmedabad. He has been the visiting scientist and visiting professor at the University of British Columbia, Vancouver, BC, and Institute of Cardiovascular Sciences, Winnipeg, Canada. In 2019 he was conferred with the Honorary Professorship at Stavropol State Medical University, Russia, and recently appointed as the Distinguished Professor at USCI University, Kula Lumpur, Malaysia. His research interest is development of newer drugs for cardiovascular and metabolic disorders through phyto-pharmaceuticals. Recently he has come up with an herbal formulation specifically targeting ACE-2 of SARC-CoV-2 virus. Dr Goyal got three patents awarded, 6 under consideration, 42 books and book chapters, over 350 full papers articles published in national and international journals. He is the recipient of 74 national and international awards. He is the fellow of eight professional bodies (FIPS, FIACS, FAMS, FIC, FICN, FNASc, FSCH, FIVSPT). He has been chairman/member of prestigious national and international bodies.

Contributors

Geeta Aggarwal Delhi Pharmaceutical Sciences and Research University, New Delhi, India

Ansab Akhtar University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh, India

Shailendra Asthana Translational Health Science and Technology Institute (THSTI), Faridabad, India

Pavithra Balakrishnan Department of Biotechnology, School of Biotechnology and Genetic Engineering, Bharathiar University, Coimbatore, India

Antara Banerjee Department of Medical Biotechnology, Faculty of Allied Health Sciences, Chettinad Academy of Research and Education (CARE), Chettinad Hospital and Research Institute (CHRI), Chennai, India

Kushal Kumar Bansal Govt. Pharmacy College, Rakkar, Distt. Kangra, Matyari, Himachal Pradesh, India

Yasir Bashir Medical Microbiology and Molecular Biology Lab., Interdisciplinary Biotechnology Unit, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

Umang Berry Virology Laboratory, Vaccine and Infectious Disease Research Centre, Translational Health Science and Technology Institute, NCR Biotech Science Cluster, Faridabad, Haryana, India

Nilam Bhusare Department of Pharmaceutical Chemistry, SPP School of Pharmacy and Technology Management, SVKM's NMIMS, Mumbai, India

Meena Chintamaneni Department of Pharmaceutical Chemistry, SPP School of Pharmacy and Technology Management, SVKM's NMIMS, Mumbai, India

Mei Nee Chiu University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh, India

Simran Chopra Department of Textile and Fibre Engineering, Indian Institute of Technology Delhi, Delhi, India

Vincent T. K. Chow NUHS Infectious Diseases Translational Research Program, Department of Microbiology and Immunology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore

Aditya Dahiya Department of Textile and Fibre Engineering, Indian Institute of Technology Delhi, Delhi, India

Mini Dahiya University Institute of Pharmaceutical Sciences (UIPS), UGC Centre of Advanced Studies (UGC-CAS), Panjab University, Chandigarh, Punjab, India
University Institute of Pharmaceutical Sciences (UIPS), UGC Centre of Advanced Studies (UGC-CAS), Panjab University, Chandigarh, Haryana, India

Prity Rani Deshwal Department of Pharmacy Practice, National Institute of Pharmaceutical Education, Mohali, Punjab, India

Pratibha Dhakla Department of Pharmacy, School of Medical & Allied Sciences, GD Goenka University, Gurgaon, Haryana, India

John Disouza Tatyasaheb Kore College of Pharmacy, Warananagar, Maharashtra, India

Nabeela Farhat Medical Microbiology and Molecular Biology Lab., Interdisciplinary Biotechnology Unit, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

Isra Ahmad Farouk School of Science, Monash University Malaysia, Subang Jaya, Selangor, Malaysia

Charlene Fong NUHS Infectious Diseases Translational Research Program, Department of Microbiology and Immunology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore

Harsha Ganesan Department of Medical Biotechnology, Faculty of Allied Health Sciences, Chettinad Academy of Research and Education (CARE), Chettinad Hospital and Research Institute (CHRI), Chennai, India

Rajat Goyal MM College of Pharmacy, Maharishi Markandeshwar (Deemed to be University), Mullana-Ambala, Haryana, India

Ramesh K. Goyal Delhi Pharmaceutical Sciences and Research University, New Delhi, India
School of Pharmaceutical Sciences, Delhi Pharmaceutical Sciences and Research University, New Delhi, India

Pooja Gupta ADR Monitoring Center, AIIMS Delhi, Pharmacovigilance Program of India (PvPI), New Delhi, India
Pharmacology, All India Institute of Medical Sciences, New Delhi, India

Viney Jain Jain Vishva Bharti University, Ladnun, Rajasthan, India

Niraj Kumar Jha Department of Biotechnology, School of Engineering and Technology (SET), Sharda University, Greater Noida, Uttar Pradesh, India
Department of Biotechnology Engineering and Food Technology, Chandigarh University, Mohali, India
Department of Biotechnology, School of Applied and Life Sciences (SALS), Uttaranchal University, Dehradun, India

Priya Joon University Institute of Pharmaceutical Sciences (UIPS), UGC Centre for Advanced Study, Panjab University, Chandigarh, India

Kajal Joshi Himachal Institute of Pharmaceutical Education & Research, Nadaun, Himachal Pradesh, India

Monika Kadian University Institute of Pharmaceutical Sciences (UIPS), UGC Centre for Advanced Study, Panjab University, Chandigarh, India

Jaspreet Kaur University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh, India

Asad U. Khan Medical Microbiology and Molecular Biology Lab., Interdisciplinary Biotechnology Unit, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

Kapil Kole Tatyasaheb Kore College of Pharmacy, Warananagar, Maharashtra, India

Anurag Kuhad Pharmacology Research Laboratory, University Institute of Pharmaceutical Sciences, UGC-Centre of Advanced Study, Panjab University, Chandigarh, Punjab, India

Anil Kumar University Institute of Pharmaceutical Sciences (UIPS), UGC Centre of Advanced Studies (UGC-CAS), Panjab University, Chandigarh, Punjab, India
University Institute of Pharmaceutical Sciences, UGC Centre of Advanced Studies (UGC-CAS), Panjab University, Chandigarh, Haryana, India

Vipin Kumar Laboratory of Applied Microbiology, Indian Institute of Technology (Indian School of Mines), Dhanbad, Jharkhand, India

Popat Kumbhar Tatyasaheb Kore College of Pharmacy, Warananagar, Maharashtra, India

Sunil K. Lal School of Science, Monash University Malaysia, Subang Jaya, Selangor, Malaysia
Tropical Medicine and Biology Platform, Monash University Malaysia, Subang Jaya, Selangor, Malaysia

Chie-Min Lim School of Science, Monash University Malaysia, Subang Jaya, Selangor, Malaysia

Zheng Yao Low School of Science, Monash University Malaysia, Subang Jaya, Selangor, Malaysia

Bharti Mangla Delhi Pharmaceutical Sciences and Research University, New Delhi, India

Arehalli Manjappa Tatyasaheb Kore College of Pharmacy, Warananagar, Maharashtra, India

Y. C. Mayur Department of Pharmaceutical Chemistry, SPP School of Pharmacy and Technology Management, SVKM's NMIMS, Mumbai, India
Somaiya Institute for Research & Consultancy, Somaiya Vidyavihar University, Mumbai, India

Velisha Mehta Department of Pharmaceutical Chemistry, SPP School of Pharmacy and Technology Management, SVKM's NMIMS, Mumbai, India

Antony Anista Michael Department of Biotechnology, School of Biotechnology and Genetic Engineering, Bharathiar University, Coimbatore, India

Archana Mishra Pharmacology, All India Institute of Medical Sciences, New Delhi, India

Lovika Mittal Delhi Pharmaceutical Sciences and Research University, New Delhi, India

Pankaj Musyuni Delhi Pharmaceutical Sciences and Research University, New Delhi, India

Ashrit Nair Department of Textile and Fibre Engineering, Indian Institute of Technology Delhi, New Delhi, India

T. Narasaraju NUHS Infectious Diseases Translational Research Program, Department of Microbiology and Immunology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore
Adichunchanagiri Institute of Medical Sciences, Adichunchanagiri Centre for Research and Innovation, Adichunchanagiri University, BG Nagara, Karnataka, India

Swati Paliwal Delhi Pharmaceutical Sciences and Research University, New Delhi, India

Naresh Kumar Panda Department of Otolaryngology and Head and Neck Surgery, Postgraduate Institute of Medical Education and Research, Chandigarh, India

Janvi Patel Centre for Infectious Disease Research, Department of Microbiology and Cell Biology, Indian Institute of Science, Bengaluru, India

Surajit Pathak Department of Medical Biotechnology, Faculty of Allied Health Sciences, Chettinad Academy of Research and Education (CARE), Chettinad Hospital and Research Institute (CHRI), Chennai, India
Departments of Oncology and Biomedical and Clinical Sciences, Linköping University, Linköping, Sweden

Ritika Patial Centre for Systems Biology and Bioinformatics, UIEAST, Panjab University, Chandigarh, India
Department of Biotechnology, Panjab University, Chandigarh, India

Vandana Patravale Department of Pharmaceutical Sciences and Technology, Institute of Chemical Technology, Mumbai, Maharashtra, India

Sourabha Kumar Patro Department of Otolaryngology and Head and Neck Surgery, Postgraduate Institute of Medical Education and Research, Chandigarh, India

Sanjeev Puri Department of Biotechnology, Panjab University, Chandigarh, India

Shah Kamranur Rahman Department of Infection Biology, London School of Hygiene and Tropical Medicine, London, UK

Shivani Raj University School of Biotechnology, Guru Gobind Singh Indraprastha University, New Delhi, India

C. T. Ranjith-Kumar University School of Biotechnology, Guru Gobind Singh Indraprastha University, New Delhi, India

Rahul Rawat Department of Pharmaceutical Sciences, Chaudhary Bansi Lal University, Bhiwani, Haryana, India

Eupa Ray University Institute of Pharmaceutical Sciences (UIPS), UGC Centre for Advanced Study, Panjab University, Chandigarh, India
Institute of Nano Science and Technology (INST), Mohali, Punjab, India

Madhurya Ray Laboratory of Applied Microbiology, Indian Institute of Technology (Indian School of Mines), Dhanbad, Jharkhand, India

Sangeeta Pilkhwal Sah University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh, India

Tammanna R. Sahrawat Centre for Systems Biology and Bioinformatics, UIEAST, Panjab University, Chandigarh, India
Department of Biotechnology, Panjab University, Chandigarh, India

M. Samatha Jain Department of Medical Biotechnology, Faculty of Allied Health Sciences, Chettinad Academy of Research and Education (CARE), Chettinad Hospital and Research Institute (CHRI), Chennai, India

Ranjana Bhandari Sanshita Pharmacology Research Laboratory, University Institute of Pharmaceutical Sciences, UGC-Centre of Advanced Study, Panjab University, Chandigarh, Punjab, India

Archana Sharma School of Pharmaceutical Sciences, Delhi Pharmaceutical Sciences and Research University, New Delhi, India

Garima Sharma University Institute of Pharmaceutical Sciences (UIPS), UGC Centre for Advanced Study, Panjab University, Chandigarh, India

Kritika Sharma University Institute of Pharmaceutical Sciences (UIPS), UGC Centre for Advanced Study, Panjab University, Chandigarh, India

Maryada Sharma Department of Otolaryngology and Head and Neck Surgery, Postgraduate Institute of Medical Education and Research, Chandigarh, India

Navneet Sharma Department of Textile and Fibre Engineering, Indian Institute of Technology Delhi, Delhi, India

Prabodh Chander Sharma School of Pharmaceutical Sciences, Delhi Pharmaceutical Sciences and Research University, New Delhi, India

Rakesh Kumar Sharma SGT University, Gurugram, Haryana, India

Sandeep Sharma I.K. Gujral Punjab Technical University, Jalandhar, Punjab, India

N. R. Shruthi Department of Medical Biotechnology, Faculty of Allied Health Sciences, Chettinad Academy of Research and Education (CARE), Chettinad Hospital and Research Institute (CHRI), Chennai, India

Ranbir Chander Sobti Centre for Systems Biology and Bioinformatics, UIEAST, Panjab University, Chandigarh, India
Department of Biotechnology, Panjab University, Chandigarh, India

Paavan Sood Pharmacology Research Laboratory, University Institute of Pharmaceutical Sciences, UGC-Centre of Advanced Study, Panjab University, Chandigarh, Punjab, India

Xiao-Feng Sun Departments of Oncology and Biomedical and Clinical Sciences, Linköping University, Linköping, Sweden

Milan Surjit Virology Laboratory, Vaccine and Infectious Disease Research Centre, Translational Health Science and Technology Institute, NCR Biotech Science Cluster, Faridabad, Haryana, India

Deeksha Thakur Pharmacology Research Laboratory, University Institute of Pharmaceutical Sciences, UGC-Centre of Advanced Study, Panjab University, Chandigarh, Punjab, India

Pramil Tiwari Department of Pharmacy Practice, National Institute of Pharmaceutical Education, Mohali, Punjab, India

Rajiv K. Tonk Delhi Pharmaceutical Sciences and Research University, New Delhi, India

Ekta Tripathi Department of Biotechnology, Faculty of Life and Allied Health Sciences, M.S. Ramaiah University of Applied Sciences, Bengaluru, India

Shiva Tushir Department of Pharmacy, Panipat Institute of Engineering and Technology (PIET), Samalkha, Panipat, Haryana, India

Thirunavukkarasu Velusamy Department of Biotechnology, School of Biotechnology and Genetic Engineering, Bharathiar University, Coimbatore, India

Rohit Verma Virology Laboratory, Vaccine and Infectious Disease Research Centre, Translational Health Science and Technology Institute, NCR Biotech Science Cluster, Faridabad, Haryana, India

Ka Heng Wong School of Science, Monash University Malaysia, Subang Jaya, Selangor, Malaysia

Monu Yadav Department of Pharmacy, School of Medical & Allied Sciences, GD Goenka University, Gurgaon, Haryana, India

Tanuja Yadav Department of Pharmaceutical Chemistry, SPP School of Pharmacy and Technology Management, SVKM's NMIMS, Mumbai, India

Vivek Yadav Delhi Pharmaceutical Sciences and Research University, New Delhi, India

Ashley Jia Wen Yip School of Science, Monash University Malaysia, Subang Jaya, Selangor, Malaysia

Nur Zawanah Zabidi School of Science, Monash University Malaysia, Subang Jaya, Selangor, Malaysia

Hong Zhang Institute of Medical Sciences, School of Medicine, Örebro University, Örebro, Sweden

Chapter 1
Drug Repurposing: An Advance Way to Traditional Drug Discovery

Priya Joon, Mini Dahiya, Garima Sharma, Kritika Sharma, Eupa Ray, Monika Kadian, Ranbir Chander Sobti, Sanjeev Puri, and Anil Kumar

Abstract Despite advancements in drug discovery techniques, developing medications for diverse ailments still remains challenging. As a result, novel techniques such as drug repurposing are required to develop therapeutics to treat both common and rare disorders. Drug repurposing is a potential approach in drug discovery for identifying new therapeutic applications for existing medications that are distinct from the original medical indication. Identification of new indications for existing drugs by drug repurposing has the potential to supplement traditional drug development by reducing the substantial monetary and time costs and hazards associated with the latter. To date, most of the repurposed drugs are a result of serendipitous discovery through careful observations by physicians, medical staffs and basic researchers. Repurposing approaches involving experimental screening and computational approaches are

P. Joon · G. Sharma · K. Sharma · M. Kadian
University Institute of Pharmaceutical Sciences (UIPS), UGC Centre for Advanced Study, Panjab University, Chandigarh, India

M. Dahiya
University Institute of Pharmaceutical Sciences (UIPS), UGC Centre of Advanced Studies (UGC-CAS), Panjab University, Chandigarh, Punjab, India

University Institute of Pharmaceutical Sciences (UIPS), UGC Centre of Advanced Studies (UGC-CAS), Panjab University, Chandigarh, Haryana, India

E. Ray
University Institute of Pharmaceutical Sciences (UIPS), UGC Centre for Advanced Study, Panjab University, Chandigarh, India

Institute of Nano Science and Technology (INST), Mohali, Punjab, India

R. C. Sobti · S. Puri
Department of Biotechnology, Panjab University, Chandigarh, India

A. Kumar (✉)
University Institute of Pharmaceutical Sciences (UIPS), UGC Centre of Advanced Studies (UGC-CAS), Panjab University, Chandigarh, Punjab, India

University Institute of Pharmaceutical Sciences, UGC Centre of Advanced Studies (UGC-CAS), Panjab University, Chandigarh, Haryana, India
e-mail: kumaruips@pu.ac.in

R. C. Sobti et al. (eds.), *Drug Repurposing for Emerging Infectious Diseases and Cancer*, https://doi.org/10.1007/978-981-19-5399-6_1

already developed to increase the speed and ease of the repurposing process. With the advancement of technologies such as proteomics, genomics, transcriptomics, metabolomics and the availability of massive databases resources such as drug omics data, disease omics data and so on, there are a plethora of opportunities to discover drugs by combining all of the above methods/approaches.

Keywords Drug repurposing · Genomics · Proteomics · Transcriptomics

1.1 Introduction

Translation of fundamental research findings into meaningful medicinal breakthroughs is an essential objective of biomedical research. Despite advancements in drug discovery techniques, developing medications for diverse ailments still remains challenging (Gribkoff and Kaczmarek 2017). As a result, novel techniques such as drug repurposing are required to develop therapeutics to treat both common and rare disorders. Attempts to create novel remedies for diseases are typically expensive and unsatisfactory, necessitating both extensive timeframes and large expenditures. The repurposing of safe existing medications to new indications, on the other hand, offers a cost-effective and time-saving alternative (Morofuji and Nakagawa 2020). Drug repurposing is a revolutionary method of discovering new applications for existing medications that are not covered by the original medical indication (Pushpakom 2022). Drug repurposing makes advantage of the adaptability of approved medications to reassign them to a new function (Nosengo 2016). Other phrases commonly employed in this context include drug repositioning, drug reprofiling and drug re-tasking, all of which have somewhat different meanings but are used interchangeably with drug repurposing. This alternate method of drug discovery fast-tracking is gaining popularity (Morofuji and Nakagawa 2020). Some of the early examples of repurposing depended on serendipity and retrospective clinical experience, resulting in the effective repurposing of previously unsuccessful medications such as thalidomide and sildenafil in a variety of illness situations. Modern repurposing methodologies, on the other hand, make use of an ever-expanding pool of drug- and disease-related data, computationally driven hypothesis development and high throughput screening methods to identify fresh applications for existing medications (Pushpakom 2022). Furthermore, systematic drug repurposing involving network analysis, data mining and machine learning is also expected to play an important role in future treatment developments.

1.2 Rationale of Drug Repurposing

Drug development is a complicated, time-consuming and expensive process with high failure rates. An average of 12–15 years is required for a drug to be approved (Wouters et al. 2020). The investment necessary to get it rises exponentially as the drug progresses through the regulatory development phase, which precedes clinical stages,

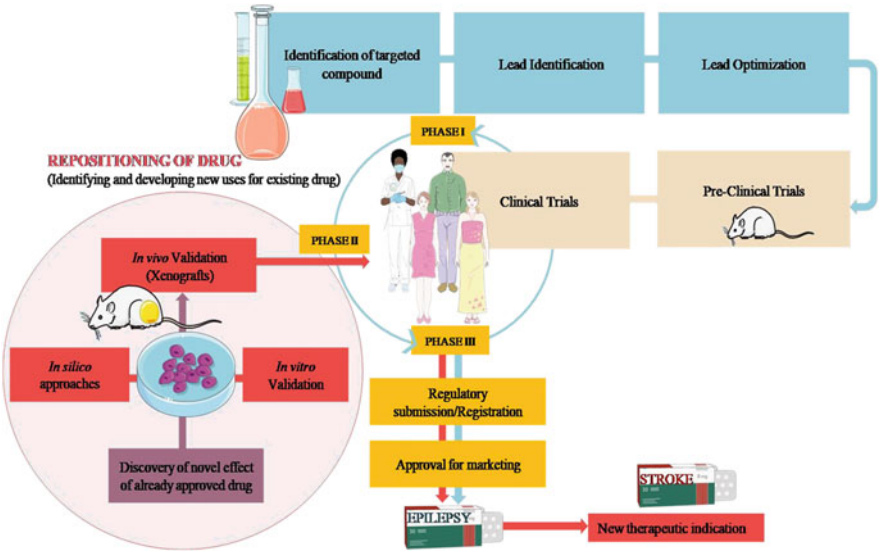


Fig. 1.1 Traditional drug discovery versus drug repurposing

until it is eventually approved for marketing by the respective regulatory bodies. Furthermore, clinical translation of results from sophisticated animal and cellular models is limited (Leenaars et al. 2019). In this context, drug repurposing has gained prominence in recent years as a means of expediting the drug development process (Pushpakom et al. 2019) (Fig. 1.1). Drug repurposing is a potential approach in drug discovery for identifying new therapeutic applications for existing medications that are distinct from the original medical indication (Cantrell et al. 2021). Drug repurposing, using known drugs and compounds for new indications, offers a number of advantages over traditional approaches to de novo drug discovery and development, as these 'old drugs' have already been proven safe in humans. The advantages include (a) Faster drug development timeline compared to the conventional method; (b) reduced healthcare cost; (c) faster regulatory approval; (d) risk reduction; (e) higher odds of success and faster investment return and (f) further understanding of disease mechanisms which may lead to development of novel entities structurally similar but more potent to the repurposed drug (Cantrell et al. 2021; Pushpakom 2022). In recent years, drug repurposing has emerged as a viable strategy to increase the overall productivity of drug discovery. According to estimates, drug repurposing can possibly make a treatment ready for usage in patients within 3–12 years at a total cost of $40–80 million, as opposed to at least 13–15 years and a cost of $2–3 billion for creating a new drug (Cha et al. 2018; Pushpakom 2022). Drug repurposing is also seen to be an acceptable strategy for discovering treatments for orphan and rare diseases, and it is predicted to play a significant role in this area in the future. Indeed, despite the fact that rare diseases impact over 350 million people globally, creating de novo therapies for their limited individual markets is not profitable enough to attract economic interest (Gelosa et al. 2020a, b).

1.3 Role of Drug Repurposing in Conventional Pharmaceutical Market

Over the last 30 years, it has been abundantly evident that the pharmaceutical business is experiencing an ever-increasing productivity gap (Scannell et al. 2012). Despite greater expenditures in cutting-edge technology and a better understanding of numerous human diseases, in conjunction with advancements in fields such as genomics and proteomics, the pharmaceutical industry has struggled to translate these into viable therapeutic outcomes. The global pharmaceutical industry is confronting a high medication attrition rate, rising drug development costs ($2–3 billion per medicine), and increased delay to bring novel chemical entities to market (average of 12 years) (Pushpakom 2022). Many lead compounds that demonstrate success in pre-clinical studies fail in later clinical trials. Rising research costs, high failure rates and an ever-increasing time to bring a molecule from bench to approval have made the pharmaceutical sector a less appealing investment. The pharmaceutical sector returns less than a $1 for every dollar invested on research and development (R&D). Drug repurposing is largely seen as a viable solution to this 'problem' (Pushpakom 2022). It is frequently appropriate to repurpose medications or failed drug candidates for new use (Naylor 2015). Most successful drug repositioning cases are aimed to repurposing drugs for a new indication (Pantziarka et al. 2018). However, most drug repositioning cases occur more by chance than a systematical design (Huang 2020). Identification of new indications for existing drugs by drug repurposing has the potential to supplement traditional drug development by reducing the substantial monetary and time costs and hazards associated with the latter (Cha et al. 2018). A prospective repurposing medicine will have a well-established safety and toxicity profile, with data previously amassed in preparation for regulatory approval. Repurposing a medicine with an existing favourable safety profile onto the market for a different indication not only saves time but also increases possible returns on investment (Ashburn and Thor 2004; Pushpakom 2022).

1.4 Roadmap to Modern Drug Repurposing

Historically, drug repurposing has largely been an unintended, fortuitous process that occurs when a medicine is discovered to have an off-target impact or a previously undetected on-target effect that might be put to another purpose. The discovery of two of the most successful medication repurposing instances, thalidomide and sildenafil citrate, was entirely inadvertent and serendipitous, and was based on retrospective clinical experience. Sildenafil, which was originally developed for angina pectoris and failed as a cardiovascular drug, has been repurposed for the treatment of erectile dysfunction and, subsequently, pulmonary arterial hypertension (Ghofrani et al. 2006). In March 1998, the FDA approved Viagra (sildenafil) for the treatment of men with erectile dysfunction. It was approved for the treatment of patients suffering from pulmonary arterial hypertension by the FDA in 2005

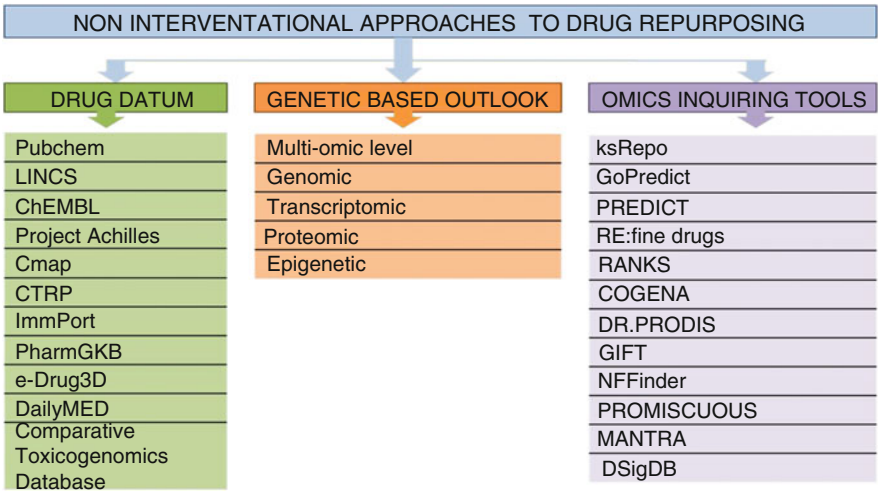


Fig. 1.2 Systematic approach to repurposing

(Pushpakom 2022). Thalidomide, which was originally developed as a sleep-inducing drug but discontinued due to foetal teratogenicity, is now repurposed for use in Erythema Nodosum Leprosum and also used against multiple myeloma (Kim and Scialli 2011). In 1998, the FDA approved thalidomide for the treatment of ENL. Thalidomide in combination with Dexamethasone was officially approved by the FDA in 2006 for the treatment of multiple myeloma (Pushpakom 2022). The need for novel approaches to medication research and development, along with the emergence of large data repositories and accompanying analytical technologies, has fuelled interest in creating systematic ways to drug repurposing in recent years (Cha et al. 2018). A systematic strategy based on drug- and disease-related data, utilizing the power of high-performance computational tools, and employing high-throughput screening procedures (termed as 'systematic repurposing'), has emerged as the way ahead in drug repurposing (Fig. 1.2). The majority of repurposing endeavours presently rely on systematic repurposing approaches, which may be broadly classified as experimental screening approaches and in silico approaches that use existing data to uncover possible novel drug–disease connections (Pushpakom 2022).

1.5 Drug Repurposing Strategies and Approaches

Through the drug repurposing approach, commercial compounds can be identified for new therapeutic uses (Tables 1.1 and 1.2) that complements the traditional drug research method by reducing time and cost. Before considering the candidate medicine for repurposing, drug repurposing procedures include three steps.

Table 1.1 Breakthrough discoveries using drug repurposing

Disease/targeted area	Drug	First marketed for	Status	Remarks
Central nervous system (CNS)	Galantamine	Paralysis	FDA approved	
	Dimethyl fumarate	Psoriasis	FDA approved	
	Verapamil	Hypertension angina pectoris arrhythmia	Phase 4	NCT03150524
	Bumetanide	Liver disease heart failure stubborn oedema acute and chronic renal failure	Phase 3	NCT04766177
	Minocycline	Anti-bacterial	Phase 3	NCT01828203
	Fenfluramine	Simple obesity diabetes hypertension	Phase 4	NCT05232630
	Propranolol	Hypertension supraventricular tachycardia prolonged Q-T interval thyrotoxicosis	FDA-approved	
	Angiotensin receptor blocker	Essential hypertension renal disease diabetes	Phase 2	NCT04574713
	Amantidine	Antiviral	FDA-approved	
	Abatacept	Rheumatoid arthritis	Phase 2	NCT01116427
	Ceftriaxone	Anti-bacterial	Phase 3	NCT00349622
	Lenalidomide	Myelodysplastic syndrome; MM, multiple myeloma	Phase 2	NCT00067743
	Pioglitazone	Type II diabetes	Phase 2	NCT00690118
	Levetiracetam	Cortical myoclonus	Phase 2	NCT01463033
	Nilotinib	Anti-cancer	Phase 2	NCT03205488
	Cysteamine bitartrate	Nephropathic cystinosis	Phase 2, 3	NCT02101957
	Laquinimod	Immunomodulatory drug in multiple sclerosis	Phase 2	NCT02215616
	Deferiprone	Thalassemia	Phase 2	NCT00897221
	Omavelexolone	Anti-cancer drug	Phase 2	NCT02255435
	Liraglutide, exenatide	Anti-diabetic	Phase 3	NCT03672812
	Edaravone	Acute ischaemic stroke	Phase 3	NCT00424463
	Ambrexol	Expectorant	Phase 2	NCT02914366
	Thalidomide	Multiple myeloma	Phase 2, 3	NCT01094340
	Daratumumab	Refractory multiple myeloma	Phase 2	NCT04070378
	Mifepristone	Abortive	Phase 3	NCT00867360
	Baclofen	Muscle relaxant	Phase 1	NCT00614328
	Tofacitinib	Rheumatoid arthritis	Phase 2	NCT04799262
	Atorvastatin	Hypercholesterolemia, hyperlipidaemia	Phase 4	NCT02225834

(continued)

Table 1.1 (continued)

Disease/targeted area	Drug	First marketed for	Status	Remarks
	Metformin	Type II diabetes	Phase 3	NCT02593097
	Ketamine	Anaesthetic	Phase 2	NCT01558063
	Mecamylamine	Malignant hypertension	Phase 4	NCT03914677
	Fingolimod	Transplant rejection	Phase 2	NCT04629872
	Limaprost	Anti-platelet agent	Phase 3	NCT02125981
	Cycloserine	Tuberculosis	Phase 2	NCT01343862
	Etanercept	Autoimmune diseases	Phase 2	NCT01068353
	Brexpiprazole	Atypical antipsychotic	Phase 3	NCT01922258
	Carmustine	Chemotherapy	Phase 2	NCT04222062
	Butylphthalide	Hypertension	Phase 4	NCT05068349
	Sevoflurane	General anaesthetic	Phase 2	NCT02946437
	Filgrastim	Anti-cancer	Phase 2	NCT03656042
Respiratory system	Tofacitinib	Rheumatoid arthritis	Phase 2	NCT04332042
	Ruxolitinib	Myelofibrosis, polycythaemia	Phase 2	NCT04354714
	Dupilumab	Anti-cancer	Phase 2	NCT03595488
	Dactosilib	Anti-cancer	Phase 3	NCT04139915
	Tamoxifen	Oestrogen-dependent breast cancer	Phase 2	NCT03528902
	Statins	Hypercholesterolemia, Hyperlipidaemia	Phase 4	NCT01151306
	Metformin	Type II diabetes	Phase 4	NCT01247870
	Pioglitazone	Type II diabetes	Phase 2	NCT00994175
	Pazopanib	Advanced/metastatic renal cell carcinoma,	Phase 2, 3	NCT03850964
		Advanced soft tissue sarcomas		
	Azithromycin	Antibiotic	Phase 3	NCT00360464
	Inhaled amphotericin B	Anti-fungal	Phase 3	NCT00425620
	Oral triazoles;	Anti-fungal	Phase 4	NCT02663674
	Glycopyrronium	Peptic ulcers	FDA approved	
	Remdesivir		Phase 3	NCT04257656
	Baricitinib	Anti-arthritis	Phase2, 3	NCT04358614
	Tocilizumab		Phase 2	NCT05181397
	Chloroquine	Anti-malarial	Phase 2	NCT04323527
	Nitazoxanide	Anthelmintic and antiviral	Phase 2, 3	NCT04463264
	Prulifloxacin	Antibiotic	Phase 4	NCT01710488
	Itolizumab	Psoriasis	Phase 2	NCT04475588
	Duvelisib	Anti-cancer	Phase 2	NCT04372602
	Bictegravir	Anti-viral	Phase 2	NCT04734652
	Favipiravir	Anti-viral	Phase 3	NCT02026349
	Ribavirin,	Anti-viral	Phase 3	NCT00014391

(continued)

Table 1.1 (continued)

Disease/targeted area	Drug	First marketed for	Status	Remarks
	Lopinavir	Anti-viral	Phase 2	NCT04307693
	Tocilizumab		Phase 2	NCT05181397
	Azathioprine		Phase 3	NCT00052039
	Ivermectin		Phase 3	NCT04703608
	Fluoroquinolones	Anti-biotics	Phase 3	NCT04879030
	Pyrimethamine	Anti-malarial	Phase 3	NCT00000727
	Clofazimine	Leprosy	Phase 2	NCT01691534
	Sildenafil	Pulmonary arterial hypertension	Phase 2	NCT05061368
	Interleukin-2	Malignant neoplasm (trachea, bronchus, lung), squamous cell carcinoma, and lymphomas	Phase 4	NCT04766307
	Meropenem	Antibiotic	Not disclosed	NCT04402359
	Cycloserine	Urinary tract infection	Not disclosed	NCT04545788
	Azacitidine	Anti-cancer	Phase 1,2	NCT03941496
	Ibuprofen	Analgesic	Phase 2	NCT02781909
	Doxycycline	Anti-biotic	Phase 2	NCT02774993
	Linezolid	Anti-biotic	Phase 2	NCT00727844
	Nitazoxanide	Anti-parasitic and antiviral	Phase 2	NCT02684240
Cardiovascular system	Meropenem	Antibiotic	Not disclosed	NCT04402359
	Cycloserine	Urinary tract infection	Not disclosed	NCT04545788
	Azacitidine	Anti-cancer	Phase 1, 2	NCT03941496
	Ibuprofen	Analgesic	Phase 2	NCT02781909
	Doxycycline	Anti-biotic	Phase 2	NCT02774993
	Linezolid	Anti-biotic	Phase 2	NCT00727844
	Nitazoxanide	Anti-parasitic and antiviral	Phase 2	NCT02684240
	Metformin	Type II diabetes mellitus	Under clinical trial	NCT03331861, NCT03514108, NCT02252081
	Exenatide	Type II diabetes mellitus	Under clinical trial	NCT02404376, NCT02673931
	Liraglutide	Type II diabetes mellitus	Under clinical trial	NCT02673931
	Sildenafil	Angina	Under clinical trial	NCT03356353, NCT01616381
	Tadalafil	Erectile dysfunction	Under clinical trial	NCT02611336, NCT02611258
	Allopurinol	Gout	Under clinical trial	NCT03700645, NCT03745729
	Nevido	Testosterone therapy	Phase 4	NCT00131183

(continued)

Table 1.1 (continued)

Disease/targeted area	Drug	First marketed for	Status	Remarks
	Cilostazol	Antiplatelet agent	Phase 3	NCT02094469
	Zibotentan	Anti-cancer	Phase 2	NCT04097314
	Rosiglitazone	Anti-diabetic	Phase 4	NCT00225355
	Sodium zirconium cyclosilicate	Hyperkalaemia	Phase 2	NCT03532009
	Finerenone	Chronic kidney disease	Phase 3	NCT04435626
	Acipimox	Hypolipedemic agent	Not disclosed	NCT00549614
	Empagliflozin	Anti-diabetic	Phase 2	NCT03128528
	Abatacept	Rheumatoid arthritis	Phase 2	NCT02232880
	Melatonin	Jet lag	Phase 2	NCT05257291
	Mirabegron	Overactive bladder	Phase 2	NCT01876433
	Rivaroxaban	Deep vein thrombosis	Phase 2	NCT03178864
	Obicetrapib	Hypolipidemic	Phase 3	NCT05202509
	Dapagliflozin	Anti-diabetic	Phase 4	NCT03030235
	Sitagliptin	Anti-diabetic	Phase 4	NCT02655757
Miscellaneous diseases				
Psoriasis	Cyclosporine	Rheumatoid arthritis	FDA approved	
	Methotrexate	Cancer	FDA approved	
Plaque psoriasis	Etanercept	Rheumatoid arthritis	FDA approved	
Rheumatoid arthritis	Methotrexate	Cancer	FDA approved	
	Rituximab	Cancer	FDA approved	
Neonatal-onset multisystem inflammatory disease	Anakinra	Rheumatoid arthritis	FDA approved	
Gastrointestinal stromal tumour	Imatinib	Chronic myelogous leukaemia	FDA-approved	
Influenza A	Amantadine	Parkinson's disease	FDA-approved	
Antiplatelet	Acetylsalicylic acid	Inflammation, pain relief	FDA-approved	
Transplant rejection	Cyclosporine	Rheumatoid arthritis	FDA-approved	
Alopecia	Minoxidil	Hypertension	FDA-approved	
Leprosy	Clofazime	Tuberculosis	FDA-approved	
Fibromyalgia	Milnacipran	Depression	FDA-approved	
	Duloxetine	Depression	FDA-approved	
	Pregabalin	Anti-convulsant and neuropathic pain	FDA-approved	
Atopic dermatitis	Doxepin	Depression	FDA-approved	
HIV/AIDS	Zidovudine	Cancer	FDA-approved	

(continued)

Table 1.1 (continued)

Disease/targeted area	Drug	First marketed for	Status	Remarks
Type II diabetes	Bromocriptine	Parkinson's disease	FDA-approved	
Diabetic neuropathic pain	Duloxetine	Depression	FDA-approved	
Multiple myeloma	Thalidomide	Morning sickness	FDA approved	
Hirutism	Eflornithine	Cancer	FDA-approved	
Pemphigus vulgaris	Rituximab	Cancer	FDA-approved	
Menopausal hot flushes	Paroxetine	Anti-depressant	FDA-approved	
Erectile dysfunction	Sildenafil	Angina	FDA-approved	
Erythema nodosum leprosum	Thalidomide	Morning sickness	FDA-approved	
Autoimmune lympho-proliferative syndrome (ALPS)	Rapamycin	Organ transplant rejection	FDA-approved	
Multiple sclerosis	Dimethyl fumarate	Psoriasis	FDA-approved	
	Clemastine	Allergic rhinitis	Under clinical trial	NCT02040298
	Quetiapine	Schizophrenia	Under clinical trial	NCT02087631
Leishmaniasis	Amphotericin	Anti-fungal	FDA-approved	
	Miltefosine	Cancer	FDA-approved	
Human African trypanosomiasis	Eflornithine	Cancer	FDA-approved	
Chagas disease	Posaconazole	Anti-fungal	Under clinical trial	NCT01377480
	Azathioprine		Phase 3	NCT00052039
	Ivermectin		Phase 3	NCT04703608
	Fluoroquinolones	Anti-biotics	Phase 3	NCT04879030
	Pyrimethamine	Anti-malarial	Phase 3	NCT00000727
	Clofazimine	Leprosy	Phase 2	NCT01691534
	Sildenafil	Pulmonary arterial hypertension	Phase 2	NCT05061368
	Interleukin-2	Malignant neoplasm (trachea, bronchus, lung), squamous cell carcinoma and lymphomas	Phase 4	NCT04766307

(continued)

Table 1.1 (continued)

Disease/targeted area	Drug	First marketed for	Status	Remarks
	Meropenem	Antibiotic	Not disclosed	NCT04402359
	Cycloserine	Urinary tract infection	Not disclosed	NCT04545788
	Azacitidine	Anti-cancer	Phase 1,2	NCT03941496
	Ibuprofen	Analgesic	Phase 2	NCT02781909
	Doxycycline	Anti-biotic	Phase 2	NCT02774993
	Linezolid	Anti-biotic	Phase 2	NCT00727844
	Nitazoxanide	Anti-parasitic and antiviral	Phase 2	NCT02684240

- Identify the candidate medicine for a specific ailment by generating hypotheses.
- Evaluation of efficacy in phase II clinical trials (assuming that adequate safety data are available from phase I trials of original indication).
- Mechanistic evaluation of the drug's therapeutic potential in preclinical studies. Furthermore, after selecting the appropriate medicine for an indication of choice, several methodologies, such as systematic approaches, can be employed for drug repurposing. The systemic method is further separated into computational and experimental approaches, both of which are used synergistically.

1.5.1 Computational Approaches

This technique is mostly data-driven, involving the systematic review of data such as chemical structure, gene expression, electronic health records, genotyping and proteomic data to generate repurposing hypotheses (Hurle et al. 2013). Below are the most often utilized computational methodologies for medication repurposing (Chen 2021).

Signature matching: It is based on comparing a drug's 'signature', or unique marker, which can be generated from a database of medications such as proteome, transcriptome (RNA) and metabolomic data, as well as adverse event profiles and chemical structures, to another disease, drug or clinical characteristic (Keiser et al. 2009). The method of matching transcriptome signatures can be used to compare drug–disease and drug–drug similarity (Iorio et al. 2013). In the first case, a drug's transcriptomic signature is determined by comparing the genetic expression of cells or tissues before and after administration of the drug; the resulting molecular signature of the drug is then compared to a disease-linked expression profile determined similarly by comparing disease versus healthy conditions. The computational technique is based on the signature reversion principle (SRP), which states that if a medicine has the ability to reverse the genetic expression that is associated with a given disorder phenotype, then that drug may also be able to reverse the disease phenotype. Furthermore, because this premise is so basic, it has been

Table 1.2 Drug repurposed for the treatment of cancer

Category	Drug name	Repurposed in	Already in use	Status	Remarks
Cardiovascular drugs	Losartan	Glioblastoma	Hypertension	Phase 2	NCT03951142
		Osteosarcoma		Phase 1	NCT03900793
		Pancreatic cancer		Phase 2	NCT03563248
	Captopril	Infantile haemangioma	Hypertension	Phase 4	NCT04288700
		Lung cancer		Phase 2	NCT00077064
	Verapamil	Hodgkin lymphoma	Hypertension	Phase 1	NCT03013933
		Brain cancer		Phase 2	NCT00706810
	Carvedilol	Glioblastoma	Hypertension	Early phase 1	NCT03861598
		Breast cancer		Phase 2	NCT02993198
	Propranolol	Gastric cancer	Hypertension	Phase 2	NCT04005365 NCT02596867
		Breast cancer		Phase 2	NCT04493489
		Bladder cancer		Phase 2	NCT03838029 NCT03152786
		Pancreatic neoplasms		Phase 2	NCT01988831
		Prostate carcinoma		Phase 2	NCT04682158
		Skin melanoma		Phase 2	NCT01056341
		Oesophageal adenocarcinoma		Phase 2	NCT03523650
		Infantile haemangioma		Phase 2/3	
		Cavernous malformations		Phase 1	
	Atenolol	Haemangioma	Hypertension	Phase 3	NCT02342275
		Infantile haemangioma		Phase 3	NCT03237637
	Digoxin	Head and neck cancer	Arrhythmias	Phase 1/2	NCT02906800
		Prostate cancer		Phase 2	NCT01162135
		Malignant melanoma		Phase 1	NCT01765569
		Melanoma		Phase 1	NCT02138292
	Pitavastatin	Breast cancer	Hypercholesterolemia	Phase 2/3	NCT04705909
		Acute myeloid leukaemia		Phase 1	NCT04512105

	Simvastatin	Breast cancer	Hypercholesterolemia	Phase 2	NCT03324425
		Pancreatic cancer		Phase 2	NCT00944463 NCT01110785
		Colorectal cancer		Phase 2	NCT02161822
		Adenocarcinoma of rectum		Phase 2	NCT01332617
		Myeloma		Phase 2	NCT00281476
		Multiple myeloma		(Phase 1/2)	NCT00828282
		Chronic lymphocytic leukaemia		(Phase 1)	
	Atorvastatin	Prostate cancer	Hypercholesterolemia	Phase 3	NCT04026230
		Prostatic neoplasms		Phase 2	NCT01821404 NCT02767362
		Endometrial cancer		Early Phase 1	NCT03560882
		Malignant disease		Phase 1	NCT00490698
		Kidney cancer		Phase 2	NCT01491958
		Acute myelogenous leukaemia		Phase 2	NCT00164086
		Myeloma		Phase 1	NCT02029573
		Glioblastoma multiforme		Phase 2	NCT02819869
		Hepatocellular carcinoma		Phase 2	
	Lovastatin	Breast cancer	Hypercholesterolemia	Phase 2	NCT00285857
		Ovarian cancer		Phase 2	NCT00585052
		Acute myeloid leukaemia		Phase 1/2	NCT00583102
		Melanoma		Phase 2	NCT00963664
		Neurofibromatosis type 1		Phase 2	NCT00853580
	Verapamil	Brain cancer	Phenylalkylamine calcium channel blocker used in the the treatment of high blood pressure	Phase 2	NCT00706810
		Recurrent Hodgkin lymphoma		Phase 1	NCT03013933

(continued)

Table 1.2 (continued)

Category	Drug name	Repurposed in	Already in use	Status	Remarks
	Sildenafil	Pancreatic cancer	Hypertension	Phase 1	NCT02106871
		Lung cancer		Phase 2/3	NCT00752115
		Solid tumour		Phase 1	NCT02466802
		Lymphatic malformations		Phase 2	NCT02335242
		Glioblastoma		Phase 2	NCT01817751
		Lymphangioma		Phase 1/2	NCT01290484
		Waldenstrom's macroglobulinemia		Phase 2	NCT00165295
Microbiological agents	Chloroquine	Lung cancer	Anti-malarial	Phase 1	NCT01575782
		Breast cancer		Phase 2	NCT02333890
		Malignant neoplasm		Phase 1	NCT02071537
		Carcinoma		Phase 1/2	NCT01023477
		Glioma		Phase 1/2	NCT02496741
		Brain metastasis		Phase 2	NCT01894633
		Glioblastoma multiforme		Phase 3	NCT00224978
		Glioblastoma		Phase 2	NCT02432417
	Artemisinin	Ovarian cancer	Anti-malarial	Phase 1	NCT04805333
	Quinacrine	Prostatic cancer	Anti-malarial	Phase 2	NCT00417274
		Lung cancer		Phase 1	NCT01839955
		Colorectal adenocarcinoma		Phase 1/2	NCT01844076
		Renal cell carcinoma		Phase 2	NCT00574483
	Itraconazole	Prostate cancer	Anti-fungal	Phase 2	NCT00887458
		Lung cancer		Phase 2	NCT03664115
		Oesophageal cancer		Early Phase 1	NCT02749513
		Ovarian cancer		Phase 1/2	NCT03081702
		Solid tumours		Phase 1	NCT01900028
		Advanced solid tumours		Phase 1	NCT02259010

	Ketoconazole	Prostate cancer	Anti-fungal	Phase 2	NCT00895310
		Breast cancer		Phase 2	NCT00212095
		Granulosa cell tumour of the ovary		Phase 2	NCT01584297
		Advanced cancer		Phase 1	NCT00708591
		Solid tumours		Phase 2	NCT00697437
	Ciprofloxa cin	Pancreatic ductal adenocarcinoma	Gram-negative bacterial infection	Phase 1	NCT04523987
		Leukaemia		Phase 1/2	NCT02773732
		Bladder cancer		Phase 3	NCT00003824
		Prostate cancer		Phase 2	NCT02252978
Antibiotics	Doxycycline	Pancreatic cancer	Anti-malarial	Phase 2	NCT02775695
		Breast carcinoma		Phase 2	NCT02874430
		Head and neck squamous cell carcinoma		Phase 2	NCT03076281
		Lymphangioleiomy omatosis		Phase 4	NCT00989742
		Cutaneous T-cell lymphoma		Phase 2	NCT02341209
		Melanoma		Phase 1/2	NCT01590082
Anti-viral drugs	Ritonavir	Breast cancer	Anti-retroviral	Phase 1	NCT01009437
	Nelfinavir	Non-Hodgkin lymphoma/Hodgkin lymphoma/gastric cancer/nasopharyn-geal cancer	Anti-retroviral	(Early Phase 1)	NCT02080416
		Colorectal cancer		Phase 1/2	NCT00704600
		Head and neck neoplasms		Phase 2	NCT01065844
		Non-small cell lung cancer		Phase 2	NCT00791336
		Glioblastoma		Phase 1	NCT00694837
		Glioma		Phase 1	NCT01020292
	Maraviroc	Metastatic colorectal cancer	Anti-retroviral	Phase 1	NCT03274804
		Hematologic malignancy		Phase 2	NCT01785810

(continued)

Table 1.2 (continued)

Category	Drug name	Repurposed in	Already in use	Status	Remarks
Anti-inflammatory drugs	Aspirin	Colorectal cancer	COX-2 inhibitor-NSAID- pain and inflammation	Phase 3	NCT02607072 NCT02813824
		Lynch syndrome		Phase 3	NCT02497820
		Lynch syndrome I		Phase 3	NCT02467582
		Colon cancer		Phase 3	NCT03170115
		Rectal cancer		Phase 2	NCT04214990
		Gastric cancer		Phase 3	NCT03491410
		Breast cancer		Phase 2/3	
		Node positive HER2 negative breast cancer		Phase 3	NCT02927249
		Fallopian tube cancer		Early Phase 1	NCT03771651
		Non-small cell lung cancer		Phase 3	NCT01058902
		Urinary bladder neoplasms		Phase 4	NCT02350543 (Phase 4)
		Oesophageal squamous cell carcinoma		Early Phase 1	NCT03900871
		Cutaneous melanoma		Phase 2	NCT03396952
		Nasopharyngeal carcinoma		Phase 2	NCT03290820
		Glioblastoma		Phase 2	NCT00790452
		Melanoma		Phase 2	NCT04062032
	Celecoxib	Endometrium cancer	COX-2 inhibitor- NSAID- pain and inflammation	Phase 2	NCT03896113
		Breast cancer		Phase 3	NCT02429427
		Metastatic cancer		Phase 2	NCT03864575
		Oral squamous cell carcinoma		Phase 2	NCT02739204
		Lung cancer		Phase 2	NCT00020878
		Prostate cancer		Phase 2	NCT00022399
		Recurrent respiratory papillomatosis		Phase 2	NCT00571701

		Cervix neoplasms		Phase 1/2	NCT00152828
		Head and neck cancer/lung cancer		Phase 2	NCT00527982
		Non-muscle invasive bladder cancer		Phase 2	NCT02343614
		Head and neck cancer		Phase 2	NCT00061906
		Smouldering multiple myeloma		Phase 2	NCT00099047
		Recurrent bladder cancer		Phase 2/3	NCT00006124
		Uterine cancer		Phase 2	NCT00231829
		Cervical carcinoma		Phase 2	NCT00081263
		Non-small cell lung cancer		Phase 1	NCT00108186
		Lymphangioleiomy omatosis		Phase 2	NCT02484664
	Memantine	Glioblastoma	Alzheimer's disease	Phase 2	NCT01260467
Antipsychotic drugs	Chlorpromazine	Glioblastoma multiforme/MGMT-Unmethylated unmethylated glioblastoma	Schizophrenia	Phase 2	NCT04224441
	Fluphenazine	Multiple myeloma and plasma cell neoplasm	Schizophrenia	Phase 1/2	NCT00335647
		Multiple myeloma		Phase 1	NCT00821301
Anti-depressants	Imipramine	Breast cancer	Enuresis and depression	Early Phase 1	NCT03122444
Miscellaneous drugs	Metformin	Breast cancer	Anti-diabetic	Phase 2/3	NCT04387630
		Prostate cancer		Phase 3	NCT03031821
		Endometrial cancer		Phase 3	NCT04792749
		Colorectal cancer		Phase 2	NCT01926769
		Colon cancer		Phase 1	NCT01440127
		Rectal cancer		Phase 2	NCT02437656
		Bladder cancer		Phase 2	NCT03379909
		Head and neck cancer		Early Phase 1	NCT02402348
		Head and neck squamous cell cancer		Early Phase 1	NCT02083692
		Non-small cell lung cancer		Phase 2	NCT01997775

(continued)

Table 1.2 (continued)

Category	Drug name	Repurposed in	Already in use	Status	Remarks
		Non-small cell lung cancer/Lung cancer		Phase 2	NCT03086733
		Well-differentiated neuroendocrine tumours hepatocellular carcinoma		Phase 2	NCT02279758
		Chronic lymphocytic leukaemia		Phase 2	NCT01750567
	Pioglitazone	Lung cancer	Anti-diabetic	Phase 2	NCT00780234
		Thyroid cancer		Phase 2	NCT01655719
		Non-small cell lung cancer		Phase 2	NCT00923949
		Pancreatic cancer		Phase 2	NCT01838317
		Prostate cancer		Early Phase 1	NCT04658849
		Skin squamous cell cancer		(Phase 2)	NCT02347813
	Mebendazole	Medulloblastoma/astrocytoma/glioblastoma/anaplastic astrocytoma/brain stem neoplasms/oligodendroblastoma/Anaplastic anaplastic oligodendroglioma/malignant glioma	Threadworms	Phase 1	NCT02644291
		High-grade glioma		(Phase 1)	NCT01729260
	Niclosamide	Colon cancer	Tapeworm infection	Phase 1	NCT02687009
		Colorectal cancer		Phase 2	NCT02519582
	Disulfiram	Metastatic breast cancer	Chronic alcoholism	Phase 2	NCT03323346
		Melanoma		Phase 1/2	NCT00256230
	Raloxifene	Endometrial cancer	Postmenopausal osteoporosis	Phase 2	NCT00004915

effectively used to identify innovative drug repurposing prospects across a wide range of therapeutic domains. Based on anticancer drug-resistance profiles, the SRP has also been successful in identifying medications that could be repositioned as chemo-sensitizers (Wagner et al. 2015; Hsieh et al. 2016; Mirza et al. 2017). Another method of medication repositioning signature matching is based on chemical structures and their therapeutical relevance. Furthermore, comparing the chemical signatures of two drugs to see if they have any molecular similarities could imply that they have similar therapeutical action. The method entails choosing a set of chemical traits for each drug and then building networks based on the shared chemical features. Chemical similarity approaches have their own set of limitations, such as errors in chemical structures and physiological activity that exists outside of the structural relationship (e.g. a metabolite of an initial drug with a modified chemical structure could be an active molecule), which may limit their use in drug repurposing (Dudley et al. 2011a, b).

Genome-wide association studies (GWAS): Following breakthroughs in genotyping methodology and the completion of the Human Genome Project, which reduced genotyping costs, GWAS research have been done primarily in the previous 10 years. GWAS aims to identify genetic variants associated with diseases' common shared mechanisms and provide deep insights into disease pathophysiology. The data generated may also be useful in identifying new drug targets, as some of the targets may be shared across diseases, resulting in therapeutic repositioning. Sanseau et al enhanced the USNHGRI (National Human Genome Research Institute) database of published GWAS traits and found that genes associated with illness characteristics are likely coded for proteins that are 'druggable' in comparison to the rest of the genome (Sanseau et al. 2012). Furthermore, Grover et al discovered that a bioinformatics approach can be used to match gene targets identified for coronary artery disease with drug information obtained from various drug–target databases such as DrugBank, PharmGKB and Therapeutic Target Database, which could be useful for identifying potential drug repositioning opportunities (Grover et al. 2015). Although there are certain limitations to using information from GWAS for drug repurposing, its utility is currently unclear.

Network/Pathway mapping: Reconstruct disease-specific pathways that give significant targets for repositioning medications using disease omics data, accessible signalling or metabolic pathways and protein interaction networks. These approaches have the advantage of being able to narrow down huge signalling networks to a specialized network with only a few proteins or target molecules. It has mostly been used to identify therapeutic targets and medicines with repurposing potential. As previously discussed, some potential targets identified using GWAS or other methods may become immediately acquiescent as drug targets; nonetheless, these genes may not always be excellent druggable targets. In such circumstances, a network/pathway-based method may provide a pool of data on genes that are either downstream or upstream of the GWAS-associated target, which can be investigated for drug repositioning potential (Greene and Voight 2016). Network analysis entails building drug or illness networks based on gene expression sequences, disease pathophysiology, drug–protein interactions and GWAS data to identify the best

repurposing molecule (Pushpakom et al. 2019). A recent study found that employing a network-wide association study (NetWAS) to explore disease–gene interaction by combining genetic variant data from GWAS with tissue-specific interaction networks is more efficient than using GWAS alone. In addition, a study found that pathway analysis of a gene expression database that covers a wide range of respiratory viruses in human host infection models identified 67 widely shared biological processes that play a crucial role in respiratory viral infections (Pushpakom et al. 2019). Furthermore, these pathways are compared to the Drug Bank, allowing numerous medicines with potential effects on host-viral targets to be tested. For instance, pranlukast is an LT-I (leukotriene 1) antagonist used to treat asthma, and amrinone is a phosphodiesterase (PDE) inhibitor used to treat congestive heart failure (CHF). Despite their propensity to modify immune cell responses, both of these medications have been shown to be effective in treating viral infections.

Computational molecular docking: Molecular docking is a structure-based computational method for predicting the ligand (drug) and target binding sites (receptor). If information about receptors and enzymes as targets that are pathologically involved in a disease already exists, various medicines could be tested against the specific target. Furthermore, drug libraries can be tested against a variety of target receptors using inverse docking, which involves numerous targets and one ligand, to identify novel interactions that can be used for repurposing. Dakshanamurthy et al used high-throughput screening to perform molecular fit computations on 3671 FDA-approved medicines versus 2335 human protein structures. According to these findings, the anti-parasitic medication mebendazole has the structural capacity to block vascular endothelial growth factor 2 (VEGFR2) is an angiogenesis mediator (Dakshanamurthy et al. 2012). However, there are a few drawbacks to using computational docking for medication repurposing. Because drug targets are typically membrane proteins, such as G protein-coupled receptors (GPCRs), 3D structures for some target proteins may not be available (Cooke et al. 2015). Furthermore, well-established macromolecular target databases that could provide correct structural information have flaws. Finally, the effectiveness of docking algorithms in predicting binding affinities has been called into question, as has variance in software predictability, such as mode of binding and entropic effects (Huang et al. 2018).

Retrospective clinical analysis: This method employed retrospective clinical data analysis to investigate the medication having repositioning potential. Furthermore, repurposing breakthrough from the retrospective clinical analyses approach is the use of aspirin in colorectal cancer, which is also helpful in preventing cardiovascular disease. Another drug, raloxifene, has been approved by the FDA for reducing the risk of breast cancer in postmenopausal females who are at a higher risk of the disease. Electronic health records (EHR), clinical trial data and post-marketing surveillance data are all good places to look for retrospective clinical data. EHRs contain a wealth of information about patient outcomes, both structured and unstructured, such as diagnostic and pathophysiological data and clinical descriptions of patient symptoms and signs, respectively (Pushpakom et al. 2019). Additionally, the data in EHRs could be used to recognize signals for drug repositioning and this

enormous EHR data also provides high statistical power. Despite the fact that there are numerous obstacles, including ethical and legal obstacles, retrieving the unstructured results available in this databank is tough. Other significant sources include post-marketing surveillance (PMS) and clinical trial data; however, access to this data is restricted for commercial and confidentiality reasons. As a result, there is a requirement to have access to this data in order to benefit future medication development research.

1.5.2 Experimental Approaches

Phenotypic screening: Compounds that have potential disease-specific effects in model systems without prior knowledge of the target are tested using this method. If the chemicals under examination are licenced or investigational, this could imply repurposing opportunities that can be pursued promptly in the context of medicine repurposing. In vitro phenotypic screens typically use a 96-well size and a range of cell-based testing. Iljin et al used four prostate cancer cell lines and two non-malignant prostate epithelial cell lines to conduct high-throughput cell-based screening of a library of 4910 drug-like small-molecule compounds, with proliferation as the primary endpoint (Iljin et al. 2009). They discovered that disulfiram, a drug used to treat alcoholism, is a selective anticancer agent, which they proved by genome-wide gene expression studies. Whole-organism phenotypic assays are also used in drug repurposing. Moreover, Cousin et al tested 39 FDA-approved medicines for tobacco addiction in a zebrafish model and discovered that apomorphine and topiramate changed nicotine- and ethanol-induced behaviour in this species (Pushpakom et al. 2019; Cousin et al. 2014).

Binding assays: Proteomic techniques like mass spectrometry and affinity chromatography have been used to investigate drug binding targets for various drugs. Analyses of drug targets and off-targets, as well as drug repurposing, have become natural bedfellows in the chemical biology era for target validation. The Cellular ThermoStability Assay (CETSA) assay, for example, has been developed to map target engagement in cells using biophysical concepts that anticipate thermal stabilization of target proteins by drug-like ligands with the proper cellular affinity. The confirmation of cellular targets for the tyrosine kinase inhibitor (TKI) crizotinib and the finding of quinone reductase 2 (NQO2) as a cellular off-target of acetaminophen (paracetamol) are two early triumphs with this technique (Pushpakom et al. 2019; Alshareef et al. 2016; Miettinen and Björklund 2014).

1.6 Opportunity and Challenges in Drug Repurposing

Drug repositioning, in contrast to traditional medication discovery which is a difficult and time-consuming procedure with high development costs and risk of failure, decreases the time and expense of drug development. Repositioning drugs is

another low-risk method. The performance of drug repositioning has been greatly enhanced using a computational or machine learning technique. Experimental procedures that provide a direct evidence-based understanding of linkages between medications and diseases are more reliable and believable than computational approaches. In recent years, however, computational tools have been frequently paired with experimental procedures to uncover novel indications for existing medications, a process known as mixed approaches. Biological experiments and clinical studies are used to validate computational approaches in this approach. The mixed approach to repositioning allows for a systematic and thorough examination of all repositioning options, taking into account enhanced access to databases and technology advancements. Furthermore, compared to traditional drug research, the R&D expenditure needed for drug repositioning is cheaper. As a result, drug repositioning allows numerous pharmaceutical companies to manufacture treatments at cheaper costs (Matthews et al. 2013). The mixed approach of DR allows for more successful and rapid development of repositioned medications. From a market standpoint, a vast number of diseases necessitate the development of novel medications, with possible market needs and economic implications. As a result, repositioning medications for the treatment of uncommon, neglected, orphan diseases or difficult-to-treat diseases is a possibility. There are about 6000 uncommon diseases for which there is no effective treatment. Approximately 5% of them are being studied. Due to the high attrition rates, high expenditure and slow process of drug discovery, repurposing already marketed drugs to treat both common and rare diseases is becoming a more appealing area of research (Talevi and Bellera 2020; de Oliveira and Lang 2018). It involves the use of drug molecules with a lower risk of failure and development time and cost. With the advancement of technologies such as proteomics, genomics, transcriptomics, metabolomics and the availability of massive databases resources such as drug omics data, disease omics data and so on, there are a plethora of opportunities to discover drugs by combining all of the above methods/approaches. Researchers now have the most up-to-date tools and data to investigate novel unknown mechanisms of action/pathways based on illness-specific target proteins, genes and specific biomarkers linked to disease development. Public databases and software for genomes, proteomics, metabolomics and pathway analysis are accessible. Researchers are currently equipped with the latest reliable tools and data to explore the novel unknown mechanism of actions/pathways based upon disease-specific target proteins/genes and specific biomarkers associated with the progression of the disease. Several computational strategies are already developed to increase the speed and ease of the repurposing process. However, opportunities come often with many challenges in drug repositioning. The identification of a new therapeutic indication for an existing drug poses a major challenge in repositioning. Drug repositioning, on the other hand, is a complicated process including a variety of aspects such as technology, patents, commercial models, and investment and market demands. Choosing the appropriate therapeutic area for the drug under investigation, as well as concerns with clinical studies, such as the necessity to start new trials from the beginning if data from preclinical or clinical trials for the existing drug or drug product is obsolete or unsatisfactory (Rudrapal et al. 2020; Pushpakom et al. 2019).

1.7 Conclusion

Despite a large rise in pharmaceutical company investment, the rate of new medication approvals stays consistent, owing mostly to high attrition rates. This, along with a significant unmet need in treating a wide range of disorders, generates an urgent need for innovation in bringing viable therapeutics to market. In recent years, drug repurposing has emerged as a feasible technique for increasing the overall productivity of drug discovery and development. Drug repurposing (also known as drug repositioning, reprofiling or re-tasking) is a promising area in drug discovery for uncovering new therapeutic applications for previously investigated medications. Drug repositioning may simply be summarized as expanding effective medications and renewing unsuccessful ones. Drug repurposing is a direct application of polypharmacology, which characterizes the drugs' potential to act on multiple targets and disease pathways. The identification of new molecular targets and pharmacological effects opens up new treatment avenues for clinically utilized medications. Drug repurposing holds the potential to bring medications with known safety profiles to new patient populations. This strategy provides for speedier drug development with less expenditure and risk than the development of a de novo drug. The majority of repurposing endeavours presently rely on systematic repurposing approaches that make use of an ever-expanding pool of drug- and disease-related data, computationally driven hypothesis development, and high-throughput screening methods to identify fresh applications for existing medications to increase the speed and ease of the repurposing process.

References

Alshareef A, Zhang HF, Huang YH, Wu C, Zhang JD, Wang P, El-Sehemy A, Fares M, Lai R (2016) The use of cellular thermal shift assay (CETSA) to study crizotinib resistance in ALK-expressing human cancers. Sci Rep 6(1):1–2. https://doi.org/10.1038/srep33710

Ashburn TT, Thor KB (2004) Drug repositioning: identifying and developing new uses for existing drugs. Nat Rev Drug Discov 3(8):673–683. https://doi.org/10.1038/nrd1468

Cantrell MS, Soto-Avellaneda A, Wall JD, Ajeti AD, Morrison BE, Warner LR, McDougal OM (2021) Repurposing drugs to treat heart and brain illness. Pharmaceuticals 14(6):573. https://doi.org/10.3390/ph14060573

Cha Y, Erez T, Reynolds IJ, Kumar D, Ross J, Koytiger G, Kusko R, Zeskind B, Risso S, Kagan E, Papapetropoulos S (2018) Drug repurposing from the perspective of pharmaceutical companies. Br J Pharmacol 175(2):168–180. https://doi.org/10.1111/bph.13798

Chen J (2021) Computational drug repurposing: a review in modern application. In: AIP conference proceedings, vol. 2350, no. 1, pp 020012. AIP Publishing LLC. https://doi.org/10.1063/5.0049200

Cooke RM, Brown AJ, Marshall FH, Mason JS (2015) Structures of G protein-coupled receptors reveal new opportunities for drug discovery. Drug Discov Today 20(11):1355–1364. https://doi.org/10.1016/j.drudis.2015.08.003

Cousin MA, Ebbert JO, Wiinamaki AR, Urban MD, Argue DP, Ekker SC, Klee EW (2014) Larval zebrafish model for FDA-approved drug repositioning for tobacco dependence treatment. PLoS One 9(3):e90467. https://doi.org/10.1371/journal.pone.0090467

Dakshanamurthy S, Issa NT, Assefnia S, Seshasayee A, Peters OJ, Madhavan S, Uren A, Brown ML, Byers SW (2012) Predicting new indications for approved drugs using a proteochemometric method. J Med Chem 55(15):6832–6348. https://doi.org/10.1021/jm300576q

de Oliveira EA, Lang KL (2018) Drug repositioning: concept, classification, methodology, and importance in rare/orphans and neglected diseases. J Appl Pharma Sci 8(8):157–165. https://doi.org/10.7324/JAPS.2018.8822

Dudley JT, Deshpande T, Butte AJ (2011a) Exploiting drug–disease relationships for computational drug repositioning. Brief Bioinform 12(4):303–311. https://doi.org/10.1093/bib/bbr013

Dudley JT, Sirota M, Shenoy M, Pai RK, Roedder S, Chiang AP, Morgan AA, Sarwal MM, Pasricha PJ, Butte AJ (2011b) Computational repositioning of the anticonvulsant topiramate for inflammatory bowel disease. Sci Transl Med 3(96):96ra76. https://doi.org/10.1126/scitranslmed.3002648

Gelosa P, Castiglioni L, Camera M, Sironi L (2020a) Drug repurposing in cardiovascular diseases: opportunity or hopeless dream? Biochem Pharmacol 177:113894. https://doi.org/10.1016/j.bcp.2020.113894

Gelosa P, Castiglioni L, Camera M, Sironi L (2020b) Repurposing of drugs approved for cardiovascular diseases: opportunity or mirage? Biochem Pharmacol 177:113895. https://doi.org/10.1016/j.bcp.2020.113895

Ghofrani HA, Osterloh IH, Grimminger F (2006) Sildenafil: from angina to erectile dysfunction to pulmonary hypertension and beyond. Nat Rev Drug Discov 5(8):689–702. https://doi.org/10.1038/nrd2030

Greene CS, Voight BF (2016) Pathway and network-based strategies to translate genetic discoveries into effective therapies. Hum Mol Genet 25(R2):R94–R98. https://doi.org/10.1093/hmg/ddw160

Gribkoff VK, Kaczmarek LK (2017) The need for new approaches in CNS drug discovery: why drugs have failed, and what can be done to improve outcomes. Neuropharmacology 120:11–19. https://doi.org/10.1016/j.neuropharm.2016.03.021

Grover MP, Ballouz S, Mohanasundaram KA, George RA, Goscinski A, Crowley TM, Sherman CD, Wouters MA (2015) Novel therapeutics for coronary artery disease from genome- wide association study data. BMC Med Genet 8(2):1. https://doi.org/10.1186/1755-8794-8-S2-S1

Hsieh YY, Chou CJ, Lo HL, Yang PM (2016) Repositioning of a cyclin-dependent kinase inhibitor GW8510 as a ribonucleotide reductase M2 inhibitor to treat human colorectal cancer. Cell Death Discov 2(1):1–8. https://doi.org/10.1038/cddiscovery.2016.27

Huang J (2020) Can drug repositioning work as a systematical business model? ACS Med Chem Lett 11(6):1074–1075. https://pubs.acs.org/doi/10.1021/acsmedchemlett.0c00122

Huang H, Zhang G, Zhou Y, Lin C, Chen S, Lin Y, Mai S, Huang Z (2018) Reverse screening methods to search for the protein targets of chemopreventive compounds. Front Chem 6:138. https://doi.org/10.3389/fchem.2018.00138

Hurle MR, Yang L, Xie Q, Rajpal DK, Sanseau P, Agarwal P (2013) Computational drug repositioning: from data to therapeutics. Clin Pharmacol Therap 93(4):335–341. https://doi.org/10.1038/clpt.2013.1

Iljin K, Ketola K, Vainio P, Halonen P, Kohonen P, Fey V, Grafström RC, Perälä M, Kallioniemi O (2009) High-throughput cell-based screening of 4910 known drugs and drug-like small molecules identifies disulfiram as an inhibitor of prostate cancer cell growth. Clin Cancer Res 15(19):6070–6078. https://doi.org/10.1158/1078-0432.CCR-09-1035

Iorio F, Rittman T, Ge H, Menden M, Saez-Rodriguez J (2013) Transcriptional data: a new gateway to drug repositioning? Drug Discov Today 18(7–8):350–357. https://doi.org/10.1016/j.drudis.2012.07.014

Keiser MJ, Setola V, Irwin JJ, Laggner C, Abbas AI, Hufeisen SJ, Jensen NH, Kuijer MB, Matos RC, Tran TB, Whaley R (2009) Predicting new molecular targets for known drugs. Nature 462(7270):175–181. https://doi.org/10.1038/nature08506

Kim JH, Scialli AR (2011) Thalidomide: the tragedy of birth defects and the effective treatment of disease. Toxicol Sci 122(1, 1):–6. https://doi.org/10.1093/toxsci/kfr088

Leenaars CH, Kouwenaar C, Stafleu FR, Bleich A, Ritskes-Hoitinga M, De Vries RB, Meijboom FL (2019) Animal to human translation: a systematic scoping review of reported concordance rates. J Transl Med 17(1):1–22. https://translational-medicine.biomedcentral.com/articles/10.1186/s12967-019-1976-2

Matthews H, Usman-Idris M, Khan F, Read M, Nirmalan N (2013) Drug repositioning as a route to anti-malarial drug discovery: preliminary investigation of the in vitro anti- malarial efficacy of emetine dihydrochloride hydrate. Malar J 12(1):1. https://doi.org/10.1186/1475-2875-12-359

Miettinen TP, Björklund M (2014) NQO2 is a reactive oxygen species generating off-target for acetaminophen. Mol Pharm 11(12):4395–4404. https://doi.org/10.1021/mp5004866

Mirza N, Sills GJ, Pirmohamed M, Marson AG (2017) Identifying new antiepileptic drugs through genomics-based drug repurposing. Hum Mol Genet 26(3):527–537. https://doi.org/10.1093/hmg/ddw410

Morofuji Y, Nakagawa S (2020) Drug development for central nervous system diseases using in vitro blood-brain barrier models and drug repositioning. Curr Pharm Des 26(13):1466–1485. https://doi.org/10.2174/1381612826666200224112534

Naylor DM (2015) Therapeutic drug repurposing, repositioning and rescue. Drug Discov. 57. https://www.ddw-online.com/therapeutic-drug-repurposing-repositioning-and-rescue-part-ii-business-review-888-201504/

Nosengo N (2016) Can you teach old drugs new tricks? Nature 534(7607):314–316. https://ideas.repec.org/a/nat/nature/v534y2016i7607d10.1038_534314a.html

Pantziarka P, Pirmohamed M, Mirza N (2018) New uses for old drugs. BMJ 361:k2701. https://doi.org/10.1136/bmj.k2701

Pushpakom S (2022) Introduction and historical overview of drug repurposing opportunities. pp 1–13. https://doi.org/10.1039/9781839163401-00001

Pushpakom S, Iorio F, Eyers PA, Escott KJ, Hopper S, Wells A, Doig A, Guilliams T, Latimer J, McNamee C, Norris A (2019) Drug repurposing: progress, challenges and recommendations. Nat Rev Drug Discov 18(1):41–58. https://www.nature.com/articles/nrd.2018.168

Rudrapal M, Khairnar SJ, Jadhav AG (2020) Drug repurposing (DR): an emerging approach in drug discovery. Drug repurposing hypothesis. Mol Aspects Ther Appl. https://www.intechopen.com/chapters/72744

Sanseau P, Agarwal P, Barnes MR, Pastinen T, Richards JB, Cardon LR, Mooser V (2012) Use of genome-wide association studies for drug repositioning. Nat Biotechnol 30(4):317–320. https://doi.org/10.1038/nbt.2151

Scannell JW, Blanckley A, Boldon H, Warrington B (2012) Diagnosing the decline in pharmaceutical R&D efficiency. Nat Rev Drug Discov 11(3):191–200. https://doi.org/10.1038/nrd3681

Talevi A, Bellera CL (2020) Challenges and opportunities with drug repurposing: finding strategies to find alternative uses of therapeutics. Expert Opin Drug Discov 15(4):397–401. https://doi.org/10.1080/17460441.2020.1704729

Wagner A, Cohen N, Kelder T, Amit U, Liebman E, Steinberg DM, Radonjic M, Ruppin E (2015) Drugs that reverse disease transcriptomic signatures are more effective in a mouse model of dyslipidemia. Mol Syst Biol 11(3):791. https://doi.org/10.15252/msb.20145486

Wouters OJ, McKee M, Luyten J (2020) Estimated research and development investment needed to bring a new medicine to market, 2009-2018. JAMA 323(9):844–853. https://jamanetwork.com/journals/jama/article-abstract/2762311

Chapter 2
Drug Polypharmacology Toward Drug Repurposing

Tammanna R. Sahrawat, Ritika Patial, and Ranbir Chander Sobti

Abstract Polypharmacology has emerged as a new paradigm in drug discovery as several drugs exert their effects through interaction with multiple targets, thereby shifting the focus from one target-one drug model to a multi-target approach. Polypharmacology uses comprehensive detailed knowledge about the composition of the drug, pathogenesis course of its mode of action, drug toxicology, pharmacodynamics, pharmacokinetics, and their side effects. Drug pharmacology has made the targeting of multiple receptors possible thereby reducing the manufacturing cost and revealing some novel-application of the already available FDA-approved drugs. It also offers manifold benefits to the pharmaceutical industry such as lower costs and reduced time for drug development as it eliminates the crucial ADMET analysis for repurposing the drug. Drug repurposing promises to provide a novel avenue for more effective but less toxic therapeutic agent designing.

Keywords Drug polypharmacology · Drug repurposing · Off-targets · Virtual screening · *In-silico*

2.1 Drug Pharmacology

Drug pharmacology is a discipline dealing with the action mechanism of the drugs within our systems and their various therapeutic approaches. It has made it possible for us to target the specific sites or the receptors through receptor molecular analysis targeting different signaling or the metabolic pathways at once or at different intervals. This would not only prevent cost failures but also offer the possibility of identification of novel applications of existing drugs through drug repurposing. There are numerous drugs available in the market which are altered for their purpose initially they were developed for. The advances in in silico approaches would help us

T. R. Sahrawat (✉) · R. Patial · R. C. Sobti
Centre for Systems Biology and Bioinformatics, UIEAST, Panjab University, Chandigarh, India

Department of Biotechnology, Panjab University, Chandigarh, India
e-mail: tammanna@pu.ac.in

R. C. Sobti et al. (eds.), *Drug Repurposing for Emerging Infectious Diseases and Cancer*, https://doi.org/10.1007/978-981-19-5399-6_2

to find various multitarget sites and to anticipate their potential in drug designing process. These in silico approaches can be classified broadly into ligand- and structure-based approaches, data mining, and network systems biology to develop some new drugs having high efficacy and lower toxicity levels.

Pharmacology include comprehensively the detailed information about drug composition and its specifications, synthesis and drug design, its cellular and molecular mechanisms, interactions, systems mechanisms, signal transduction, chemical biology, medicinal and therapeutic application, and antipathogenesis course of its action. These details information about the drug can be used if we need to find its alternating target, or its different targeting pathway mechanism.

2.2 Drug Repurposing

In past years, the instances for drug design were "one drug and one target." This classic approach of drug design was to develop a drug targeting specific receptors without affecting other pathways of the systems. But this approach is now modified to "one drug multiple targets" and is emerging field of pharmacology popularly known as drug repurposing or reorientation. It is based on valuable available knowledge of several different pathways opted by single gene. A single gene may have two or more signaling pathway to express itself or there can be some common pathway between different genes involved in any kind disease. In either way, drug repurposing is highly beneficial. We can either target a single pathway for inhibiting multiple genes connected in their course of action or can specifically target multiple targets at a time. Diseases like cancer, neuron degeneration involves different pathways and targeting only one pathway is not enough to control its progression. So there is a need to find some multitargeting drugs in our treatments. The successful example of drug repurposing includes duloxetine initially developed for treating depression but now is FDA-approved drug in the United States for treating multiple myeloma and leprosy (Ashburn and Thor 2004) (Fig. 2.1).

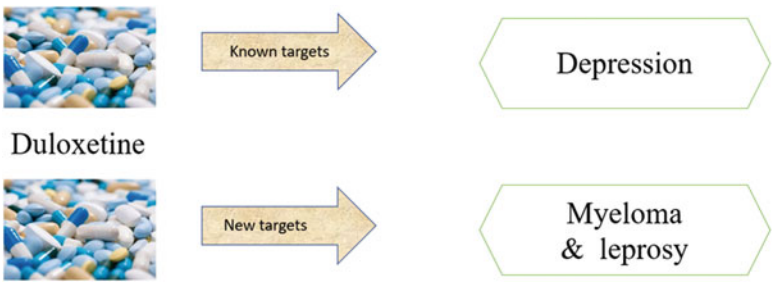


Fig. 2.1 The successful example of drug repurposing includes duloxetine initially develop for treating depression but now is FDA-approved drug in the United States for treating multiple myeloma and leprosy

2.3 Need for Drug Repurposing

The classical approach of drug design is time consuming, it takes about 10–12 years for a drug to get approved and launched in the market if it falls under the significance parameters of FDA. The approval rate for drugs has declined and many launched had been taken off the market because of their side-effects. They have shown their ill effects due to their unwanted interactions with off-targets. Drugs repurposing would help to find these off-targets in the early stages of the drug design to prevent these roll backs steps of the drugs (Rothman et al. 2000; Connolly et al. 1997). The drug market today is worth of billions of dollars, and quite of investment is made in drug designing process. The average cost for a drug design is 600–800 millions. Out of two either, we can go for new drug development or we can find new purposes to already available drugs which is more significant and resource saving. The finding of new targeting site for available drugs makes use of the already available data or research to benefit pharmaceutical industry by lowering drug manufacturing cost, limiting the decades time for drug discovery to few years to entirely reposition the available existing drug to new applications (DeBusk et al. 2004). Numerous drugs are known for their multitargeting activities. An illustrative example is Aspirin that has been clinically used as an analgesic or antipyretic has been found to acts as an anti-inflammatory medication to treat rheumatoid arthritis, pericarditis, and Kawasaki diseases. Additionally, it has been also used in the prevention of transient ischemic attacks, strokes, heart attacks, pregnancy loss, and even cancer (Reddy and Zhang 2013). The advances in technology to find multiple treatments from single drug is increasing the investment in drug development so the pharmaceutical companies are shifting their area of interest to find ways to repurpose already available drug to bring billions of benefit to the market. It is a low-risk approach with max-fold benefit to use the available on shelf drug. Only the data to evaluate are its other targeting receptors because existing drugs are up to mark for safety parameters (Fig. 2.2).

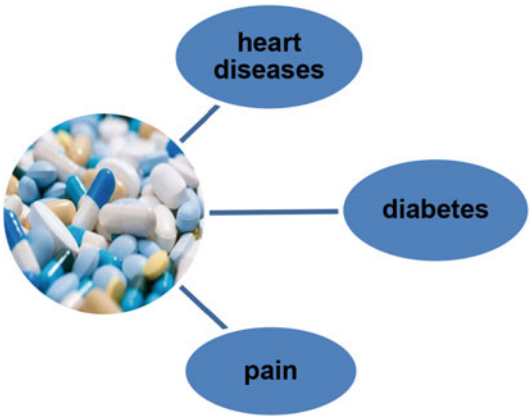
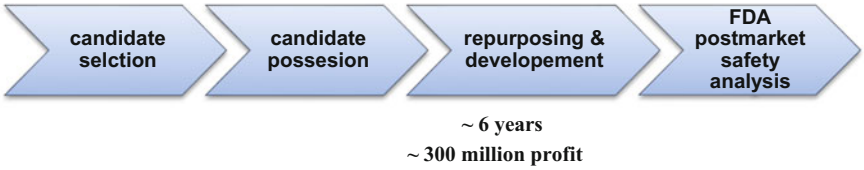


Fig. 2.2 The need for drug repurposing is to validate the present effective drug for different disease targeting. Like there maybe instances that the drug working on diabetic patient would be effective and show significant potential on some heart disease symptoms and pain

2.4 Challenges of Drug Repurposing

The idea of drug repurposing seems so easy but it has its own limitation. Here, we are finding the different targets for the drug which are already part of our treatment approaches. Now here comes the big task say we need to target gene "A" which is alternative target, but that same target is working in some other pathway which is having positive regulatory effect on the body. The major challenges in drug repurposing are to find the suitable candidate and to fulfill the missing data required to entirely repurpose the existence of already approved drug, so that we can derive some beneficial and potential instances.



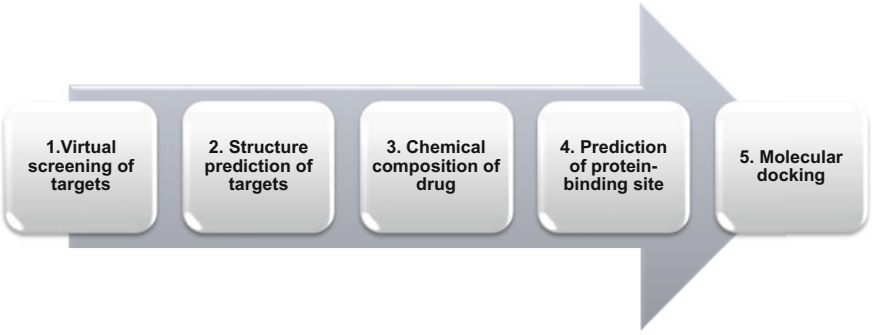
2.5 Different Strategies of Drug Repurposing

The strategies for drug repurposing are based on the action mechanisms of the drug on its target site. Mostly the data about the mechanism of drug working are either unknown or lesser known. Either way there is a missing link in the data that is essential for changing the purpose of the existing data. The gap between the available knowledge and the one that can be deduced will help us to find the off-targets of drugs. Donepezil, drug used in AD treatment through its repurposing, is proposed to have two different off-targets n-alpha acetyl transferase 60 and Heparasulfate n-deacetylase which were earlier reported to be associated with gastrointestinal hemorrhage, bleeding, and peptic ulcers (Sahrawat and Batra 2020).

These kinds of off-target would allow us to add our already valuable and working drug to some new purpose. The current approach being used in the drug repurposing is the "drug action spectrum" which is based on three major criteria: target-based, drug-based, and diseases-based repurposing (Akhoon et al. 2019). Target-based approach implicates for identification of new indications for the already established targets. Drug-based approach implicates on finding new target indications for the existing drug. It makes use of techniques such as ligand- and structural-based computational method, and off-target screening for finding targets, and disease-based repurposing approach implicates for identification of co-occurring pathophysiological nature between different diseases. The different kind tools which are being used for drug-based approach are phenotypic screening, clinical observation, side-effect analysis, network, and data mining (Fig. 2.3).

Fig. 2.3 The most accepted approach being used in drug repurposing makes use of three criteria: target-based, drug-based, and disease-based approach

2.6 Methodology for Drug Repurposing



2.6.1 Virtual Screening

The basic hypothesis on which drug purposing is based is that drugs not only have interactions with their associated targets but also interact with other off-targets with high specificity. There are several chemical libraries available that have information regarding the off-targets details for several drugs. In VLS, every molecule in the library is tested against an ideal model of activity and they are ranked by assigning each compound a predicted activity score. Only the top-ranking fractions are analyzed using further testing (Jenwitheesuk et al. 2008). The available in silico programs for virtual screening of the drugs off-targets include PubChem, ZINC which reduces the screening time to few days. Once the screening is done, the target with high specificity or affinity is selected based on the low toxicity and potency of the binding pocket (Fig. 2.4).

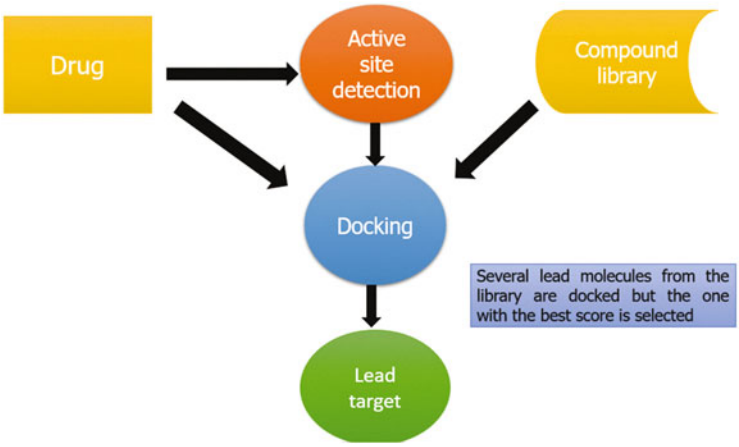
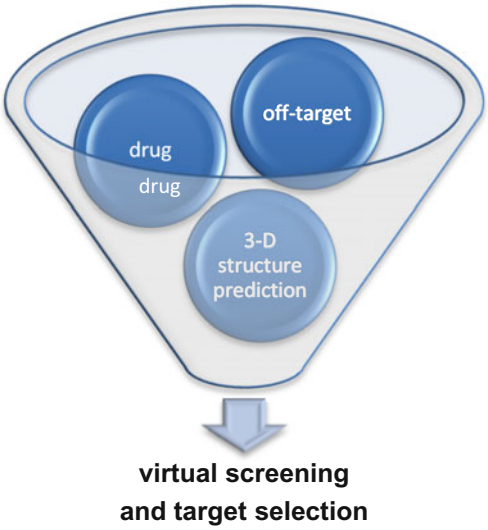


Fig. 2.4 Virtual screening involves finding of the best scored lead molecules which shows best docking result with the selected drug

2.6.2 Structure Prediction of Target

The selection of the target is accompanied by its structure evaluation. Predicted target structures are easily available on PDB with different formats. The structures can be downloaded in any format according to the format required, but if our selected target does not have any target, then its structure prediction can be done using various in silico software's such as I-TASSER, 3-D prediction, homology modeling, and fold recognition. The most preferred method of structure prediction is homology modeling, which makes use of the similar sequence having $\geq$50% similarity as a template for target structure prediction. Protein folding or threading is used when targets do not have similar sequences; here, we find the similar fold between sequences and use them as template.



2.7 Chemical Composition of Drug

To understand the binding of the drug to its targets sites, the chemical composition or the chemical structure of the drug is to be well known. With the relevant information about the chemical composition, the target binding site predictions can be easily done with the use of PubChem (www.PubChem.ncbi.nlm.nih.gov n.d.) chemical repository maintained by National Institute of Health (NIH) and DrugBank (www.drugbank.ca n.d.).

2.8 Prediction of Protein Binding Site

Now to find the exact site where our drug will bind to its particular off-targets target, the collective data about the target of interest and the drugs chemical information are further evaluated in predicting the site where the drug will bind its off-targets. Freely online available in silico tools such as Intfold3, SP-Align, HHPredA, 3DLigandsite & Atom2, etc. will help us to locate the binding site with which the drug will bind with the highest binding affinity. These tools will provide many binding sites but the one with the highest affinity is of higher significance. It shows that the drug binding will change the target property and its working of causing illness will be blocked. Higher the energy more is the affinity of the drug for its off-target. In order to calculate the energies of the docked complex, several in silico tools available to

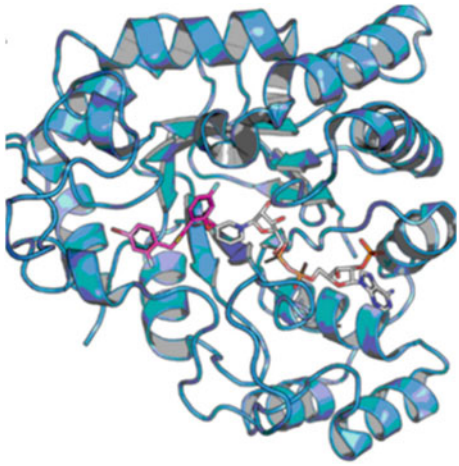


Fig. 2.5 The interaction between the lead molecules and the drug moiety is calculated and the best docked molecule with the highest scored is selected for further experimentation

calculate the docking energy are AutoDock, AutoDock Vina, GOLD, ITscore, etc (Fig. 2.5).

2.9 Successful Example of Drug Repurposing

Drug	Original indication	New indication
Amphotericin B	Fungal infection	Leishmaniasis
Aspirin	Inflammation, pain	Antiplatelets
Bromocriptine	Parkinson's diseases	Diabetes mellitus
Finasteride	Prostate hyperplasia	Hair loss
Gemcitabine	Viral infection	Cancer
Methotrexate	Cancer	Psoriasis, rheumatoid arthritis
Minoxidil	Hypertension	Hair loss
Raloxifene	Cancer	Osteoporosis
Taloxifenethalidomide	Morning sickness	Leprosy, multiple myeloma
Seldenafil	Angianse	Erectile dysfunctionality, pulmonary hypertension

Source: Second medical uses for existing drugs—Geneva Network—Patents for Secondary Indications (geneva- b network.com)

Ormeloxifene (ORM) is used as an oral conceptive targeting selective estrogen receptor but its recent investigation lighted upon its potential as an anticancerous agent (Aubé 2012).

A commonly used commercial drug Aspirin, is known for its efficacy in decreasing the mortality late up to 31% in case of cancer patients initially developed as anti-inflammatory and pain killer (Aubé 2012). It is also known for its clot dissolving

potential in blood vessels carrying blood toward the heart (Regular aspirin intake and acute myocardial infarction 1974).

Methotrexate: Available as generic medicine, Methotrexate was first launched in year 1947 as a chemotherapy drug for breast cancer, lung cancer, lymphoma, leukemia, osteosarcoma as it showed less side-effect than already available treatment for cancer. Repurposing of this anticancerous drug leads to the discovery of its off-targets for treating rheumatoid arthritis (Kremer et al. 1994).

Gemcitabine has been known for its chemotherapeutic potential in treating breast cancer, ovarian cancer, testicular cancer, pancreatic, and even gall bladder cancer. Its initial purpose was to treat viral infections such as Heparitis virus by inducing interferon-like mechanism but its repurposing discovered cancer cells as its off-targets (Li et al. 2020).

Raloxifene initially discovered for cancer treatments is now part of osteoporosis treatment in women. It lowers the risk of breast cancer invasion by estrogen receptors present on breast tissue by their inhibition (Morello et al. 2003). New indication of raloxifene in treating osteoporosis in women after menopause targets the decreased bone density, making bones strong with Ca absorption and preventing it from sudden fractures (Xu et al. 2016).

Phentolamine is an antihypertensive drug, developed for treating hypertension (high blood pressure) condition is now used as anesthesia reversal agents in dental. It has vasodilating property due to α-receptors blockage which lowers the raised blood pressure (Jewell et al. 2003).

In time duration of less than two decades, seldenafil, antianginase, drug has found its purposing role in treating erectile functionality in males and recently to treat pulmonary hypertension. It has phosphodiesterase-inhibiting property that increases the relaxation of smooth muscle cells, which makes it a potential targeting drug to pulmonary hypertension and erectile functionality (Ghofrani et al. 2006).

References

Akhoon BA, Tiwari H, Nargotra A (2019) In silico drug design methods for drug repurposing. In: In silico drug design. Academic, pp 47–84

Ashburn TT, Thor KB (2004) Drug repositioning: identifying and developing new uses for existing drugs. Nat Rev Drug Discov 3(8):673

Aubé J (2012) Drug repurposing and the medicinal chemist. ACS Med Chem Lett 3(6):442–444

Connolly HM, Crary JL, McGoon MD, Hensrud DD, Edwards BS, Edwards WD, Schaff HV (1997) Valvular heart disease associated with fenfluramine–phentermine. N Engl J Med 337(9): 581–588

DeBusk RF, Pepine CJ, Glasser DB, Shpilsky A, DeRiesthal H, Sweeney M (2004) Efficacy and safety of sildenafil citrate in men with erectile dysfunction and stable coronary artery disease. Am J Cardiol 93(2):147–153

Ghofrani HA, Osterloh IH, Grimminger F (2006) Sildenafil: from angina to erectile dysfunction to pulmonary hypertension and beyond. Nat Rev Drug Discov 5(8):689–702

Jenwitheesuk E, Horst JA, Rivas KL, Van Voorhis WC, Samudrala R (2008) Novel paradigms for drug discovery: computational multitarget screening. Trends Pharmacol Sci 29(2):62–71

Jewell JR, Longworth DL, Stoller JK, Casey D (2003) The Cleveland Clinic internal medicine case reviews. Lippincott Williams & Wilkins, Hagerstown, p 32. ISBN 0-7817-4266-8. The Phentolamine injection is designed to reverse the local vasoconstrictor properties used in many local anesthetics to perpetuate anesthesia. Malamed S (2009). "What's new in local anaesthesia?". SAAD Digest. 25:4–14. PMID 19267135

Kremer JM, Alarcón GS, Lightfoot RW Jr, Willkens RF, Furst DE, Williams HJ, Dent PB, Weinblatt ME (1994) Methotrexate for rheumatoid arthritis. Suggested guidelines for monitoring liver toxicity. American College of Rheumatology. Arthritis Rheum 37(3):316–328

Li Y, Li P, Li Y, Zhang R, Yu P, Ma Z, Kainov DE, de Man RA, Peppelenbosch MP, Pan Q (2020) Drug screening identified gemcitabine inhibiting hepatitis E virus by inducing interferon-like response via activation of STAT1 phosphorylation. Antivir Res 184:104967

Morello KC, Wurz GT, DeGregorio MW (2003) Pharmacokinetics of selective estrogen receptor modulators. Clin Pharmacokinet 42(4):361–372. https://doi.org/10.2165/00003088-200342040-00004. ISSN 0312-5963. PMID 12648026. S2CID 13003168

Reddy AS, Zhang S (2013) Polypharmacology: drug discovery for the future. Expert Rev Clin Pharmacol 6(1):41–47

Regular aspirin intake and acute myocardial infarction. Br Med J. 1974;1(5905):440–3. https://doi.org/10.1136/bmj.1.5905.440. PMC 1633212. PMID 4816857

Rothman RB, Baumann MH, Savage JE, Rauser L, McBride A, Hufeisen SJ, Roth BL (2000) Evidence for possible involvement of 5-HT2B receptors in the cardiac valvulopathy associated with fenfluramine and other serotonergic medications. Circulation 102(23):2836–2841

Sahrawat TR, Batra M (2020) Investigation of polypharmacological targets of cholinesterase inhibitor drug donepezil: a structure based systems biology approach. J Adv Sci Res 11(4): 315–320

www.drugbank.ca

www.PubChem.ncbi.nlm.nih.gov

Xu B, Lovre D, Mauvais-Jarvis F (2016) Effect of selective estrogen receptor modulators on metabolic homeostasis. Biochimie 124:92–97. https://doi.org/10.1016/j.biochi.2015.06.018. ISSN 0300-9084. PMID 26133657. In healthy postmemopausal women, raloxifene treatment for one year prevented body weight gain and abdominal adiposity by promoting a shift from an android to gynoid fat distribution [46]

Chapter 3
Pharmacovigilance-Based Drug Repurposing

Pooja Gupta and Archana Mishra

Abstract Pharmacovigilance involves evaluation of adverse effects of drugs in the interest of patient safety. Large-scale application of pharmacovigilance generates big datasets that are mined to identify previously unknown drug–event combinations, and, as an extension, may help in identifying new indications for old drugs. The therapeutic potential of a drug using pharmacovigilance-based drug repurposing can be assessed in one of the four ways—serendipity, mechanistic profiling, signature matching, and inverse signaling. Serendipity is the phenomenon of discovery of some valuable information for an already known drug, by chance, like minoxidil. Mechanistic profiling proposed the use of sulfonylureas for diabetes mellitus, based on the observation of their hypoglycemic effect. Signature matching is puzzling out new indications of drugs based on similarity of characteristics in a network of other drugs which are already approved for any condition. Inverse signaling approach takes cues from data mining approaches, applied to pharmacovigilance databases. Currently, this approach is being tried to evaluate existing compounds for Raynaud's phenomenon, COVID-19, Alzheimer' disease, etc. In this chapter, we discuss these pharmacovigilance-based methods as they have immense translational potential for drug repurposing.

Keywords Pharmacovigilance · Drug repurposing · Serendipity · Inverse signaling · Mechanistic profiling · Signature matching

Most fruitful basis for discovery of new drug is to start with an old drug—Sir James Black

P. Gupta (✉)
ADR Monitoring Center, AIIMS Delhi, Pharmacovigilance Program of India (PvPI), New Delhi, India

Pharmacology, All India Institute of Medical Sciences, New Delhi, India
e-mail: drgupta.pooja@aiims.edu

A. Mishra
Pharmacology, All India Institute of Medical Sciences, New Delhi, India

R. C. Sobti et al. (eds.), *Drug Repurposing for Emerging Infectious Diseases and Cancer*, https://doi.org/10.1007/978-981-19-5399-6_3

3.1 Drug Development and Pharmacovigilance

Drug development is the science of identification of a novel molecule and its journey through in vitro, preclinical and clinical studies to become a safe and efficacious medication for clinical use. The new product, whether drug, medical device, or vaccine, undergoes a rigorous process for establishment of efficacy and safety before the grant of marketing authorization. Conventionally, it takes 12–15 years and billions of dollars to bring one new drug to the market. As per USFDA statistics, 70% molecules move from phase 1 to phase 2; 33% of these undergo phase 3 and a further 30% attain marketing authorization. It may be concluded that only 1 out of 10,000 molecules, which enter preclinical stage, reach the shelf. Many molecules fail in phases 2 and 3 due to lack of efficacy and/or presence of serious adverse events. Identification of potential new benefits of already marketed drugs may save some time and money, as the safety profile would already be established. The repurposing of sildenafil for erectile dysfunction is one of the many such examples of drug repurposing which was identified from its adverse effect profile. Sildenafil was originally proposed for hypertension and angina pectoris in 1989. The clinical trials were, however, disappointing. The drug was on the verge of being abandoned when it was discovered that even though coronary vasodilation was modest, sildenafil-dilated penile vessels. At the time when erectile dysfunction could be treated with prosthetic implants or injections only, sildenafil became the wonder blue pill and was approved by USFDA in 1998.

During the clinical development stages, the product is tested in a few thousand of homogenous patient pool. Some adverse effects may be missed which are rare, limited to a specific population, or occur after prolonged use. Thus, adverse effects of drugs need to be monitored in a continuous process, which helps us understand safety performance of the drug in real-world therapeutic settings. Pharmacovigilance literally means "Pharmaco = medicine" and "Vigilare = to watch." WHO defines Pharmacovigilance as "*the science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other medicine/vaccine related problem.*"

3.1.1 Need for Pharmacovigilance

- Clinical trials in developmental phases of a drug are statistically powered for efficacy rather than safety endpoints.
- Inclusion/exclusion criteria of preapproval trials are strict and a larger population with comorbidities are exposed to the new drug only when it is marketed.
- Rare adverse effects may be missed out in clinical trials.
- Adverse effects which may occur on prolonged exposure of the drug may be missed out in developmental stages.

- Treating adverse drug reactions may cost more than treating the disease.
- Promotion of rational therapeutics among practitioners.

Pharmacovigilance is not limited to gathering case reports regarding safety but is one step toward inception of previously unknown or inadequately characterized drug–event combination.

3.2 Pharmacovigilance and Drug Repurposing

Absolute selectivity of a drug for one single target is a myth. Whenever a drug is administered, there are multiple interactions with different targets in human body which results in desired pharmacodynamic effect along with unintended adverse effects which may or may not be beneficial. Pharmacovigilance data can be used to identify therapeutic potential of a drug in one of the four ways:

(a) Signature matching
(b) Mechanistic profiling
(c) Inverse signaling
(d) Serendipity

3.2.1 Serendipity

Serendipity is described as "the occurrence and development of events by chance in a happy or beneficial way" by Oxford medical dictionary. Basically, it is a happy coincidence of finding something good when one is not looking for it. Minoxidil and Finasteride are good examples of serendipitous discovery for alopecia. Minoxidil initially attained emergency use approval as an effective oral antihypertensive agent in 1971 but the enthusiasm for use faded with severe cosmetic concerns of hypertrichosis in some female patients. Later, this adverse effect of minoxidil was studied explicitly and a topical formulation of minoxidil got approval from USFDA for alopecia in August 1988. Similar is the story of finasteride which was initially used in 1992 for prostatic enlargement.

3.2.2 Signature Matching

Signature matching is the process of matching unique characteristics of one drug with another, which may be a proven therapeutic agent in the disease area of interest. These signatures could be driven by chemical structure, pharmacodynamics, or adverse effect profile of the drug. With respect to pharmacovigilance, every drug has its own set of adverse effects which could be used as a surrogate for therapeutic

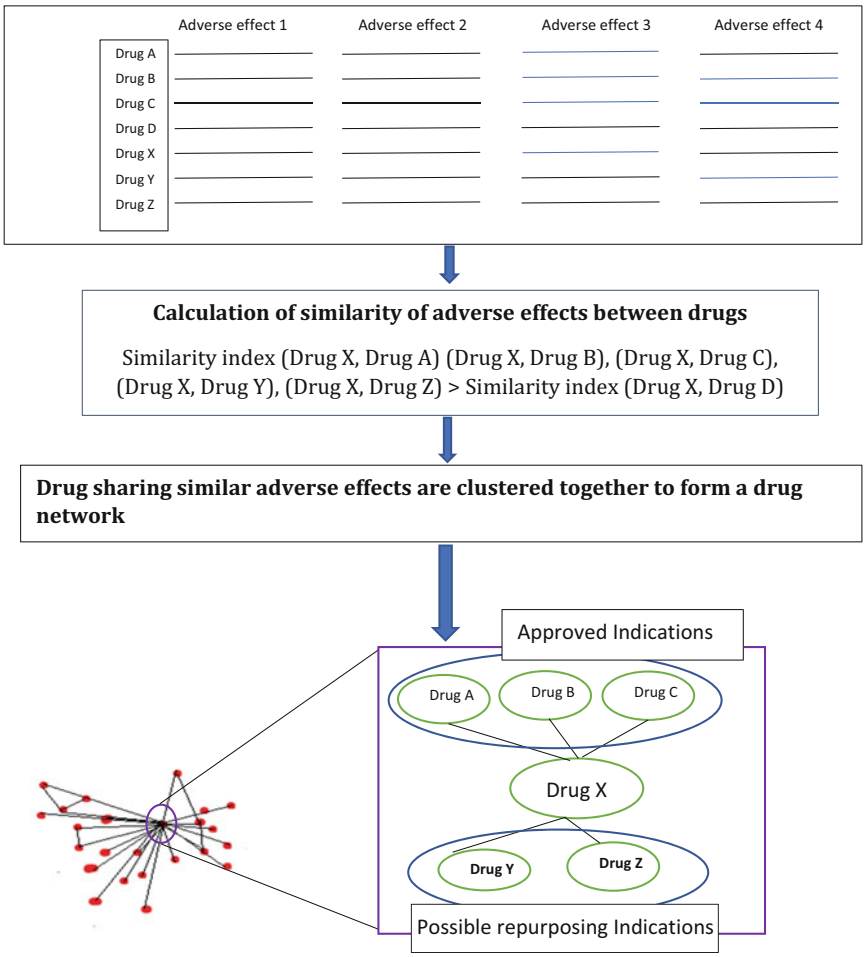


Fig. 3.1 Exploring subnetwork of drug X and puzzling out the similarities of adverse effects with other drugs. (Adapted from: Ye H, Liu Q, Wei J (2014) Construction of drug network based on adverse effects and its application for drug repositioning. PLoS One 9(2):e87864)

activity based on the same mechanisms of action. Similarities in clinical adverse effect fingerprints of the drugs are used to construct a drug network, keeping in mind that drug pairs sharing the same indication should be maximum. The clue for a new indication of a drug could be found in the approved or known functions of nearest neighbors in its class of drugs. Signature matching for adverse effects, done in a stepwise manner, can lead to a new indication for any existing drug. This approach for drug repurposing is in experimental stages where new indications of already existing drugs are being figured out based on similar adverse event profile. Network has been built for tramadol and it has been found to be in subnetwork with drugs for depression and Parkinson's disease (Fig. 3.1).

3.2.3 Mechanistic Profiling

Mechanistic profiling, in simple terms, is discovering new mechanism of action of a drug based on clinical observations. It involves searching for new indications of a drug based on observation of their mechanism of action through multiple pathways. For a disease where etiology and pathophysiological pathway has been deciphered well, adverse effects which are opposite to that of pathophysiological mechanism of the disease in question can be listed out; they may be considered to reverse the pathology of disease. Drugs matching these adverse effect profiles can be sorted and scanned for other desirable attributes to further evaluations. For example, drugs causing hypoglycemia as an adverse event may be considered for diabetes mellitus. The story of sulfonylureas starts with observation of hypoglycemic effect of synthetic sulfur compounds in 1937. In the early 1940s, hypoglycemia was observed in some typhoid patients being treated with the antibiotic para-amino-sulfonamide-isopropyl-thiodiazole. After a few years, it was confirmed that aryl sulfonylureas stimulated pancreas to release insulin and sulfonylureas became the first-line agents for treatment of type 2 diabetes mellitus.

3.2.4 Inverse Signals

To understand application of inverse signals approach in drug repurposing, let us first understand what a signal in pharmacovigilance is.

3.2.4.1 Signal

CIOMS VIII Working group defines signal in pharmacovigilance as "*Information that arises from one or multiple sources (including observations and experiments), which suggest a new potentially causal association, or a new aspect of a known association, between an intervention and an event or set of related events, either adverse or beneficial, that is judged to be of sufficient likelihood to justify verificatory action*."

Traditionally, signal detection was dependent on case-series or aggregate analysis of case reports in pharmacovigilance database or medical literatures. This approach may miss detection of an early signal owing to large volume and complexity of data. In recent times, automated signal generation software(s) use data mining and statistical approaches to assess disproportionality of adverse effects associated with a drug which are more prevalent than expected.

To simplify this concept, let us consider a 2×2 contingency table to depict the numbers associated with drug–event combinations.

	Reports of events of interest	Reports of all other events	Total
Reports of drug of interest	a	b	a + b (Total events for target drug)
Reports of all other drugs	c	d	c + d (Total events for other drugs)
Total	a + c (Total target events)	b + d (Total other events)	

The associations can be calculated by following formulae but not limited to these:

Traditional	Relative reporting (RR)	(Probability of adverse events with specific drug) / (Probability of all adverse events in database)	a*[(a + b + c + d)/ (a + b) (a + c)]
Bayesian	Information component	Log_2 RR	Log_2 a * [(a + b + c + d)/ (a + b) (a + c)]

Odd's ratio and proportional reporting ratio have also been used traditionally to elucidate drug–event associations. There is a higher probability of positive association between the drug–event combination if numbers in cells *a* and *d* are higher. Conventionally, a signal is generated, if lower limit of confidence (traditional approaches)/credible interval (Bayesian approaches) crosses the chosen threshold for detection of disproportionality in the drug–event combination. If the association does not cross a given threshold or the relation between drugs and adverse effects are inverse, the claim of signal generation is disregarded. Thresholds selected to identify signals are generally trade-offs between too low to avoid generating too many false-positive signals and too high to avoid missing probable signals.

3.2.4.1.1 Inverse Signal

The reverse of traditional drug safety signal generation may indicate a new therapeutic application. This inverse signaling is done by obtaining a list of drug–adverse effect combinations which are less frequently observed than expected (Fig. 3.2). These pharmacovigilance data are being utilized for hypothesizing new therapeutic applications for already existing drugs. After obtaining drugs with inverse signals, further shortlisting can be done based on desirable attributes. For example, if a systemic effect is desired, topical formulations, known cytotoxic drugs, etc. could be excluded when preparing final list of drugs. Let us elaborate this for Alzheimer's disease. Assuming we found anticancer drugs, antidiabetic drugs, analgesic patches, and antiparkinsonian drugs to have an inverse signal association for Alzheimer's disease. Anticancer drugs would be excluded because of cytotoxicity and analgesic patches would also be excluded as the need is for a drug that has minimal systemic effect but crosses blood–brain barrier. Antidiabetic drugs and antiparkinsonian drugs would then be taken further in drug development for Alzheimer's diseases.

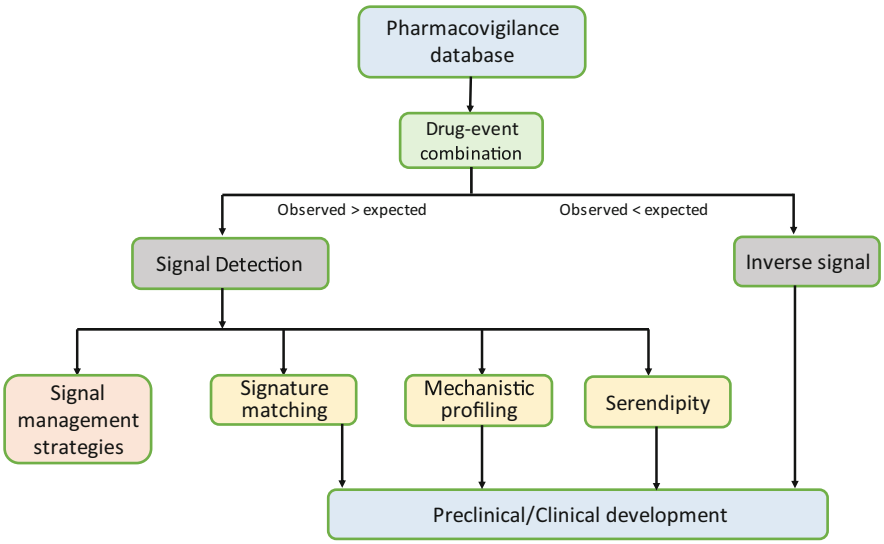


Fig. 3.2 Classification of drug repurposing approaches based on pharmacovigilance

3.3 The Process of Drug Repurposing

Apart from a few serendipitous successes, the potential of pharmacovigilance-based approach has not been fully utilized for drug repurposing. Pharmacovigilance data are obtained from either WHO pharmacovigilance database or national pharmacovigilance databases like Food and Drug Administration (FDA) Adverse Event Reporting System (FAERS) and Japanese Adverse Drug Event Report (JADER) database, etc. Adverse event reporting and retrieval systems use preferred terms for adverse events as specified by medical dictionary for regulatory activities (MedDRA), a subscription-based product of International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). This ensures uniformity in terminology and avoids unnecessary ambiguity and duplication of terms, while retrieving data.

The drugs retrieved, based on any of the parameters discussed above, are then scanned and screened for the desirable attributes like other toxicity profiles, available or possible formulations, dosage needed, etc. to prepare a list of drugs which could be tested further in drug development process.

The drugs thus shortlisted can be directed toward high throughput screening pathway to enable quick automated testing for desired target. Leads thus identified can be brought to patient's rescue in very less time as safety profile of the drug is already within agreeable limits and pharmacokinetics is well understood. This applies to all drug repurposing strategies alike (Fig. 3.3).

Fig. 3.3 Process of development for repurposed drugs

3.3.1 Present Scenario

Pharmacovigilance approaches are increasingly being used in research studies. Researchers have been trying to repurpose drugs for various diseases, where therapeutic options are not available or are limited; for example, Alzheimer's disease, Raynaud's disease, COVID-19, etc. do not have curative therapeutic options.

3.3.1.1 Alzheimer's Disease

Drug candidates with inhibitory activity obtained from VigiBase were compared with labeled cholinesterase inhibitor drugs like rivastigmine, galantamine, and donepezil. Twenty-two drug candidates were identified as having safety profile similar to medicines available for Alzheimer's disease. Four of these drugs (antipsychotics like aripiprazole, clozapine, antidepressants like sertraline, and S-duloxetine) showed a high butyryl cholinesterase inhibition rate and serotonin reuptake inhibition and presented multitarget-directed ligand therapeutic strategy.

3.3.1.2 Raynaud's Phenomenon (RP)

Drugs available for Raynaud's have moderate efficacy. Repurposing of drugs was done for this phenomenon by screening VigiBase for at least one case of erythromelalgia (an adverse effect opposite to that of RP). Along with this, adverse effect signature of the drugs used in secondary RP was also constructed. Thereafter, hierarchical analysis was done, and two drugs (alemtuzumab and fumaric acid) were hypothesized to have therapeutic potential for treatment of Raynaud's phenomenon.

3.3.1.3 COVID-19

In a noble endeavor to find treatment for COVID-19, a group of researchers extracted data of last 15 years for drugs acting on virus-borne respiratory diseases in US FAERS database. The drugs with significant inverse association, i.e., reporting odds ratio <1, were shortlisted. A check for implausibility is done, e.g., topical only formulations or other known adverse effects, e.g., cytotoxic drugs were excluded. What was studied further was a manually curated list of 112 candidate drugs such as antiviral drugs, antidiabetics, peptidase inhibitors, tyrosine kinase inhibitors,

neuropharmacological sigma-receptor modulators, female sex hormones, and nicotinic acetylcholine receptor agonists as putatively antiviral. Some of these drugs were already known for their antiviral activity (e.g., antivirals for respiratory infections). For others, further exploration with respect to their mechanism of action for antiviral activity was recommended.

3.4 Future Perspectives

Pandemics might become a new routine as already experienced by frequent breakthrough infections of SARS-CoV1 followed by MERS and SARS-CoV2. There is an increase in virulence, morbidity, and mortality with each new strain. Drug repurposing can be the only solution for the search of cure in such unforeseen situations. Pharmacovigilance-based drug repurposing will be an ideal tool as a suitable adverse effect profile decreases burden of proving druggable attributes of a molecule manifold.

Pharmacovigilance approaches can also be tried in cancer chemotherapy. A few studies have already shown efficacy of metformin, pioglitazone, dabigatran, nitroglycerine, antiepileptic drugs, propranolol, etc. in multiple cancers. These drugs have been shown to have potential to prevent occurrence and have impact on survival in patients with cancers. Balance between therapeutic effect and toxicity of drugs used for oncotherapy is very delicate; pharmacovigilance approaches would help in recognizing and repurposing relatively safer drugs for therapy. Inverse signaling and mechanistic profiling can be done for other drugs with theoretical chances of benefit and these drugs can be further studied for use in patients.

Further Reading

Chrétien B, Jourdan JP, Davis A, Fedrizzi S, Bureau R, Sassier M, Rochais C, Alexandre J, Lelong-Boulouard V, Dolladille C, Dallemagne P (2021) Disproportionality analysis in VigiBase as a drug repositioning method for the discovery of potentially useful drugs in Alzheimer's disease. Br J Clin Pharmacol 87(7):2830–2837

CIOMS (2010) Practical aspects of signal detection in pharmacovigilance. Report of CIOMS Working Group VIII. Council for International Organizations of Medical Sciences (CIOMS), CIOMS

Mishra A, Jena M (2019) Signal detection and management. In: Maiti R (ed) Postgraduate topics in pharmacology, 3rd edn. Paras Medical Books, Hyderabad, pp 236–243

Takada M, Fujimoto M, Motomura H, Hosomi K (2016) Inverse association between sodium channel-blocking antiepileptic drug use and cancer: data mining of spontaneous reporting and claims databases. Int J Med Sci 13(1):48–59

Ye H, Liu Q, Wei J (2014) Construction of drug network based on side effects and its application for drug repositioning. PLoS One 9(2):e87864

Zaza P, Matthieu R, Jean-Luc C, Charles K (2020) Drug repurposing in Raynaud's phenomenon through adverse event signature matching in the World Health Organization pharmacovigilance database. Br J Clin Pharmacol 86(11):2217–2222

Chapter 4
In Silico Analysis of Cellular Interactors of PQBP1 for Potential Drug Repurposing

Shah Kamranur Rahman

Abstract PQBP1, or polyglutamine tract binding protein 1, is a 265-amino-acid protein. It is known to be expressed in neurons of the human brain, with a higher level of expression in the cerebellar cortex and hippocampus. According to cellular protein–protein interactor analysis, it appears to play a role in transcription control. Protein–protein interactions play fundamental roles in physiological roles such as cell proliferation, gene regulation, cell survival, survival and programmed cell death. This research briefly attempts to identify crucial cellular interactors that may be impacted by mutant PQBP1 in patients with neurological disorders. The key interactors predicted are POLR2A, MED31 and POU3F2, which may be used as targets for drug repurposing. Additionally, this work was also significant since PQBP1 plays a direct role in virus-infected cells.

Keywords Polyglutamine tract binding protein 1 · Drug repurposing; protein–protein interaction

4.1 Introduction

In recent times, clinical reports on mutations in the pqbp1 gene and their causative symptoms of intellectual disability (Germanaud et al. 2011) have become the focus of investigations by medical and biophysical researchers. Given the severity of mutation with the clinical features in patients, this neurological disorder poses another big challenge in medicine. Polyglutamine tract binding protein 1 (PQBP1) is a 265-amino-acid long protein, expressed mainly in neurons throughout the brain, with abundant expression levels in the cerebellar cortex and hippocampus (Qi et al. 2005). Since PQBP1 is known for binding to the polyglutamine tracts, initial studies were carried out on its role in the pathogenesis of polyglutamine expansion-related diseases, such as spin-cerebellar ataxia type 1, Huntington disease, etc. (Okazawa

S. K. Rahman (✉)
Department of Infection Biology, London School of Hygiene and Tropical Medicine, London, UK
e-mail: Shah.Rahman@lshtm.ac.uk

R. C. Sobti et al. (eds.), *Drug Repurposing for Emerging Infectious Diseases and Cancer*, https://doi.org/10.1007/978-981-19-5399-6_4

et al. 2002; Waragai 1999). However, with reports on disability-causing mutations, the search for the exact function of PQBP1 intensified, which remains unclear (Nizon et al. 2015). Analysis of cellular interactors of PQBP1 (Fig. 4.1) suggests it plays a role in transcription regulation (Iwasaki and Thomsen 2014). For instance, PQBP1 binding to the phosphorylated C terminal domain (CTD) of RNA polymerase II, a platform to which many transcription regulatory nucleoproteins bind, implies it regulates levels of RNA Pol II-dependent gene transcription (Okazawa et al. 2002).

The normal functioning of every living cell is brought about by the coherent interactions of many biomolecules within the cell and their communication with the extracellular matrix. These interactions are categorized broadly as protein–protein, protein–nucleic acid, protein–lipid and nucleic acid–nucleic acid interactions. Although all these interactions play some role in vital cellular processes, the protein–protein interaction is believed to play a major role and is therefore most extensively studied. The understanding of vital cellular processes like metabolism, gene regulation, cell signalling, cytoskeleton organization, cell proliferation, survival and even programmed cell death suggests a very critical role for protein–protein interactions (Pawson and Nash 2000). In the last few decades, there has been a considerable increase in the publication of studies demonstrating the detailed mechanisms by which protein–protein interactions are reported to influence important cellular signalling pathways and thus the physiological fates of cells. Many of these studies highlighted the critical importance of the three-dimensional structure of proteins bearing some common docking platforms necessary for interaction. For instance, a set of common docking domains in p38 MAPK (Mitogen-activated protein kinase) and Jnk (c-Jun N-terminal kinase) family members apparently decide interactions with substrate and regulators (Holland and Cooper 1999). Also, some interactions were found to be specific in the presence of signature structural domains (SH2, SH3 domain) present in the interacting proteins (Lu et al. 1997).

In this context, different sets of mutations in PQBP1 are also predicted to change its structure; some of these are validated (Rahman et al. 2019). This change has the potential to show abnormal interaction with cognate cellular interactors. Therefore, an in-silico study was conducted to find out the most relevant cellular interactors of PQBP1. This study was also important as PQBP1 has a direct role in cells infected with infectious viruses.

4.2 Material and Methods

The complete updated list of cellular interactors of PQBP1 was gathered from the BioGRID database (https://thebiogrid.org/) web interface (Stark 2006). PQBP1 was given as a gene of interest to get this list (organism Homo sapiens). The list was downloaded in two-column format once it displayed on the screen. The cytoscape utility (Shannon et al. 2003) version 3.4.0 was installed on a MacBook Pro computer and used to evaluate the downloaded list (PC). The downloaded file was opened in

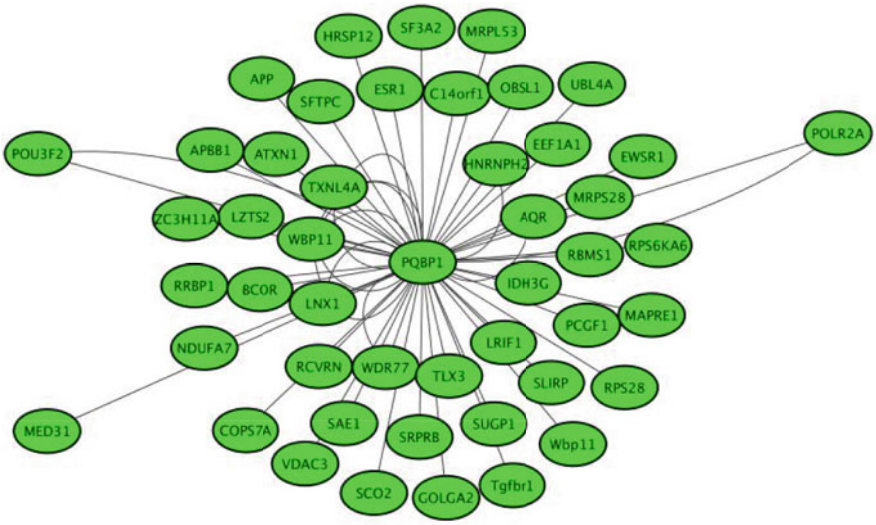


Fig. 4.1 The protein–protein interaction network of PQBP1 is shown. The interaction map was generated using cytoscape tool and BioGRID database

the cytoscape interface, and two columns were assigned and analysed: one for the protein of interest (PQBP1) and another for interactors. The user interface command 'network style' built an interaction network when the file was loaded. Figure 4.1 depicts the network map that was created.

4.3 Results and Discussion

The BioGRID (Stark 2006) database records 47 unique cellular interactors of PQBP1 reported by different studies. These sets of protein–protein interactions in the database were validated through yeast two-hybrid systems and pull-down assays (Stark 2006). Upon analysis of these interactors, it was found that PQBP1 interacts with cellular proteins that have a role in gene regulation and are important components of the transcription machinery (Table 4.1). The interaction network of the PQBP1 protein, created using the cytoscape tool, is presented in Fig. 4.1. PQBP1 interacts with the viral transcription factor POU3F2 (Brn-2) in the human brain, influencing transcription activation induced by the latter (Waragai 1999). In addition, interactions of PQBP1 with well-known splicing factors like U5–15KD and WBP11 suggest that it affects regulation of pre-mRNA processing (Waragai et al. 2000; Komuro et al. 1999a, b; Mizuguchi 2014). The presence of PQBP1 in liquid–liquid phase separated (LLPS) RNA granules and early spliceosomes further confirms its role in pre-mRNA processing and transportation (Makarova 2004; Kunde 2011). Three interactors of PQBP1, namely POU3F2 (Brn-2), MED31 and

Table 4.1 Cellular interactors of PQBP1 (Source: BioGrid https://thebiogrid.org/115393/summary/homo-sapiens/pqbp1.html)

Abbreviations of interactors of PQBP1	Full names of cellular interactors of PQBP1
WBP11	Splicing factor that interacts with PQBP1 and PP1. Activates pre-mRNA splicing.May inhibit PP1 phosphatase activity
POLR2A	DNA-directed RNA polymerase II largest subunit, RNA polymerase II 220 kDsubunit
TXNL4A	Spliceosomal U5 snRNP-specific 15 kDa protein
POU3F2 (Brn-2)	Nervous system-specific octamer-binding transcription factor N-Oct-3. Also known as Brn-2.
LNX1	ligand of numb-protein X 1, E3 ubiquitin protein ligase
COPS7A	COP9 signalosome subunit 7A
APBB1	Amyloid beta (A4) precursor protein-binding, family B, member 1 (Fe65).Transcription co-regulator that can have both co-activator and co-repressor functions
PCGF1	Polycomb group ring finger 1
IDH3G	Isocitrate dehydrogenase 3 (NAD+) gamma
MRPS28	Mitochondrial ribosomal protein S28
SUGP1	SURP and G patch domain containing 1
AQR	Aquarius intron-binding spliceosomal factor
WDR77	WD repeat domain 77
HNRNPH2	heterogeneous nuclear ribonucleoprotein H2 (H-prime)
GOLGA2	Golgin A2
TGFBR1	transforming growth factor, beta receptor I
MAPRE1	microtubule-associated protein, RP/EB family, member 1
LZTS2	leucine zipper, putative tumor suppressor 2
SCO2	SCO2 cytochrome c oxidase assembly protein
OBSL1	Obscurin-Like 1
ESR1	Estrogen receptor 1
EWSR1	EWS RNA binding protein 1
SAE1	SUMO1 activating enzyme subunit 1
RRBP1	Ribosome binding protein 1
SLIRP	SRA stem-loop interacting RNA binding protein
UBL4A	Ubiquitin-like 4A
RCVRN	Recoverin
C14ORF1	Probable ergosterol biosynthetic protein 28
LRIF1	ligand dependent nuclear receptor interacting factor 1
MED31	Mediator of RNA polymerase II transcription, subunit 31 homolog.
EEF1A1	Eukaryotic translation elongation factor 1 alpha 1-like 14.
ATXN1	Spinocerebellar ataxia type 1 protein. Chromatin-binding factor that repress Notch signaling during neurogenesis and neural differentiation.
TLX3	T-cell leukemia homeobox 3
SFTPC	Pulmonary surfactant-associated proteolipid SPL (Val). Pulmonary surfactantassociated proteins promote alveolar stability.
SF3A2	Splicing factor 3a, subunit 2, 66kDa
APP	Amyloid beta (A4) precursor protein
HRSP12	Heat-responsive protein 12
VDAC3	Voltage-dependent anion channel 3
ZC3H11A	Zinc finger CCCH-type containing 11A
NDUFA7	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 7, 14.5kDa
RBMS1	RNA binding motif, single stranded interacting protein 1
MRPL53	Mitochondrial ribosomal protein L53
RPS28	Ribosomal protein S28
RPS6KA6	Ribosomal protein S6 kinase, 90kDa, polypeptide 6
SRPRB	Signal recognition particle receptor, B subunit
BCOR	BCL6 corepressor

POLR2A, have a direct role in the process of transcription regulation in cells (shaded in grey, Table 4.1). These interactors can be studied for aberrant interaction with mutant PQBP1 and serve as potential drug screening targets for the set of purified multi-protein complexes. These drug candidates may compensate for the cell signalling pathways affected by the mutation in PQBP1 and can be repurposed to alleviate the adverse effects of mutant PQBP1.

References

Germanaud D et al (2011) The Renpenning syndrome spectrum: new clinical insights supported by 13 new PQBP1-mutated males. Clin Genet 79:225–235

Holland PM, Cooper JA (1999) Protein modification: docking sites for kinases. Curr Biol 9:R329–R331

https://thebiogrid.org/

Iwasaki Y, Thomsen GH (2014) The splicing factor PQBP1 regulates mesodermal and neural development through FGF signaling. Development 141(19):3740–3751

Komuro A et al (1999a) Association of two nuclear proteins, Npw38 and NpwBP, via the interaction between the WW domain and a novel proline-rich motif containing glycine and arginine. J Biol Chem 274:36513–36519

Komuro A et al (1999b) Npw38, a novel nuclear protein possessing a WW domain capable of activating basal transcription. Nucleic Acids Res 27(9):1957–1965

Kunde SA (2011) The X-chromosome-linked intellectual disability protein PQBP1 is a component of neuronal RNA granules and regulates the appearance of stress granules. Hum Mol Genet 20(24):4916–4931

Lu WS et al (1997) Activation of Pak by membrane localization mediated by an SH3 domain from the adaptor protein Nck. Curr Biol 7:85–94

Makarova OV (2004) A subset of human 35S U5 proteins, including Prp19, function prior to catalytic step 1 of splicing. EMBO J 23:2381–2391

Mizuguchi M (2014) Mutations in the PQBP1 gene prevent its interaction with the spliceosomal protein U5-15 kD. Nat Commun 30(5):3822

Nizon M et al (2015) Phenotype-genotype correlations in 17 new patients with an Xp11.23p11.22 microduplication and review of the literature. Am J Med Genet A 167A(1):111–122

Okazawa H et al (2002) Interaction between mutant ataxin-1 and PQBP1 affects transcription and cell death. Neuron 34(5):701–713

Pawson T, Nash P (2000) Protein-protein interactions define specificity in signal transduction. Genes Dev 14(9):1027–1047

Qi Y et al (2005) PQBP1 is expressed predominantly in the central nervous system during development. Eur J Neurosci 22(6):1277–1286

Rahman SK et al (2019) Frameshift PQBP-1 mutants K192S fs*7 and R153S fs*41 implicated in X-linked intellectual disability form stable dimers. J Struct Biol 206(3):305–313

Shannon P et al (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 13(11):2498–2504

Stark C (2006) BioGRID: a general repository for interaction datasets. Nucleic Acids Res 34:D535–D539

Waragai M (1999) PQBP1, a novel polyglutamine tract-binding protein, inhibits transcription activation by Brn-2 and affects cell survival. Hum Mol Genet 8(6):977–987

Waragai M, Junn E, Kajikawa M, Takeuchi S, Kanazawa I, Shibata M, Mouradian MM, Okazawa H (2000) PQBP-1/Npw38, a nuclear protein binding to the polyglutamine tract, interacts with U5-15kD/dim1p via the carboxyl-terminal domain. Biochem Biophys Res Commun 273:592–595

Chapter 5
Drug Repurposing Opportunities in Cancer

Popat Kumbhar, Kapil Kole, Arehalli Manjappa, Niraj Kumar Jha, John Disouza, and Vandana Patravale

Abstract Cancer is the second most vital health concern across the globe. Chemotherapy has long been a popular option for the treatment of different sorts of cancer. Chemotherapy, on the other hand, has a number of pitfalls, including non-specificity, resistance by tumor cells, fatal toxicities, and high drug costs. Furthermore, developing new therapeutics is an extensive and expensive process with poor chances of success. As a result, drug repurposing (DR) has transpired as a new path in which a medicine previously licensed for one condition can now be used to treat another condition, such as cancer. This avenue is superior to de novo with respect to the time and money. In addition, there is less probability of failure of repurposed therapeutics in clinical trials. Large numbers of studies have reported the potential of repurposed therapeutics and phytochemicals in various cancers. In the present chapter, we have discussed the importance of repurposed therapeutics, barriers in DR, and computational approaches in DR.

The opportunities of repurposing small-molecules non-oncology therapeutics and phytochemicals against assorted cancers are also discussed. Furthermore, repurposed cargos in clinical trials and future prospects of DR are briefed. In the current scenario, the drug repurposing appears to be an optimistic way and so is

P. Kumbhar · K. Kole · A. Manjappa · J. Disouza (✉)
Tatyasaheb Kore College of Pharmacy, Warananagar, Maharashtra, India
e-mail: jidisouza@tkcpwarana.ac.in

N. K. Jha
Department of Biotechnology, School of Engineering and Technology (SET), Sharda University, Greater Noida, Uttar Pradesh, India

Department of Biotechnology Engineering and Food Technology, Chandigarh University, Mohali, India

Department of Biotechnology, School of Applied and Life Sciences (SALS), Uttaranchal University, Dehradun, India

V. Patravale (✉)
Department of Pharmaceutical Sciences and Technology, Institute of Chemical Technology, Mumbai, Maharashtra, India
e-mail: vb.patravale@ictmumbai.edu.in

R. C. Sobti et al. (eds.), *Drug Repurposing for Emerging Infectious Diseases and Cancer*, https://doi.org/10.1007/978-981-19-5399-6_5

likely to offer proficient treatment at a fair price in debilitating diverse sorts of cancers.

Keywords Cancer · Drug repurposing · Computational tools · Small-molecules · Phytochemicals · Clinical trials

5.1 Introduction

Cancer is a pressing health issue and serves as the second major cause of death across the globe. In 2025, it is estimated that above 20 million of individuals will be diagnosed with cancer across the globe. The cancers like breast, prostate, lung, and colorectal that are difficult to cure at advanced stages will cause significant mortality (Sleire et al. 2017). Therefore, there is an unmet need of new effective anticancer therapeutics to tackle this major health concern.

The new drug discovery and development process by de novo approach is a complicated and highly regulated process as it involves lead identification and optimization as well as preclinical and clinical testing. Moreover, it requires more time (10–17 years) and huge cost (161–1800 million dollars) (Sleire et al. 2017). The success rate with new drug discovery is very low (only 2.01%). Besides, new drug discovery by de novo approach may fail to control the cancer mortality due to their huge cost which may not be affordable for cancer patients in low- and middle-income countries (LMICs) resulting higher mortality. In addition, this approach may not be able to meet the demands of anticancer therapeutics (Kumbhar et al. 2022a, b).

Drug repurposing (DR) is an alternative approach to de novo approach wherein drug previously approved for other indications can be used for the treatment of cancer (Kumbhar et al. 2022a, b). The range of advantages associated with DR includes well established pharmacokinetics, pharmacodynamics, and toxicity profile of the drug and thereby reductions in time span and cost for drug to reach the market. In addition, threat of failure is minimal because the safety of drugs has previously been confirmed in preclinical and early-stage clinical studies (Zhang et al. 2020a, b). Therefore, the researchers and healthcare practitioners are massively adopting this strategy to address the problem of drug shortage in the search of new therapeutics for cancer.

The advancement in genomic and proteomic methods for the evaluation of particular biological pathways linked to cancers has resulted in various novel therapeutic targets, opening up an ample of opportunities in DR. Nearly all therapeutics employed for human use have ability to address several targets. Thus, if the targets of repurposed therapeutics are closely linked to the cancers, these therapeutics can be employed for the effective treatment of cancer (Huang et al. 2020). In this chapter, we have discussed about the DR and its need in the cancer treatment. Different DR approaches are also briefed. The various drug candidates from different pharmacological class repurposed for cancer treatment are discussed. Further, we have described barriers in DR and pathways to address these barriers and briefed the conclusion and future perspectives of DR.

5.2 Drug Repurposing

Drug repurposing (DR) recognized also as drug reprofiling or repositioning is the use of formerly approved drug for new indications other than their original approved indications (Ashburn and Thor 2004). The relevant non-oncology drugs having the clinical potential can be tested for their anticancer potential via different mechanisms. DR serves as a substitute for the classical pharmacology approach. DR required less expenditure and less time span because of the availability of information related to the dosing, pharmacokinetics, pharmacodynamics, drug interactions, toxicity, and safety (Pushpakom et al. 2019). The time span required for the new drug discovery and development process might be 10–17 years, in contrast drug repurposing may require only 3–12 years. Furthermore, prior evidence of these medications' effects in preclinical models and humans lowers the likelihood of failure in subsequent clinical trials. Various non-oncology drugs approved for various diseases have shown great promise against different types of cancers. For instance, the anti-HIV drug zidovudine was first introduced as a repurposed drug for cancer (Clouser et al. 2010). Besides, the drugs such as thalidomide, sildenafil, rituximab, and azidothymidine have shown promise in cancer treatment (Pushpakom et al. 2019; Abbruzzese et al. 2017).

5.3 Drug Repurposing Barriers

The several barriers in the DR are legal issues and patent, organizational barriers, and market exclusivity. The new indication of a repurposed therapeutics which is non-obvious can be safeguarded via the patent. If the new indication is already been published in the literature, innovator needs to propose a new claim indicating the non-obviousness of repurposed therapeutics (Pushpakom et al. 2019). The aforementioned barriers in the path of repurposed therapeutics can be overcome via development of their new chemical derivatives showing similar activity like original (Talevi and Bellera 2020). In addition, the repurposed drug delivery via advanced dosage forms and their endorsement in new geographical area is another way to get over the obstacles. In the case of regulatory aspects of repurposed drugs, the regulatory features may differ from country to country. In the United States, for example, only 3 years of data exclusivity criteria is given for the new indication of formerly approved drug which is not sufficient to recover the expenditure incurred by the innovator (Breckenridge and Jacob 2019). The regulatory agencies need to relook into this aspect.

An additional issue with DR is the scarcity of information about drug substances owing to the restricted public access to the clinical data, and discontinued drugs (Pushpakom et al. 2019). Furthermore, the companies are not ready to repurpose the approved cargos for new purpose if they do not fit within their core disease area (Talevi and Bellera 2020).

5.4 Computational Approaches in Drug Repurposing

Various computational tools play an imperative role in DR in cancer. DR predictions in cancer can be made via the computational tools. There are main two sorts of computational analysis chiefly classical data type and modern data type. The former is mainly associated with structure and physicochemical properties of drugs and molecular targets, while later deals with drug-induced metabolic simulations or transcriptional responses (omics data types). The disease-drug interaction based computational tools can suggest new therapeutic application of the drug for analogous disease phenotypes. In addition, chemical structure similarities can be employed to select substitutes to existing therapeutics. Furthermore, these computational tools can help in DR by identifying unrevealed cancer molecular targets of existing therapeutics (Mottini et al. 2021). The interpretation of the outcomes is the key element of computational approaches. Complex models can normally advance predictive recital, however are not able to easily impart biological insights into the mechanisms underlying the predictions. Besides, simpler and quickly interpretable tools are unable to apprehend significantly non-linear associations in data. Thus, furthermore research is needed on the interpretability via machine learning approach (Mottini et al. 2021; Camacho et al. 2018).

Different computational molecular docking software can be employed in DR to study the ligand–receptor interactions. These include AutoDock Vina (open-access), Glide, GOLD (commercial based). The docking results need to be confirmed by molecular dynamic (MD) simulation to check the stability of interaction between the ligand and receptor. In addition, this simulation study aids in the precise determination of the thermodynamics and kinetics allied with drug target recognition and binding. Desmond and GROningen MAchine for Chemical Simulations (GROMACS) are employed for MD simulation via GUI interface or command line (Gurung et al. 2021).

5.5 Drug Repurposing in Cancer

5.5.1 Importance of Drug Repurposing in Cancer Treatment

Cancer is the principal cause of the death globally that caused around 10.0 million cancer mortalities in 2020. Amongst the cancers, female breast cancer (11.7%) is an enormously noticed cancer than lung (11.4%), colorectal (10.0%), prostate (7.3%), and stomach (5.6%) cancers. Furthermore, in the LMICs and higher-income countries, the extent of lung cancer was found to be enormous with an estimated 1.8 million deaths (18%), followed by colorectal (9.4%), liver (8.3%), stomach (7.7%), and female breast (6.9%) (Sung et al. 2021). Thus, global impact of various cancers is a major worry, and this trend is expected to continue.

The prime critical worry in oncology practice is the origin and changeability of clones towards cargos and radiations, existence of cancer stem cells, rapid angiogenesis and metastasis, and relapse after therapy. Surgery is highly recommended for several early-stage cancers like lung cancer; yet, patient's low tolerance limits its applications. Furthermore, gene therapy is known to be promising in cancer treatment when compared to chemotherapy. Nevertheless, it is associated with a range of problems like non-specificity, less efficiency, increased health issues due to the mutagenesis. Besides, poor success to gene therapy is owing to the worries allied with biosafety, ethical, and financial issues. As a result, for cancer detected at an advanced stage, chemotherapy is the crucial line of treatment, either alone or in combination with other treatments (Kumbhar et al. 2022a, b). However, the enormous rate of marketed chemotherapeutics resulting in elevated death in LMICs is also documented. Moreover, the existing chemotherapeutic applications are restricted owing to multidrug resistance (MDR), poor therapeutic performance, and fatal side effects. Furthermore, new anticancer cargos development via de novo approach to tackle the aforementioned problem is a tedious and lengthy and pricey process with sturdy regulatory barriers, and a higher frequency of failure (a success rate of only 2.01%) (Kumbhar et al. 2022a, b; Das et al. 2015; Fojo and Parkinson 2010; Hay et al. 2014; Sleire et al. 2017). Therefore, to conquer the challenges in the new drug development by de novo approach and to meet the existing need for anticancer cargos, there is an unmet need to use repurposed drugs. These cargos save time and cost because they are formerly approved for indications other than cancer and lots of them are available as generic. Moreover, several repurposed non-oncology dugs are reported to show effectivity against MDR cancers. Thus, DR can provide cancer patients with a novel therapy alternative at a low cost, thereby reducing cancer-related mortality in LMICs.

5.5.2 Repurposing Small-Molecule Non-Oncology Drugs

The small-molecule non-oncology therapeutics from different pharmacological classes repurposed against assorted cancers are depicted in Fig. 5.1 and summarized along with molecular mechanisms in Table 5.1. These all therapeutics repurposed are further discussed below in brief.

5.5.2.1 Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

5.5.2.1.1 Aspirin

Aspirin is a common NSAID and it has been observed to be effective in cardiovascular illness by inhibiting cyclooxygenase-1 (COX-1), which is crucial in the manufacture of thromboxane A2, a critical component in platelet aggregation (Raber et al. 2019). After rigorously evaluating randomized trials with cohort and

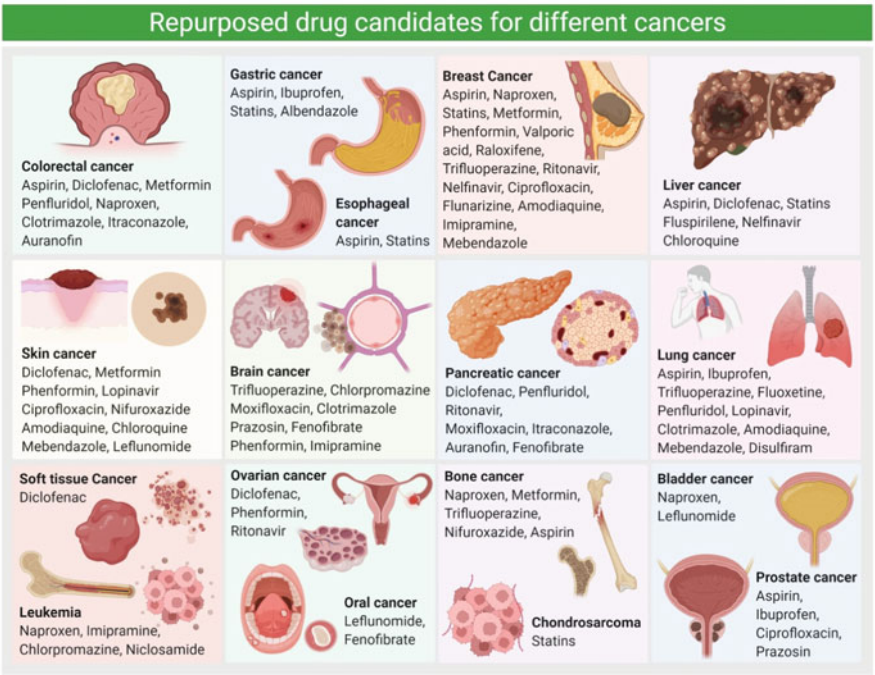


Fig. 5.1 Repurposed small-molecule non-oncology drug candidates for various cancers

case reports, Algra and his team revealed that frequent consumption of aspirin considerably decreased the risk of colorectal, esophageal, gastric, liver, and breast cancer (Algra and Rothwell 2012). It was capable to stop hepatocellular carcinoma cells from growing by cell cycle arrest, induction of apoptosis, and changes in mRNA expression (Shi et al. 2020). In colorectal malignancy, it induces adenosine monophosphate kinase (AMP kinase) (Steinberg et al. 2013), G0/G1 cell cycle arrest, and apoptosis (Goel et al. 2003). Furthermore, it suppresses mTOR signaling (Din et al. 2012), Wnt-signaling, and β-Catenin phosphorylation in colon cancer (Bos et al. 2006). Aspirin was also reported to inhibit osteosarcoma development and metastasis via inhibiting the nuclear factor kappa B (NF-κB) network (Liao et al. 2015). Moreover, it prevented inhibition of nuclear factor-κB kinase (IKK-β)-mediated prostate cancer cell invasion by attacking matrix metalloproteinase-9 (MMP-9) and urokinase-type plasminogen activators (Shi et al. 2017). Furthermore, it also inhibited inflammation-related stemness gene activity (particularly $ICAM_3$) and histone demethylase (KDM6A/B) activity, which slows tumor development, spread and lengthens life in breast cancer cells (Zhang et al. 2020a, b).

Table 5.1 Repurposed small-molecule non-oncology drugs for the treatment of various cancers

Category	Drug	Cancers against which drug is repurposed	Molecular mechanisms
Non-steroidal anti-inflammatory drugs	Aspirin	Colorectal, esophageal, gastric, liver, & breast	Induced cell cycle arrest, apoptosis, changes in mRNA expression and AMP kinase, inhibited mTOR signaling, Wnt-signaling, β-Catenin phosphorylation, NF-κB network, matrix metalloproteinase-9 and urokinase-type plasminogen activator, GRP78 protein, $ICAM_3$, and KDM6A/B (Shi et al. 2020; Goel et al. 2003; Din et al. 2012; Bos et al. 2006; Liao et al. 2015; Shi et al. 2017; Zhang et al. 2020a, b)
	Diclofenac	Ovarian, pancreatic, skin colorectal, soft tissue, & liver	Induced apoptosis, ↑ arginase activity, ↓ VEGF levels, ↓ vascularization levels (Sleire et al. 2017; Albano et al. 2013; Falkowski et al. 2003; Mayorek et al. 2010)
	Ibuprofen	Lung, prostate, & gastric	Induced apoptosis, inhibited the heat shock protein 70, cell proliferation, and angiogenesis (Endo et al. 2014; Kim and Chung 2007; Akrami et al. 2015)
	Naproxen	Bone, bladder, colon, melanoma, leukemia, & breast	Activated cell cycle arrest and apoptosis, inhibited immunosuppressive state, ↑ TNF-alpha, ↓ PGE2 (Deb et al. 2014; Kim et al. 2014)
Statins	Simvastatin	Esophageal, liver, gastric, breast, & chondrosarcoma	↓ AKT and ERK signals, activated cell cycle arrest and apoptosis, blocked cell proliferation (Nagayama et al. 2021; Kirtonia et al. 2021)
Biguanides	Metformin	Skin, bone, breast, & colorectal	Induced G0-G1 phase cell cycle arrest, apoptosis, AMPK pathway and autophagy, inhibited cell proliferation and mobility (Tomic et al. 2011; Park et al. 2019)
	Phenformin	Ovary, glioma, breast, & skin	Induced AMPK activation, cellular stress and G1 phase cell cycle arrest, inhibited mTOR cascade, cellular proliferation, adhesion, and invasion (Appleyard et al. 2012; Jackson et al. 2017; Yuan et al. 2013)

(continued)

Table 5.1 (continued)

Category	Drug	Cancers against which drug is repurposed	Molecular mechanisms
Selective estrogen receptor modulators	Raloxifene	Breast	Inhibited MMP-2 enzyme tumor invasion and angiogenesis (Ağardan et al. 2016)
Antidepressants	Imipramine	Breast, myeloid leukemia, & brain	Induced apoptosis, inhibited FoxM1-mediated DNA repair, ROS, invasion, and metastasis (Rajamanickam et al. 2016; Metts et al. 2017; Munson et al. 2012)
	Trifluoperazine	Breast & skin	Induced mitochondria-mediated apoptosis and G0/G1 cell cycle arrest, blocked cyclin-dependent kinase D1/CDK4 and cyclin E/CDK2 (Feng et al. 2018; Xia et al. 2021)
	Fluoxetine	Breast, lung, & skin	Induced cytotoxic endoplasmic reticulum stress, apoptosis and autophagy, inhibited DNA repair and metastatic, ↓ mitogen-induced T cell proliferation (Bowie et al. 2015; Grygier et al. 2013)
Antipsychotic	Chlorpromazine	Brain, leukemia, & breast	Induced autophagic cell death, inhibited Akt/mTOR pathway, FLT3-ITD, KIT-D816V, and YAP signaling (Abbruzzese et al. 2020; Rai et al. 2020; Yang et al. 2019)
Anticonvulsant	Valproic acid	Head and neck squamous cell cancer & breast	↑ p21 level, induced G0/G1 cell cycle arrest (Gan et al. 2012; Aztopal et al. 2018)
Antiviral drugs	Ritonavir	Breast, ovaries, & pancreas	Induced apoptosis, inhibited Hsp90, RB/E2F-1 and AKT signaling (Srirangam et al. 2006; Kumar et al. 2009; Batchu et al. 2014)
	Nelfinavir	Liver & breast	Induced apoptosis and cell cycle arrest, inhibited Hsp90 & ROS generation (Sun et al. 2012; Shim et al. 2012; Soprano et al. 2016)
	Lopinavir	Skin, lung, & urological	Induced apoptosis, autophagy, and cell cycle arrest, ↑ ER stress (Marima et al. 2020; Okubo et al. 2019; Paskas et al. 2019)
Antibiotics	Ciprofloxacin	Skin, breast, & prostate	Induced apoptosis, p53/Bax/Bcl-2 signaling cascade and cell cycle arrest at S phase (Beberok et al. 2018a, b, c)

(continued)

Table 5.1 (continued)

Category	Drug	Cancers against which drug is repurposed	Molecular mechanisms
	Nifuroxazide	Thyroid & skin	Induced apoptosis, ↓ MMP-2, MMP-9 and ALDH1, ↑ CC-3 and Bax proteins, inhibited cell proliferation, migration, and invasion (Luo et al. 2019; Hu et al. 2019; Sarvi et al. 2018)
	Moxifloxacin	Brain & pancreas	Induced apoptosis, ERK, S-phase cell cycle arrest and caspase-3/7, ↓ glutathione levels (Beberok et al. 2018a, b, c; Yadav et al. 2015)
Antifungals	Clotrimazole	Lung, brain, & breast	Induced apoptosis and cell cycle arrest at the G0/G1 phase, inhibited glycolysis (Furtado et al. 2012; Khalid et al. 2005; Penso and Beitner 2002)
	Itraconazole	Colon, pancreas, & skin	Induced autophagy and BAK-1 protein, inhibited Hedgehog, Wnt, and PI3K/mTOR signaling cascades [Dend et al. 2020, Jiang et al. 2019, Liang et al. 2017]
Antiepileptic drug	Flunarizine	Skin	Induced autophagy, ↓ N-Ras protein (Zheng et al. 2018)
Antihypertensive drug	Prazosin	Brain, prostate	Induced caspase3, DNA damage stress, and G2 phase cell cycle arrest, inhibited AKT cascade, ↓ PKC-δ and Cdk-1 (Lin et al. 2007)
Antimalarials	Amodiaquine	Lung, breast, & skin	Induced apoptosis and S-phase cell cycle arrest (Parvathaneni et al. 2020; Salako 2021; Qiao et al. 2013)
	Chloroquine	Liver & skin	Activated G0/G1 cell cycle arrest, DNA damage and apoptosis, inhibited PUMA, ↓ HIF-1 and VEGF (Hu et al. 2016a, b; Lakhter et al. 2013; Thongchot et al. 2015)
Anthelmintics	Mebendazole	Breast, skin, & lung	Activated apoptosis and cell cycle arrest at the G2/M phase, ↓ XIAP, inhibited tumor cell proliferation (Zhang et al. 2019; Doudican et al. 2013; Sasaki et al. 2002)
	Niclosamide	Skin, leukemia, & renal cell cancer	Induced mitochondrial apoptotic cascade, inhibited FOXM1/-catenin and Wnt/-catenin (Zhu et al. 2019; Jin et al. 2017; Zhao et al. 2016)

(continued)

Table 5.1 (continued)

Category	Drug	Cancers against which drug is repurposed	Molecular mechanisms
	Albendazole	Ovary, gastric, & ocular	↓ VEGF-A, VEGF-C, TNF-alpha, and VEGFR-2 levels, induced STAT-3 and STAT-5, inhibited angiogenesis (Yang et al. 2021; Cho et al. 2019)
Antirheumatics	Leflunomide	Bladder, skin, & oral	Activated apoptosis, cell cycle arrest at the G1 and S phase, inhibited PI3K/Akt signaling cascade and cell proliferation, elevated cyclin A protein levels (Cheng et al. 2020; Hanson et al. 2018; Ren et al. 2017)
	Auranofin	Skin, colon, & pancreas	↓ cAMP, hexokinase, ROS and ATP production, inhibited TxnRd1 and HIF-1 (Goenka and Simon 2020; Han et al. 2019; Rios Perez et al. 2019)
Alcohol antagonist drug	Disulfiram	Breast, skin, & lungs	Induced cell cycle arrest at the G2/M phase and mitochondria-dependent apoptotic (Yang et al. 2016; Cen et al. 2004; Duan et al. 2014)
Antilipidemic	Fenofibrate	Pancreas, oral	Induced G0/G1 phase cell cycle arrest, p53, PPAR/FoxO1/p27 kip cascade, inhibited mTOR activity (Han et al. 2015; Hu et al. 2016a, b; Jan et al. 2016)

5.5.2.1.2 Diclofenac

It is an extensively prescribed NSAID that has antagonist activity on COX-2 and prostaglandin E2 production (Pantziarka et al. 2016). It has shown anticancer activity against ovarian, pancreatic, skin, colorectal, and liver cancer (Sleire et al. 2017). In skin and colorectal cancer cells, diclofenac promoted apoptosis (Albano et al. 2013; Falkowski et al. 2003). It slowed the development of pancreatic cancer cells by lowering vascular endothelial growth (VEGF) levels and increasing arginase activity (Mayorek et al. 2010).

5.5.2.1.3 Ibuprofen

Ibuprofen has displayed anti-tumor effectiveness in variety of cancers such as lung, prostate, and gastric cancer. It has caused apoptosis in lung carcinoma cells by retarding the heat shock protein 70 (Endo et al. 2014). In prostate cancer, ibuprofen

showed programmed cell death (Kim and Chung 2007). Furthermore, it blocked tumor development in gastric cancer via triggering apoptosis and decreasing cell proliferation and angiogenesis (Akrami et al. 2015).

5.5.2.1.4 Naproxen

Naproxen is a propionic-acid analog that is a non-selective COX-blocker (Han and Küçükgüzel 2020). It has exhibited anticancer activity towards bone, bladder, colon, melanoma, leukemia, and breast malignancy. Tumor growth was reduced by naproxen via inducing apoptosis and inhibiting migration in breast carcinoma (Deb et al. 2014). In human urinary bladder cancer, exhibited anticancer activity through the arrest of cell cycle progression and programmed cell death (Kim et al. 2014).

5.5.2.2 Statins

5.5.2.2.1 Simvastatin

Statins like simvastatin are a kind of cholesterol-lowering medication commonly employed in the treatment of atherosclerosis. It works by inhibiting the active site of 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMG-CoA reductase), the first and most important rate-limiting enzyme in the mevalonate cascade (Ward et al. 2019). Statins were reported to show anticancer activity against esophageal, breast, gastric, and liver cancer. Statins have inhibited tumor growth in esophageal carcinoma via downregulating AKT and extracellular signal-regulated kinase (ERK) signals (Nagayama et al. 2021). Also, statins proved to inhibit proliferation and promote conversion in primary liposarcoma cells in culture as well as growth arrest and differentiation in breast cancer cells and apoptosis in malignancies such as chondrosarcoma and gastric and liver tumors (Kirtonia et al. 2021).

5.5.2.3 Antidiabetic Drugs

5.5.2.3.1 Metformin

Metformin, a biguanide analog, is a medication used for the treatment of type 2 diabetes (Tomic et al. 2011). It has shown chemotherapeutic properties against a variety of malignancies including skin, bone, breast, and colorectal. It has demonstrated anti-tumor action against skin cancer cells by inducing autophagy, arresting the cell cycle (G0-G1) phase, and activating the AMP-activated protein kinase (AMPK) pathway (Tomic et al. 2011). Further, it has prevented tumor development in rectal cancer cells by decreasing cell proliferation, mobility, and penetration, lowering clonogenic capacity, and promoting apoptotic cell death (Park et al. 2019).

5.5.2.3.2 Phenformin

It is also an antidiabetic drug belonging to the biguanide class (Jackson et al. 2017). It has demonstrated antineoplastic activity against ovarian, glioma, breast, and skin cancer. It has hindered the growth of breast cancer cells via AMPK activation (Appleyard et al. 2012). In ovarian cancer, phenformin decreased cellular proliferation, produced cell cycle G1 arrest and death, caused cellular stress, prevented adhesion and invasion, and activated AMPK while inhibiting the mTOR cascade (Jackson et al. 2017). Moreover, it induced programmed cell death in glioma stem cells (Jiang et al. 2016). In melanoma cells, it has significantly reduced tumor growth via inhibiting cell viability, mTOR signaling, and inducing apoptosis (Yuan et al. 2013).

5.5.2.4 Selective Estrogen Receptor Modulators (SERMs)

5.5.2.4.1 Raloxifene

Raloxifene is a SERM member approved by the food and drug administration (FDA) against osteoporosis. It showed promise against breast cancer. The underlying mechanism of anti-tumor activity of raloxifene is obstruction of the matrix metalloproteinase-2 (MMP-2) enzyme, which is recognized to be important for tumor invasion and angiogenesis during tumorigenesis (Ağardan et al. 2016).

5.5.2.5 Antidepressants

5.5.2.5.1 Imipramine

Imipramine is FDA approved drug for treating depression via inhibition of the reuptake of serotonin and norepinephrine. It reported effectiveness against breast cancer, leukemia, and glioma. It suppressed breast cancer growth and metastasis via inhibition of forkhead box protein M1 (FoxM1) and FoxM1-mediated DNA repair (Rajamanickam et al. 2016). Furthermore, it induced apoptosis and inhibited reactive oxygen species (ROS) in myeloid leukemia cells (Metts et al. 2017). In the glioma, imipramine caused inhibition of invasion of glioma cells (Munson et al. 2012).

5.5.2.5.2 Trifluoperazine

It belongs to the phenothiazine derivative that blocks dopaminergic D1 and D2 receptors in the brain and is extensively prescribed as an antidepressant. Several studies reported its anti-tumor potential against breast cancer and melanoma (skin cancer). It enhanced mitochondria-mediated apoptosis in breast cancer by inducing G0/G1 cell cycle arrest and lowering the levels of both cyclinD1/CDK4 and cyclin

E/CDK2 (Feng et al. 2018). Furthermore, trifluoperazine inhibited melanoma cell growth and migration to the lungs, bones, and brain by causing G0/G1 cell cycle seize and mitochondrial-dependent intrinsic apoptosis (Xia et al. 2021).

5.5.2.5.3 Fluoxetine

Fluoxetine was the first licensed selective serotonin reuptake antagonist for depression, blocking serotonin 5-hydroxytryptamine (5-HT) reuptake at the presynaptic membrane while boosting 5-HT effects on serotonin receptors at the post-synaptic neuron. It demonstrated promise against cancers like breast, lung, and melanoma. In triple-negative breast cancer, fluoxetine promoted cytotoxic endoplasmic reticulum stress and autophagy (Bowie et al. 2015). In non-small cell lung cancer (NSCLC), it caused apoptosis and hindered DNA repair and metastatic potential (Wu et al. 2018). Furthermore, it reduced melanoma progression via augmenting the mitogen-induced T cell proliferation (Grygier et al. 2013).

5.5.2.6 Antipsychotic

5.5.2.6.1 Chlorpromazine

Chlorpromazine, a tricyclic antipsychotic belonging to phenothiazine derivatives is employed to treat mental problems (Abbruzzese et al. 2020). It has shown its anticancer activity against cancers like brain, leukemia, breast, and glioma. Inhibition of the Akt/mTOR pathway caused autophagic cell death in human glioma cells (Abbruzzese et al. 2020). Furthermore, by disrupting the subcellular organization of FMS-like tyrosine kinase 3 (FLT3-ITD) and KIT-D816V, chlorpromazine was able to eradicate acute myeloid leukemia and slow tumor development (Rai et al. 2020). It reduced tumor development by suppressing yes-associated protein (YAP) signaling and stemness features in breast cancer cells (Yang et al. 2019).

5.5.2.7 Anticonvulsant

5.5.2.7.1 Valproic Acid

It is a short-chain fatty acid used as an anticonvulsant and mood stabilizer in humans, notably in the long-term therapy of epilepsy. It mainly acts on GABA (-γ-aminobutyric acid) levels in the central nervous system, blocking voltage-gated ion channels and suppressing histone deacetylase, among other things. It exhibited anticancer action in squamous cell carcinoma of the head and neck and breast cancer. It showed antineoplastic activity via suppression of cell growth both acutely and chronically by promoting p21 expression and cell cycle arrest at G0/G1 (Gan et al. 2012; Aztopal et al. 2018).

5.5.2.8 Antiviral Drugs

5.5.2.8.1 Ritonavir

Ritonavir is an antiretroviral protease blocker utilized in HIV transmission treatment and prophylaxis (Meini et al. 2020). It showed anti-tumor activity against cancers like breast, ovarian, and pancreatic cancer. Ritonavir decreased cell growth in breast cancer via blocking heat shock protein-90 (Hsp90) substrates, including Akt (Srirangam et al. 2006). Moreover, it reduced migration and invasion in ovarian cancer cells by blocking AKT signaling and activating apoptosis (Kumar et al. 2009). In the pancreatic cancer, it has displayed anticancer activity through protein/E2 transcription factor-1 (RB/E2F-1) and AKT pathways mediated apoptosis (Batchu et al. 2014).

5.5.2.8.2 Nelfinavir

Nelfinavir is another antiretroviral protease inhibitor used in the treatment and prevention of HIV acquisition. It showed promise against various cancers like liver and breast cancer. In liver cancer, nelfinavir suppressed the development of tumor cells by inducing apoptosis and cell cycle arrest (Sun et al. 2012). It blocked the growth of breast tumor cells via suppression of Hsp90 (Shim et al. 2012). In addition, it also exhibited antiproliferative activity in breast cancer by ROS generation (Soprano et al. 2016).

5.5.2.8.3 Lopinavir

Lopinavir is an antiviral peptidomimetic human immunodeficiency virus (HIV) protease inhibitor used for the treatment of acquired immunodeficiency syndrome (AIDS) (Marima et al. 2020). Apart from antiviral potential, it has shown anti-tumor effects in skin, lung, and urological cancers. It disrupted cell cycle progression in lung cancer cells, resulting in slower tumor development (Marima et al. 2020). The combination of lopinavir and ritonavir has a combinatorial effect on urological cancer cells by generating endoplasmic reticulum (ER) stress (Okubo et al. 2019). Furthermore, lopinavir showed anti-melanoma activity via induction of apoptosis, autophagy, and reduction of cell growth (Paskas et al. 2019).

5.5.2.9 Antibiotics

5.5.2.9.1 Ciprofloxacin

Ciprofloxacin is a fluoroquinolone-class second-generation antibiotic used to combat respiratory and urinary tract infections. It stops cell growth by blocking topoisomerase II (DNA gyrase) and topoisomerase IV, which are required for bacterial deoxyribonucleic acid (DNA) separation (Zhang et al. 2018). It has also been proven to have anticancer properties in skin, breast, and prostate malignancies. Ciprofloxacin induced apoptosis and cell cycle arrest at S phase in melanoma cells (Beberok et al. 2018a, b, c). It activated the p53/BCL2-associated x protein (Bax)/B-cell lymphoma-2 (Bcl-2) signaling cascade, which led to the death of human breast cancer cells (triple-negative) (Beberok et al. 2018a, b, c). Besides, it inhibited the growth of prostate cancer cells through the apoptosis and cell cycle arrest (Beberok et al. 2018a, b, c).

5.5.2.9.2 Nifuroxazide

Nifuroxazide (nitrofuran antibiotic) is used orally to treat colitis and diarrhea in humans and other animals (Luo et al. 2019). It has demonstrated an anti-tumor activity against bone, thyroid, and skin malignancy. In bone cancer, it triggered apoptosis and reduced cell migration and invasion (Luo et al. 2019). In thyroid malignancy, it caused cell death by potentiating the expressions of CC-3 and Bax proteins and hindering cell proliferation, migration, and invasion by lowering the protein expressions of MMP-2 and MMP-9 (Hu et al. 2019). It inhibited the aldehyde dehydrogenase-1 (ALDH1) enzyme, which inhibited tumor development in melanoma cells (Sarvi et al. 2018).

5.5.2.9.3 Moxifloxacin

Moxifloxacin is a fourth-generation fluoroquinolone highly effective against gram-positive bacteria. It works by blocking DNA gyrase, topoisomerase II and IV (Beberok et al. 2018a, b, c). Moxifloxacin reported antineoplastic activity toward the brain and pancreatic cancer. It caused apoptosis and S-phase arrest in brain tumors by depleting glutathione, breaking down mitochondrial membranes, activating caspase-3/7, and fragmenting DNA (Beberok et al. 2018a, b, c). Furthermore, it triggered S-phase cell cycle arrest and induced apoptosis in human pancreatic cancer cells through activation of ERK (Yadav et al. 2015).

5.5.2.10 Antifungals

5.5.2.10.1 Clotrimazole

Clotrimazole is an antifungal drug belonging to the azole analog family. It has shown anticancer potential in the cancers like lung, brain, breast, and adenocarcinoma. It decreased cell growth and survival in breast cancer cells via cell cycle arrest and inhibition of glycolysis (Furtado et al. 2012). In brain cancer, it caused apoptosis and cell cycle seize at the G0/G1 phase (Khalid et al. 2005). Clotrimazole has impaired the survival of lung carcinoma and colon adenocarcinoma cells by inhibition of glycolysis (Penso and Beitner 2002).

5.5.2.10.2 Itraconazole

This antifungal medicine is mainly employed to treat systemic fungal infections. Besides its antifungal potential, itraconazole also exhibited anti-tumor activity against colon and pancreatic cancer and melanoma. It blocked the hedgehog signaling cascade, causing colon cancer cells to die by autophagy (Deng et al. 2020). Furthermore, it activated the Bak-1 protein and reduced the development of pancreatic cancer cells (Jiang et al. 2019). Itraconazole also displayed potency against melanoma via suppression of Hedgehog, Wnt, and phosphoinositide 3-kinase (PI3K)/mTOR signaling cascades (Liang et al. 2017).

5.5.2.11 Antiepileptic Drug

5.5.2.11.1 Flunarizine

Flunarizine is a calcium channel blocker employed to cure headaches and seizures. Antineoplastic effects have been reported against breast cancer. In breast malignancy, its antineoplastic activity was noticed by activating the autophagy process by degrading the neuroblastoma-RAS (N-Ras) protein (Zheng et al. 2018).

5.5.2.11.2 Prazosin

Prazosin is an antihypertensive medication belonging to the α-1 receptor antagonist family and intended to cure hypertension. Its anticancer activity is observed in the cancers such as glioblastoma and prostate cancer. Prazosin caused cell death in glioblastoma by inhibiting the AKT cascade via protein kinase C-delta (PKC-δ), resulting in caspase3 activation (Assad Kahn et al. 2016). It also caused cell death in prostate cancer cells by inducing DNA damage stress, which resulted in cyclin-dependent kinase-1 (Cdk-1) inactivation and G2-checkpoint arrest (Lin et al. 2007).

5.5.2.12 Antimalarials

5.5.2.12.1 Amodiaquine

Amodiaquine is an antimalarial medication of the 4-aminoquinoline class. It displayed anticancer activity against lung cancer, breast cancer, and skin cancer. In NSCLC and breast cancer, it caused apoptosis and blocked autophagy (Parvathaneni et al. 2020; Salako 2021). Besides, it reduced cell growth and triggered S-phase cell cycle arrest in skin cancer cells (Qiao et al. 2013).

5.5.2.12.2 Chloroquine

It is the 4-aminoquinoline antiviral licensed by the FDA for malaria prophylaxis and therapy. It exhibited anticancer potential against several cancers like hepatic cancer, skin cancer, and bile duct cancer. In hepatocarcinoma, it caused G0/G1 cell cycle arrest, DNA damage, and death (Hu et al. 2016a, b). It augmented apoptosis in skin cancer cells by blocking the p53 upregulated modulator of apoptosis (PUMA) degradation driven by the BH3 domain (Lakhter et al. 2013). It inhibited metastasis in bile duct carcinoma via downregulating hypoxia-inducible factor (HIF-1) and factor VEGF (Thongchot et al. 2015).

5.5.2.13 Anthelmintics

5.5.2.13.1 Mebendazole

Mebendazole (benzimidazoles) are tubulin-disrupting medications employed to heal parasitic infections in people. Several studies reported its promise against breast, skin, and lung cancer. It displayed anti-tumor activity in breast cancer via induction of apoptosis and cell cycle arrest at the G2/M phase (Zhang et al. 2019). Moreover, mebendazole showed anti-melanoma activity via suppression of the X-linked inhibitor of apoptosis (XIAP) (Doudican et al. 2013). In NSCLC, it caused depolymerization of tubulin that resulted in mitotic arrest and death of tumor cells (Sasaki et al. 2002).

5.5.2.13.2 Niclosamide

It is used to treat tapeworm infection for over 50 decades. Despite anti-helminthic use, it showed activity against melanoma, leukemia, and renal cancer. It promoted apoptosis and reduced tumor cell development in melanoma cells via the mitochondrial apoptotic cascade (Zhu et al. 2019). By inhibiting the association of p65 with (FoxM1)/-catenin, it prevented leukemia stem cells from surviving and

self-renewing (Jin et al. 2017). Besides, it hindered Wnt/-catenin and caused mitochondrial malfunction, suppressing renal cell cancer (Zhao et al. 2016).

5.5.2.13.3 Albendazole

Albendazole is a carbamate anthelminthic benzimidazole analog, which can inhibit worm cell growth by disrupting microtubule formation and altering glucose absorption. It has displayed effectiveness in gastric and ocular cancer. It has anti-tumor effects on gastric cancer cells via influencing the activity of signal transducer and activator of transcription-3 (STAT-3) and (STAT-5) by a pleiotropic pathway (Yang et al. 2021). Furthermore, it inhibited angiogenesis in ocular carcinoma by lowering VEGF-A, VEGF-C, TNF-alpha, and VEGFR-2 levels (Cho et al. 2019).

5.5.2.14 Antirheumatics

5.5.2.14.1 Leflunomide

Leflunomide is an immunoregulatory medication that has been authorized in the clinic for the management of rheumatoid arthritis and allograft refusal. It is also noticed to be effective in bladder cancer, skin cancer, and head and neck cancer. It reduced growth and promoted death in human bladder malignant cells through decreasing autophagy and the PI3K/Akt signaling cascade (Cheng et al. 2020). In skin cancer, it caused apoptosis, cell cycle arrest at the G1 phase, and decreased cell proliferation (Hanson et al. 2018). Similarly, it prevented cell cycle arrest during the S phase and cell proliferation in oral squamous cell tumor cells with an elevation of cyclin A protein levels (Ren et al. 2017).

5.5.2.14.2 Auranofin

Auranofin is an FDA approved organogold medication employed to treat rheumatism. It has also been re-profiled for a variety of pharmacological purposes due to its bactericidal, fungicidal, and anti-inflammatory properties. Apart from the aforementioned activities, it also demonstrated anticancer activity in melanoma, colon cancer, and ductal carcinoma. The underlying mechanisms of anticancer activity of auranofin are reduced cyclic adenosine monophosphate (cAMP) levels and cellular ROS (Goenka and Simon 2020). Moreover, it generated significant oxidative stress on colon cancer cells, resulting in ROS-mediated blockage of hexokinase and disruption of mitochondrial redox equilibrium, eventuating in reduced ATP production and tumor cell growth (Han et al. 2019). Similarly, it slowed pancreatic ductal adenocarcinoma development via inhibition of thioredoxin reductase-1 (TxnRd1) and HIF-1 (Rios Perez et al. 2019).

5.5.2.15 Antilipidemic

5.5.2.15.1 Fenofibrate

Fenofibrate is an antilipidemic agent used to lower cholesterol levels. It is found to be effective against glioblastoma, pancreatic cancer, and oral cancer. In human glioblastoma cells, it triggered G0/G1 phase arrest via regulating the proliferator-activated receptor alpha (PPAR)/FoxO1/p27 kip cascade (Han et al. 2015). The anticancer effect in the pancreatic malignancy was observed by activating p53 through the overexpression of long non-coding RNA (MEG-3) (Hu et al. 2016a, b). Furthermore, it inhibited tumor development in oral malignancy by inhibiting mTOR activity (Jan et al. 2016).

5.5.2.16 Alcohol Antagonist Drug

5.5.2.16.1 Disulfiram

Disulfiram is a wide-range anti-alcoholism agent that inhibits aldehyde dehydrogenase, a key enzyme in the physiological purification of ethyl alcohol. Several research studies reported its antineoplastic potential against breast cancer, melanoma, and lung cancer. In breast cancer, disulfiram caused cell cycle arrest at the G2/M phase and mitochondria-dependent apoptotic cascade (Yang et al. 2016). In human melanoma cells, it accelerated intracellular copper (Cu) absorption and induced apoptosis (Cen et al. 2004). Furthermore, in NSCLC, produced cell cycle arrest at the G2/M phase, reduced lung cancer cells spheroid formation, and mRNA levels of lung cancer stem cell genes (Duan et al. 2014).

5.5.3 Repurposing Phytochemicals

The different kinds of phytochemicals repurposed against the cancers are depicted in Fig. 5.2 and are summarized with their molecular mechanisms in Table 5.2. These various phytochemicals repurposed are further discussed below.

5.5.3.1 Resveratrol

Resveratrol is a kind of polyphenol derived from peanuts, grapes (red wine), tiger cane, mulberries, and other plants. It is crucial in disorders including aging, neurological impairment, and inflammation. It has also been proven to have cytotoxic activity in lung, prostate, and breast malignancies. It increased doxorubicin sensitivity in breast carcinoma by suppressing epithelial-mesenchymal transition and

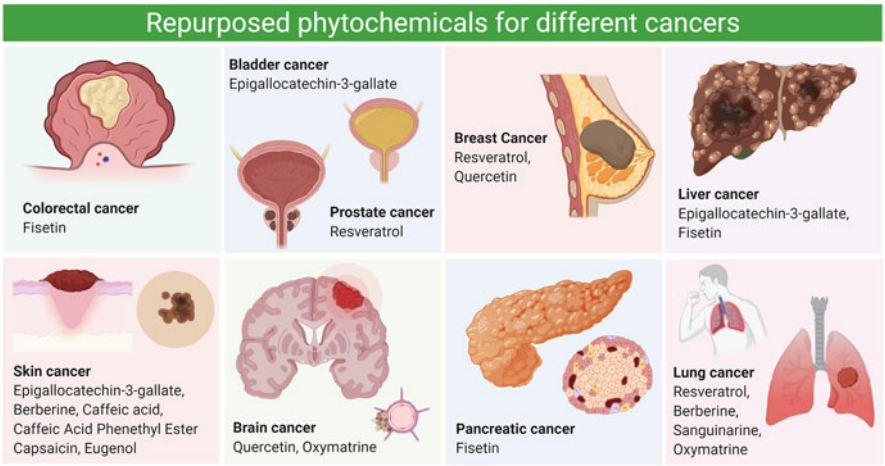


Fig. 5.2 Repurposed phytochemicals against various sorts of cancers

altering the sirtuin-1(SIRT1)/-catenin signaling pathway (Jin et al. 2019). By controlling intrinsic apoptosis, it increased the susceptibility of small-cell lung cancer cells to cisplatin (Li et al. 2018a, b). It used the p53/ $p21^{WAF1/CIP1}$ and $p27^{KIP1}$ pathways to cause cell cycle arrest and death in prostate malignancy (Singh et al. 2017).

5.5.3.2 Quercetin

Quercetin is a pigment found in plants (flavonoid). It possesses anti-inflammatory and antioxidant properties that may assist to decrease edema, destroying tumor cells, regulating blood sugar, and avoiding heart disease. In addition to the aforementioned activities, it has also shown anti-tumor efficacy against brain and breast malignancies. In human glioma cells, it induced cell death and inhibited the production of matrix metallopeptidase-9 (MMP-9) and fibronectin via the AKT and ERK signaling pathways (Pan et al. 2015). It removed breast cancer stem cells via removing Y-box binding protein 1 nuclear translocation and reduced multidrug resistance in breast cancer cells by downregulating P-GP levels (Li et al. 2018a, b).

5.5.3.3 Epigallocatechin-3-Gallate

Green tea has a high concentration of polyphenol epigallocatechin-3-gallate. It is reported to be cytotoxic in skin, liver, and bladder cancers. It induced apoptosis and slowed the development of bladder tumor cells by inhibiting the sonic hedgehog pathway (Zhang et al. 2015). In liver cancer cells, it caused apoptosis, lowered mitochondrial membrane potential, and increased G0/G1 phase cell cycle arrest (Sun

Table 5.2 Repurposed phytochemicals for the treatment of various cancers

Phytochemical	Cancers against which phytochemical is repurposed	Molecular mechanisms
Resveratrol	Lung, prostate & breast	Altered SIRT1/-catenin signaling pathway, induced cell cycle arrest, p53/ $p21^{WAF1/CIP1}$ and $p27^{KIP1}$ pathways (Jin et al. 2019; Li et al. 2018a, b; Singh et al. 2017)
Epigallocatechin-3-gallate	Bladder, skin, & liver	Activated G0/G1 phase cell cycle arrest and apoptosis, inhibited sonic Hedgehog pathway, tumor cell proliferation and NF-κB, ↓ mitochondrial membrane potential and IL-1 production (Zhang et al. 2015; Sun et al. 2019; Ellis et al. 2011)
Caffeic acid	Skin	Activated apoptosis inhibited the mTOR/PI3K/AKT signaling cascade (Zeng et al. 2018)
Quercetin	Brain & breast	↓ P-GP levels, induced cell cycle arrest, inhibited MMP-9, fibronectin, AKT and ERK signaling pathways (Pan et al. 2015; Li et al. 2018a, b)
Fisetin	Liver, colon & pancreas	Induced apoptosis, oxidative stress response, CDK-5, NRF-2, glucocorticoid signaling, and the ERK/MAPK signaling cascade (Khan et al. 2013)
Berberine	Skin & lung	Induced caspase-3 and apoptosis antiangiogenic activity, ↓ hypoxia-inducible factor-1, VEGF, Bcl-2, Bcl-xl and proinflammatory mediators (Hamsa and Kuttan 2012; Katiyar et al. 2009)
Sanguinarine	Lung	Activated cell cycle at the S phase and apoptosis, ↑ Fas-associated factor 1 (Wei et al. 2017)
Oxymatrine	Brain & lung	Induced cell cycle arrest in the G0/G1 stage, EGFR/PI3K/Akt/mTOR signaling cascade and STAT3 (Dai et al. 2018; Zhou et al. 2019)
Caffeic acid phenethyl ester	Skin	Induced apoptosis, ↑ ROS, ↓ glutathione (Kudugunti et al. 2011)
Capsaicin	Skin	Inhibited phosphoinositide 3-kinases/Akt/Rac1 signal cascade (Shin et al. 2008)
Eugenol	Skin	Induced apoptosis, ↓ c-Myc, H-ras, and Bcl2, ↑ P53, Bax, and active Caspase-3 (Pal et al. 2010)

et al. 2019). It inhibited tumor cell proliferation by decreasing interleukin 1 beta (IL-1) production and nuclear factor kappa B (NF-κB) activity in skin cancer (Ellis et al. 2011).

5.5.3.4 Fisetin

Fisetin, one of the most widespread bioactive flavonoids found in vegetables and fruits, has been widely demonstrated to have antioxidant and anti-inflammatory effects due to its polyphenol structure (Khan et al. 2013). It also exhibited an anti-tumor effect in liver, colon, and pancreatic cancer. In all aforementioned cancer, fisetin inhibited growth and triggered via the cyclin-dependent kinase 5 (CDK-5) signaling cascade, the nuclear factor erythroid 2–related factor 2 (NRF-2) facilitated oxidative stress response, glucocorticoid signaling, and the ERK/mitogen-activated protein kinase (MAPK) signaling cascade (Khan et al. 2013).

5.5.3.5 Berberine

Berberine is an isoquinoline alkaloid derivative utilized to alleviate bacterial diarrhea. It also has anti-tumor properties over skin and lung malignancies. It displayed anti-melanoma efficacy by downregulating hypoxia-inducible factor-1, VEGF, and proinflammatory mediators (Hamsa and Kuttan 2012). It caused apoptosis in human lung cancer cells by disrupting mitochondrial membrane potential, lowering Bcl-2, B-cell lymphoma-extra large (Bcl-xl) levels, and activating caspase-3 (Katiyar et al. 2009).

5.5.3.6 Sanguinarine

Sanguinarine is a benzo phenanthridine alkaloid derived from plants in the Papaveraceae family. It is commonly used to treat inflammation conditions. It observed to be effective in the lung by upregulating Fas-associated factor 1, inducing programmed cell death, and arresting the cell cycle at the S phase (Wei et al. 2017).

5.5.3.7 Caffeic Acid Phenethyl Ester

It is a caffeic acid ester analog which is an effective ingredient of propolis. It has numerous actions, comprising bactericidal, anti-inflammatory, antiviral, and anti-cancer. It decreased glutathione levels, raised ROS, and caused programmed cell death in skin cancer resulting in a decrease in the growth of cancer cells (Kudugunti et al. 2011).

5.5.3.8 Capsaicin

Capsaicin is one of the key pungent compounds present in red pepper and used as spices. It showed promising activity in melanoma. Capsaicin stopped melanoma

cells from migrating by hindering the phosphoinositide 3-kinases/Akt/Rac1 signal cascade (Shin et al. 2008).

5.5.3.9 Eugenol

Eugenol is the active ingredient found in clove essential oil (Syzygium aromaticum). It possesses antimutagenic, antigenotoxic, and anti-inflammatory activity. Despite, eugenol demonstrated anti-melanoma potential. It inhibited melanoma cell growth by downregulating the master regulator of cell cycle entrance and proliferative metabolism (c-Myc), H-ras, and Bcl2 expression and inducing apoptosis by upregulating P53, Bax, and active caspase-3 expression (Pal et al. 2010).

5.5.3.10 Caffeic Acid

Caffeic acid is a phenolic chemical found in coffee, wine, tea, and popular medications like propolis. It has antioxidant, anti-inflammatory, and anticancer properties. Caffeic acid also showed activity in skin cancer. Through activation of programmed cell death and suppression of the mTOR/PI3K/AKT signaling cascade, it has exhibited an anti-tumor effect in skin cancer (Zeng et al. 2018).

5.5.3.11 Oxymatrine

Oxymatrine is a naturally occurring quinolizidine alkaloid reported for wide range of activity like anti-inflammatory, antiviral, and immunostimulatory. It is also reported to be efficacious in the glioblastoma and lung cancer. It inhibited the invasion of human glioblastoma cells by inducing cell cycle arrest and death via the EGFR/PI3K/Akt/mTOR signaling cascade and STAT3 (Dai et al. 2018). It stopped lung cancer cells from proliferating and triggered cell cycle arrest in the G0/G1 stage (Zhou et al. 2019).

5.6 Repurposed Non-Oncology Drugs in Clinical Trials for the Treatment of Different Cancers

An ample of drugs from different pharmacological classes effective in diverse types of cancers are under diverse stages of clinical trials. Various non-oncology drugs which are under different phases of clinical trials against diverse kinds of cancer are summarized in Table 5.3. For instance, itraconazole (antifungal) medicine approved for fungal infections (toenail, fingernail) has been tested for its efficacy against prostate cancer. In prostate cancer, itraconazole exhibited anticancer activity via

Table 5.3 List of repurposed drugs undergoing clinical trials against various cancers

Drug(s)	Clinical identifier	Phase of study	Type/stage of cancer	Status
Itraconazole and hydroxychloroquine	NCT03513211	Phase I &II	Prostate cancer	Recruiting
	NCT03081702	Phase I & II	Platinum-resistant epithelial ovarian cancer	Completed
Itraconazole	NCT04081831	–	Gastrointestinal cancer	Completed
	NCT02357836	Early phase I	NSCLC	Completed
	NCT00887458	Phase II	Prostate cancer	Completed
	NCT01108094	Phase II	Skin cancer	Completed
	NCT04018872	Phase II	Esophagus squamous cell cancer	Recruiting
Aspirin & Vitamin D3	NCT03103152	Phase II & III	Prostate cancer	Completed
Aspirin	NCT04081831	–	Gastrointestinal cancer	Completed
	NCT02301286	Phase III	Colon cancer	Recruiting
Diclofenac, diclofenac, & calcitriol	NCT01358045	Phase II	Basal cell carcinoma	Completed
Diclofenac epolamine	NCT01380353	Early phase I	Breast cancer	Completed
Statin	NCT01813994	–	Gastric adenoma	Completed
Simvastatin & aspirin	NCT02285738	Early phase I	Solid tumor cancer	Recruiting
Simvastatin	NCT01099085	Phase III	Gastric cancer	Completed
	NCT04457089	Early phase I	Recurrent ovarian cancer	Recruiting
Atorvastatin	NCT05103644	Phase II & III	Breast cancer	Recruiting
	NCT01988571	Phase II	Breast cancer & lymphoma	Completed
	NCT01759836	Phase II	Prostatic neoplasms	Completed
Metformin hydrochloride & ritonavir	NCT02948283	Phase I	Multiple myeloma lymphocytic leukemia	Completed
Metformin	NCT04947020	–	Rectal cancer	Recruiting
	NCT01266486	Phase II	Breast cancer	Completed
	NCT01243385	Phase II	Prostate cancer	Completed
	NCT01205672	Early phase I	Endometrial cancer	Completed
	NCT01677897	Phase II	Metastatic prostate cancer	Completed
Raloxifene and tamoxifen	NCT00003906	Phase III	Breast cancer	Completed
Imipramine	NCT03122444	Early phase I	Breast cancer	Recruiting

(continued)

Table 5.3 (continued)

Drug(s)	Clinical identifier	Phase of study	Type/stage of cancer	Status
Temozolomide	NCT04224441	Phase II	Glioblastoma	Recruiting
Ritonavir	NCT01009437	Phase I	Breast cancer	Completed
	NCT00002366	Phase II	Kaposi sarcoma	Completed
Nelfinavir	NCT00704600	Phase I & II	Colorectal cancer	Completed
	NCT01445106	Phase I	Solid tumors	Completed
	NCT01065844	Phase II	Head and neck cancer	Completed
Ciprofloxacin	NCT02173262	Phase IV	Breast cancer	Completed
Chloroquine	NCT01023477	Phase I & II	Ductal carcinoma	Completed
Mebendazole	NCT02644291	Phase I	Medulloblastoma	Recruiting
	NCT01729260	Phase I	High-grade glioma	Completed
Leflunomide	NCT03709446	Phase I & II	Breast cancer	Recruiting
Auranofin	NCT01747798	Early phase I	Ovarian epithelial cancer & fallopian tube cancer	Completed
	NCT01737502	Phase I & II	NSCLC	Recruiting
Disulfiram & copper gluconate	NCT00742911	Phase I	Solid tumor	Completed
Disulfiram	NCT00256230	Phase I & II	Stage IV melanoma	Completed
	NCT01118741	NA	Prostate cancer	Completed
	NCT03323346	Phase II	Breast cancer	Recruiting

inhibition of Hh pathway and angiogenesis [NCT00887458]. Furthermore, in another trial, the itraconazole efficacy was tested in NSCL. They studied the effect of itraconazole on the inhibition of angiogenesis and Hh pathway. Investigator gave itraconazole (600 mg PO) for 7–10 days to the 15 patients diagnosed with NSCLC. Finally, they observed the anticancer potency of itraconazole at 600 mg in the NSCLC via inhibition of Hh pathways (NCT02357836).

5.7 Future Prospects

In the recent years, large number of small-molecule non-oncology therapeutics and phytochemicals showing anticancer indication has increased. These therapeutics may have ability to interact with many targets therefore understanding of its polypharmacology is of huge importance. There is necessity to screen the suitable therapeutics having a better affinity with cancer targets via different computational tools to achieve better anticancer effect. Moreover, these computational avenues can

also help to identify the novel cancer targets of repurposed therapeutics. The in vitro analysis like proteins expression is essential to investigate the molecular mechanisms of these therapeutics. The preclinical studies are essential to ascertain the safety and efficacy of repurposed therapeutics. Furthermore, compiling a new dossier for the new pharmacology of repurposed therapeutics is essential.

The combined delivery of repurposed therapeutics alone and repurposed therapeutics with approved antineoplastic drugs could be useful to achieve superior anticancer efficacy and reduce the dose-related toxicities. However, more research is expected to confirm the drug-drug interactions, safety, and efficacy. Besides, only few therapeutics are noticed to be repurposed for several cancers in the clinical trials and there are enormous opportunities to take the leftover therapeutics to the clinical level.

Furthermore, the prime challenge when the repurposed drug is delivered in the conventional way is its non-specific distribution and toxicities allied to their approved indication. Therefore, the delivery of repurposed therapeutics as nanoparticulate drug using an appropriate route of administration is of great significance. There is a lack of repurposed drug-loaded nanoformulations at the commercial level. Therefore, there is a great opportunity to engineer efficient nanoformulation laden with repurposed therapeutics against a variety of cancers and their commercialization.

5.8 Concluding Remarks

Drug repurposing has gained more interest from the pharmaceutical industry, academia, and other public sector as a speedier and less expensive way to expand the armory of licensed cancer therapeutics. In this chapter, the importance of repurpose therapeutics, drug repurposing approaches, repurposed small-molecule non-oncology drugs, and phytochemicals for different cancer is briefed. Various therapeutic (small-molecule non-oncology and phytochemicals) candidates repurposed for range of cancers are mentioned along with their molecular mechanisms. However, many of these therapeutics are still at the laboratory scale and so present new prospects for pharmaceutical scientist to take it to the clinical and commercial level.

Acknowledgements The authors would like to acknowledge the support from their respective institutes throughout the writing process.

References

Abbruzzese C, Matteoni S, Signore M, Cardone L, Nath K, Glickson JD, Paggi MG (2017) Drug repurposing for the treatment of glioblastoma multiforme. J Exp Clin Cancer Res 36(1):169. https://doi.org/10.1186/s13046-017-0642-x

Abbruzzese C, Matteoni S, Persico M, Villani V, Paggi MG (2020) Repurposing chlorpromazine in the treatment of glioblastoma multiforme: analysis of literature and forthcoming steps. J Exp Clin Cancer Res 39(1):26. https://doi.org/10.1186/s13046-020-1534-z

Ağardan NBM, Değim Z, Yılmaz Ş, Altıntaş L, Topal T (2016) The effectiveness of raloxifene-loaded liposomes and cochleates in breast cancer therapy. AAPS PharmSciTech 17(4):968–977. https://doi.org/10.1208/s12249-015-0429-3

Akrami H, Aminzadeh S, Fallahi H (2015) Inhibitory effect of ibuprofen on tumor survival and angiogenesis in gastric cancer cell. Tumor Biol 36(5):3237–3243. https://doi.org/10.1007/s13277-014-2952-3

Albano F, Arcucci A, Granato G, Romano S, Montagnani S, De Vendittis E, Ruocco MR (2013) Markers of mitochondrial dysfunction during the diclofenac-induced apoptosis in melanoma cell lines. Biochimie 95(4):934–945. https://doi.org/10.1016/j.biochi.2012.12.012

Algra AM, Rothwell PM (2012) Effects of regular aspirin on long-term cancer incidence and metastasis: a systematic comparison of evidence from observational studies versus randomised trials. Lancet Oncol 13(5):518–527. https://doi.org/10.1016/s1470-2045(12)70112-2

Appleyard ML, Murray KE, Coates PJ, Wullschleger S, Bray SE, Kernohan NM, Fleming S, Alessi DR, Thompson AM (2012) Phenformin as prophylaxis and therapy in breast cancer xenografts. Br J Cancer 106(6):1117–1122. https://doi.org/10.1038/bjc.2012.56

Ashburn TT, Thor KB (2004) Drug repositioning: identifying and developing new uses for existing drugs. Nat Rev Drug Discov 3(8):673–683. https://doi.org/10.1038/nrd1468

Assad Kahn S, Costa SL, Gholamin S, Nitta RT, Dubois LG, Fève M, Zeniou M, Coelho PC, El-Habr E, Cadusseau J, Varlet P, Mitra SS, Devaux B, Kilhoffer MC, Cheshier SH, Moura-Neto V, Haiech J, Junier MP, Chneiweiss H (2016) The anti-hypertensive drug prazosin inhibits glioblastoma growth via the PKC δ-dependent inhibition of the AKT pathway. EMBO Mol Med 8(5):511–526. https://doi.org/10.15252/emmm.201505421

Aztopal N, Erkisa M, Erturk E, Ulukaya E, Tokullugil AH, Ari F (2018) Valproic acid, a histone deacetylase inhibitor, induces apoptosis in breast cancer stem cells. Chem Biol Interact 280:51–58. https://doi.org/10.1016/j.cbi.2017.12.003

Batchu RB, Gruzdyn OV, Bryant CS, Qazi AM, Kumar S, Chamala S, Kung ST, Sanka RS, Puttagunta US, Weaver DW, Gruber SA (2014) Ritonavir-mediated induction of apoptosis in pancreatic cancer occurs via the RB/E2F-1 and AKT pathways. Pharmaceuticals 7(1):46–57. https://doi.org/10.3390/ph7010046

Beberok A, Rzepka Z, Respondek M, Rok J, Sierotowicz D, Wrześniok D (2018a) GSH depletion, mitochondrial membrane breakdown, caspase-3/7 activation and DNA fragmentation in U87MG glioblastoma cells: new insight into the mechanism of cytotoxicity induced by fluoroquinolones. Eur J Pharmacol 835:94–107. https://doi.org/10.1016/j.ejphar.2018.08.002

Beberok A, Wrześniok D, Minecka A, Rok J, Delijewski M, Rzepka Z, Respondek M, Buszman E (2018b) Ciprofloxacin-mediated induction of S-phase cell cycle arrest and apoptosis in COLO829 melanoma cells. Pharmacol Rep 70(1):6–13. https://doi.org/10.1016/j.pharep.2017.07.007

Beberok A, Wrześniok D, Rok J, Rzepka Z, Respondek M, Buszman E (2018c) Ciprofloxacin triggers the apoptosis of human triple-negative breast cancer MDA-MB-231 cells via the p53/Bax/Bcl-2 signaling pathway. Int J Oncol 52(5):1727–1737. https://doi.org/10.3892/ijo.2018.4310

Bos CL, Kodach LL, van den Brink GR, Diks SH, van Santen MM, Richel DJ, Peppelenbosch MP, Hardwick JH (2006) Effect of aspirin on the Wnt/beta-catenin pathway is mediated via protein phosphatase 2A. Oncogene 25(49):6447–6456. https://doi.org/10.1038/sj.onc.1209658

Bowie M, Pilie P, Wulfkuhle J, Lem S, Hoffman A, Desai S, Petricoin E, Carter A, Ambrose A, Seewaldt V, Yu D, Ibarra Drendall C (2015) Fluoxetine induces cytotoxic endoplasmic reticulum stress and autophagy in triple negative breast cancer. J Clin Oncol 6(6):299–311. https://doi.org/10.5306/wjco.v6.i6.299

Breckenridge A, Jacob R (2019) Overcoming the legal and regulatory barriers to drug repurposing. Nat Rev Drug Discov 18(1):1–2. https://doi.org/10.1038/nrd.2018.92

Camacho DM, Collins KM, Powers RK, Costello JC, Collins JJ (2018) Next-generation machine learning for biological networks. Cell 173(7):1581–1592. https://doi.org/10.1016/j.cell.2018.05.015

Cen D, Brayton D, Shahandeh B, Meyskens FL, Farmer PJ (2004) Disulfiram facilitates intracellular Cu uptake and induces apoptosis in human melanoma cells. J Med Chem 47(27):6914–6920. https://doi.org/10.1021/jm049568z

Cheng L, Wang H, Wang Z, Huang H, Zhuo D, Lin J (2020) Leflunomide inhibits proliferation and induces apoptosis via suppressing autophagy and PI3K/Akt signaling pathway in human bladder cancer cells. Drug Des Devel Ther 14:1897–1908. https://doi.org/10.2147/DDDT.S252626

Cho YK, Shin EY, Uehara H, Ambati B (2019) Antiangiogenesis effect of albendazole on the cornea. J Ocul Pharmacol Ther 35(4):254–261. https://doi.org/10.1089/jop.2018.0103

Clouser CL, Patterson SE, Mansky LM (2010) Exploiting drug repositioning for discovery of a novel HIV combination therapy. J Virol 84(18):9301–9309. https://doi.org/10.1128/JVI.01006-10

Dai Z, Wang L, Wang X, Zhao B, Zhao W, Bhardwaj SS, Ye J, Yin Z, Zhang J, Zhao S (2018) Oxymatrine induces cell cycle arrest and apoptosis and suppresses the invasion of human glioblastoma cells through the EGFR/PI3K/Akt/mTOR signaling pathway and STAT3. Oncol Rep 40(2):867–876. https://doi.org/10.3892/or.2018.6512

Das SK, Menezes ME, Bhatia S, Wang XY, Emdad L, Sarkar D, Fisher PB (2015) Gene therapies for cancer: strategies, challenges and successes: gene therapies for cancer. J Cell Physiol 230(2):259–271. https://doi.org/10.1002/jcp.24791

Deb J, Majumder J, Bhattacharyya S, Jana SS (2014) A novel naproxen derivative capable of displaying anti-cancer and anti-migratory properties against human breast cancer cells. BMC Cancer 14:567. https://doi.org/10.1186/1471-2407-14-567

Deng H, Huang L, Liao Z, Liu M, Li Q, Xu R (2020) Itraconazole inhibits the Hedgehog signaling pathway thereby inducing autophagy-mediated apoptosis of colon cancer cells. Cell Death Dis 11(7):539. https://doi.org/10.1038/s41419-020-02742-0

Din FN, Valanciute A, Houde VP, Zibrova D, Green KA, Sakamoto K, Alessi DR, Dunlop MG (2012) Aspirin inhibits mTOR signaling, activates AMP-activated protein kinase, and induces autophagy in colorectal cancer cells. Gastroenterology 142(7):1504–15.e3. https://doi.org/10.1053/j.gastro.2012.02.050

Doudican NA, Byron SA, Pollock PM, Orlow SJ (2013) XIAP downregulation accompanies mebendazole growth inhibition in melanoma xenografts. Anticancer Drugs 24(2):181–188. https://doi.org/10.1097/cad.0b013e32835a43f1

Duan L, Shen H, Zhao G, Yang R, Cai X, Zhang L, Jin C, Huang Y (2014) Inhibitory effect of Disulfiram/copper complex on non-small cell lung cancer cells. Biochem Biophys Res Commun 446(4):1010–1016. https://doi.org/10.1016/j.bbrc.2014.03.047

Ellis LZ, Liu W, Luo Y, Okamoto M, Qu D, Dunn JH, Fujita M (2011) Green tea polyphenol epigallocatechin-3-gallate suppresses melanoma growth by inhibiting inflammasome and IL-1β secretion. Biochem Biophys Res Commun 414(3):551–556. https://doi.org/10.1016/j.bbrc.2011.09.115

Endo H, Yano M, Okumura Y, Kido H (2014) Ibuprofen enhances the anticancer activity of cisplatin in lung cancer cells by inhibiting the heat shock protein 70. Cell Death Dis 5(1):e1027. https://doi.org/10.1038/cddis.2013.550

Falkowski M, Skogstad S, Shahzidi S, Smedsröd B, Sveinbjörnsson B (2003) The effect of cyclooxygenase inhibitor diclofenac on experimental murine colon carcinoma. Anticancer Res 23(3B):2303–2308

Feng Z, Xia Y, Gao T, Xu F, Lei Q, Peng C, Yang Y, Xue Q, Hu X, Wang Q, Wang R, Ran Z, Zeng Z, Yang N, Xie Z, Yu L (2018) The antipsychotic agent trifluoperazine hydrochloride suppresses triple-negative breast cancer tumor growth and brain metastasis by inducing G0/G1 arrest and apoptosis. Cell Death Dis 9(10):1006. https://doi.org/10.1038/s41419-018-1046-3

Fojo T, Parkinson DR (2010) Biologically targeted cancer therapy and marginal benefits: are we making too much of too little or are we achieving too little by giving too much? Clin Cancer Res 16(24):5972–5980. https://doi.org/10.1158/1078-0432.CCR-10-1277

Furtado CM, Marcondes MC, Sola-Penna M, de Souza MS, Zancan P (2012) Clotrimazole preferentially inhibits human breast cancer cell proliferation, viability and glycolysis. PLoS One 7(2):e30462. https://doi.org/10.1371/journal.pone.0030462

Gan CP, Hamid S, Hor SY, Zain RB, Ismail SM, Wan Mustafa WM, Teo SH, Saunders N, Cheong SC (2012) Valproic acid: growth inhibition of head and neck cancer by induction of terminal differentiation and senescence. Head Neck 34(3):344–353. https://doi.org/10.1002/hed.21734

Goel A, Chang DK, Ricciardiello L, Gasche C, Boland CR (2003) A novel mechanism for aspirin-mediated growth inhibition of human colon cancer cells. Clin Cancer Res 9(1):383–390

Goenka S, Simon SR (2020) Organogold drug Auranofin exhibits anti-melanogenic activity in B16F10 and MNT-1 melanoma cells. Arch Dermatol 312(3):213–221. https://doi.org/10.1007/s00403-019-01974-1

Grygier B, Arteta B, Kubera M, Basta-Kaim A, Budziszewska B, Leśkiewicz M, Curzytek K, Duda W, Lasoń W, Maes M (2013) Inhibitory effect of antidepressants on B16F10 melanoma tumor growth. Pharmacol Rep 65(3):672–681. https://doi.org/10.1016/s1734-1140(13)71045-4

Gurung AB, Ali MA, Lee J, Farah MA, Al-Anazi KM (2021) An updated review of computer-aided drug design and its application to COVID-19. Biomed Res Int 2021:8853056. https://doi.org/10.1155/2021/8853056

Hamsa TP, Kuttan G (2012) Antiangiogenic activity of berberine is mediated through the downregulation of hypoxia-inducible factor-1, VEGF, and proinflammatory mediators. Drug Chem Toxicol 35(1):57–70. https://doi.org/10.3109/01480545.2011.589437

Han Mİ, Küçükgüzel ŞG (2020) Anticancer and antimicrobial activities of naproxen and naproxen derivatives. Mini-Rev Med Chem 20(13):1300–1310. https://doi.org/10.2174/1389557520666200505124922

Han DF, Zhang JX, Wei WJ, Tao T, Hu Q, Wang YY, Wang XF, Liu N, You YP (2015) Fenofibrate induces G0/G1 phase arrest by modulating the PPARα/FoxO1/p27 kip pathway in human glioblastoma cells. Oncodev Biol Med 36(5):3823–3829. https://doi.org/10.1007/s13277-014-3024-4

Han Y, Chen P, Zhang Y, Lu W, Ding W, Luo Y, Wen S, Xu R, Liu P, Huang P (2019) Synergy between auranofin and celecoxib against colon cancer in vitro and in vivo through a novel redox-mediated mechanism. Cancers 11(7):931. https://doi.org/10.3390/cancers11070931

Hanson K, Robinson SD, Al-Yousuf K, Hendry AE, Sexton DW, Sherwood V, Wheeler GN (2018) The anti-rheumatic drug, leflunomide, synergizes with MEK inhibition to suppress melanoma growth. Oncotarget 9(3):3815–3829. https://doi.org/10.18632/oncotarget.23378

Hay M, Thomas DW, Craighead JL, Economides C, Rosenthal J (2014) Clinical development success rates for investigational drugs. Nat Biotechnol 32(1):40–51. https://doi.org/10.1038/nbt.2786

Hu T, Li P, Luo Z, Chen X, Zhang J, Wang C, Chen P, Dong Z (2016a) Chloroquine inhibits hepatocellular carcinoma cell growth in vitro and in vivo. Oncol Rep 35(1):43–49. https://doi.org/10.3892/or.2015.4380

Hu D, Su C, Jiang M, Shen Y, Shi A, Zhao F, Chen R, Shen Z, Bao J, Tang W (2016b) Fenofibrate inhibited pancreatic cancer cells proliferation via activation of p53 mediated by upregulation of LncRNA MEG3. Biochem Biophys Res Commun 471(2):290–295. https://doi.org/10.1016/j.bbrc.2016.01.169

Hu Y, Liang LB, Zhang Q, Lan F, Lin K, He H, Li SQ (2019) Effect of nifuroxazide on proliferation, migration, and invasion of thyroid papillary carcinoma cells. Sichuan Da Xue Xue Bao Yi Xue Ban 50(1):48–54

Huang A, Garraway LA, Ashworth A, Weber B (2020) Synthetic lethality as an engine for cancer drug target discovery. Nat Rev Drug Discov 19(1):23–38. https://doi.org/10.1038/s41573-019-0046-z

Jackson AL, Sun W, Kilgore J, Guo H, Fang Z, Yin Y, Jones HM, Gilliam TP, Zhou C, Bae-Jump VL (2017) Phenformin has anti-tumorigenic effects in human ovarian cancer cells and in an orthotopic mouse model of serous ovarian cancer. Oncotarget 8(59):100113–100127. https://doi.org/10.18632/oncotarget.22012

Jan CI, Tsa MH, Chiu CF, Huang YP, Liu CJ, Chang NW (2016) Fenofibrate suppresses oral tumorigenesis via reprogramming metabolic processes: potential drug repurposing for oral cancer. Int J Biol Sci 12(7):786–798. https://doi.org/10.7150/ijbs.13851

Jiang W, Finniss S, Cazacu S, Xiang C, Brodie Z, Mikkelsen T, Poisson L, Shackelford DB, Brodie C (2016) Repurposing phenformin for the targeting of glioma stem cells and the treatment of glioblastoma. Oncotarget 7(35):56456–56470. https://doi.org/10.18632/oncotarget.10919

Jiang F, Xing HS, Chen WY, Du J, Ruan YL, Lin AY, Zhou CZ (2019) Itraconazole inhibits proliferation of pancreatic cancer cells through activation of Bak-1. J Cell Biochem 120(3):4333–4341. https://doi.org/10.1002/jcb.27719

Jin B, Wang C, Li J, Du X, Ding K, Pan J (2017) Anthelmintic niclosamide disrupts the interplay of p65 and FOXM1/β-catenin and eradicates leukemia stem cells in chronic myelogenous leukemia. Clin Cancer Res 23(3):789–803. https://doi.org/10.1158/1078-0432.CCR-16-0226

Jin X, Wei Y, Liu Y, Lu X, Ding F, Wang J, Yang S (2019) Resveratrol promotes sensitization to Doxorubicin by inhibiting epithelial-mesenchymal transition and modulating SIRT1/β-catenin signaling pathway in breast cancer. Cancer Med 8(3):1246–1257. https://doi.org/10.1002/cam4.1993

Katiyar SK, Meeran SM, Katiyar N, Akhtar S (2009) p53 cooperates berberine-induced growth inhibition and apoptosis of non-small cell human lung cancer cells in vitro and tumor xenograft growth in vivo. Mol Carcinog 48(1):24–37. https://doi.org/10.1002/mc.20453

Khalid MH, Tokunaga Y, Caputy AJ, Walters E (2005) Inhibition of tumor growth and prolonged survival of rats with intracranial gliomas following administration of clotrimazole. J Neurosurg 103(1):79–86. https://doi.org/10.3171/jns.2005.103.1.0079

Khan N, Syed DN, Ahmad N, Mukhtar H (2013) Fisetin: a dietary antioxidant for health promotion. Antioxid Redox Signal 19(2):151–162. https://doi.org/10.1089/ars.2012.4901

Kim MH, Chung J (2007) Synergistic cell death by EGCG and ibuprofen in DU-145 prostate cancer cell line. Anticancer Res 27(6B):3947–3956

Kim MS, Kim JE, Lim DY, Huang Z, Chen H, Langfald A, Lubet RA, Grubbs CJ, Dong Z, Bode AM (2014) Naproxen induces cell-cycle arrest and apoptosis in human urinary bladder cancer cell lines and chemically induced cancers by targeting PI3K. Cancer Prev Res 7(2):236–245. https://doi.org/10.1158/1940-6207.capr-13-0288

Kirtonia A, Gala K, Fernandes SG, Pandya G, Pandey AK, Sethi G, Khattar E, Garg M (2021) Repurposing of drugs: an attractive pharmacological strategy for cancer therapeutics. Semin Cancer Biol 68:258–278. https://doi.org/10.1016/j.semcancer.2020.04.006

Kudugunti SK, Vad NM, Ekogbo E, Moridani MY (2011) Efficacy of caffeic acid phenethyl ester (CAPE) in skin B16-F0 melanoma tumor bearing C57BL/6 mice. Invest New Drugs 29(1):52–62. https://doi.org/10.1007/s10637-009-9334-5

Kumar S, Bryant CS, Chamala S, Qazi A, Seward S, Pal J, Steffes CP, Weaver DW, Morris R, Malone JM, Shammas MA, Prasad M, Batchu RB (2009) Ritonavir blocks AKT signaling, activates apoptosis and inhibits migration and invasion in ovarian cancer cells. Mol Cancer 8(1):26. https://doi.org/10.1186/1476-4598-8-26

Kumbhar P, Kole K, Yadav T, Bhavar A, Waghmare P, Bhokare R, Manjappa A, Jha NK, Chellappan DK, Shinde S, Singh SK, Dua K, Salawi A, Disouza J, Patravale V (2022a) Drug

repurposing: an emerging strategy in alleviating skin cancer. Eur J Pharmacol 926:175031. https://doi.org/10.1016/j.ejphar.2022.175031

Kumbhar P, Manjappa A, Shah R, Jha NK, Singh SK, Dua K, Disouza J, Patravale V (2022b) Inhalation delivery of repurposed drugs for lung cancer: approaches, benefits and challenges. J Control Release 341:1–15. https://doi.org/10.1016/j.jconrel.2021.11.015

Lakhter AJ, Sahu RP, Sun Y, Kaufmann WK, Androphy EJ, Travers JB, Naidu SR (2013) Chloroquine promotes apoptosis in melanoma cells by inhibiting BH3 domain-mediated PUMA degradation. J Invest Dermatol 133(9):2247–2254. https://doi.org/10.1038/jid.2013.56

Li W, Shi Y, Wang R, Pan L, Ma L, Jin F (2018a) Resveratrol promotes the sensitivity of small-cell lung cancer H446 cells to cisplatin by regulating intrinsic apoptosis. Int J Oncol 53(5): 2123–2130. https://doi.org/10.3892/ijo.2018.4533

Li S, Zhao Q, Wang B, Yuan S, Wang X, Li K (2018b) Quercetin reversed MDR in breast cancer cells through down-regulating P-gp expression and eliminating cancer stem cells mediated by YB-1 nuclear translocation: the chemosensitization of quercetin. Phytother Res 32(8): 1530–1536. https://doi.org/10.1002/ptr.6081

Liang G, Liu M, Wang Q, Shen Y, Mei H, Li D, Liu W (2017) Itraconazole exerts its anti-melanoma effect by suppressing Hedgehog, Wnt, and PI3K/mTOR signaling pathways. Oncotarget 8(17): 28510–28525. https://doi.org/10.18632/oncotarget.15324

Liao D, Zhong L, Duan T, Zhang RH, Wang X, Wang G, Hu K, Lv X, Kang T (2015) Aspirin suppresses the growth and metastasis of osteosarcoma through the NF-κB pathway. Clin Cancer Res 21(23):5349–5359. https://doi.org/10.1158/1078-0432.CCR-15-0198

Lin SC, Chueht SC, Hsiao CJ, Li TK, Chen TH, Liao CH, Lyu PC, Guh JH (2007) Prazosin displays anticancer activity against human prostate cancers: targeting DNA, cell cycle. Neoplasia 9(10):830–839. https://doi.org/10.1593/neo.07475

Luo Y, Zeng A, Fang A, Song L, Fan C, Zeng C, Ye T, Chen H, Tu C, Xie Y (2019) Nifuroxazide induces apoptosis, inhibits cell migration and invasion in osteosarcoma. Invest New Drugs 37(5):1006–1013. https://doi.org/10.1007/s10637-019-00724-4

Marima R, Hull R, Dlamini Z, Penny C (2020) Efavirenz and lopinavir/ritonavir alter cell cycle regulation in lung cancer. Front Oncol 10:1693. https://doi.org/10.3389/fonc.2020.01693

Mayorek N, Naftali-Shani N, Grunewald M (2010) Diclofenac inhibits tumor growth in a murine model of pancreatic cancer by modulation of VEGF levels and arginase activity. PLoS One 5(9): e12715. https://doi.org/10.1371/journal.pone.0012715

Meini S, Pagotto A, Longo B, Vendramin I, Pecori D, Tascini C (2020) Role of lopinavir/ritonavir in the treatment of covid-19: a review of current evidence, guideline recommendations, and perspectives. J Clin Med 9(7):2050. https://doi.org/10.3390/jcm9072050

Metts J, Bradley HL, Wang Z, Shah NP, Kapur R, Arbiser JL, Bunting KD (2017) Imipramine blue sensitively and selectively targets FLT3-ITD positive acute myeloid leukemia cells. Sci Rep 7(1):4447. https://doi.org/10.1038/s41598-017-04796-1

Mottini C, Napolitano F, Li Z, Gao X, Cardone L (2021) Computer-aided drug repurposing for cancer therapy: approaches and opportunities to challenge anticancer targets. Semin Cancer Biol 68:59–74. https://doi.org/10.1016/j.semcancer.2019.09.023

Munson JM, Fried L, Rowson SA, Bonner MY, Karumbaiah L, Diaz B, Courtneidge SA, Knaus UG, Brat DJ, Arbiser JL, Bellamkonda RV (2012) Anti-invasive adjuvant therapy with imipramine blue enhances chemotherapeutic efficacy against glioma. Sci Transl Med 4(127): 127ra36. https://doi.org/10.1126/scitranslmed.3003016

Nagayama D, Saiki A, Shirai K (2021) The anti-cancer effect of pitavastatin may be a drug-specific effect: subgroup analysis of the TOHO-LIP study. Vasc Health Risk Manag 17:169–173. https://doi.org/10.2147/VHRM.S306540

Okubo K, Isono M, Asano T, Sato A (2019) Lopinavir-ritonavir combination induces endoplasmic reticulum stress and kills urological cancer cells. Anticancer Res 39(11):5891–5901. https://doi.org/10.21873/anticanres.13793

Pal D, Banerjee S, Mukherjee S, Roy A, Panda CK, Das S (2010) Eugenol restricts DMBA croton oil induced skin carcinogenesis in mice: downregulation of c-Myc and H-ras, and activation of

p53 dependent apoptotic pathway. J Dermatol Sci 59(1):31–39. https://doi.org/10.1016/j.jdermsci.2010.04.013

Pan HC, Jiang Q, Yu Y, Mei JP, Cui YK, Zhao WJ (2015) Quercetin promotes cell apoptosis and inhibits the expression of MMP-9 and fibronectin via the AKT and ERK signalling pathways in human glioma cells. Neurochem Int 80:60–71. https://doi.org/10.1016/j.neuint.2014.12.001

Pantziarka P, Sukhatme V, Bouche G, Meheus L, Sukhatme VP (2016) Repurposing drugs in oncology (ReDO)-diclofenac as an anti-cancer agent. Ecancermedicalscience 10:610. https://doi.org/10.3332/ecancer.2016.610

Park JH, Kim YH, Park EH, Lee SJ, Kim H, Kim A, Lee SB, Shim S, Jang H, Myung JK, Park S, Lee SJ, Kim MJ (2019) Effects of metformin and phenformin on apoptosis and epithelial-mesenchymal transition in chemoresistant rectal cancer. Cancer Sci 110(9):2834–2845. https://doi.org/10.1111/cas.14124

Parvathaneni V, Kulkarni NS, Chauhan G, Shukla SK, Elbatanony R, Patel B, Kunda NK, Muth A, Gupta V (2020) Development of pharmaceutically scalable inhaled anti-cancer nanotherapy—repurposing amodiaquine for non-small cell lung cancer (NSCLC). Mater Sci Eng 115:111139. https://doi.org/10.1016/j.msec.2020.111139

Paskas S, Mazzon E, Basile MS, Cavalli E, Al-Abed Y, He M, Rakocevic S, Nicoletti F, Mijatovic S, Maksimovic-Ivanic D (2019) Lopinavir-NO, a nitric oxide-releasing HIV protease inhibitor, suppresses the growth of melanoma cells in vitro and in vivo. Invest New Drugs 37(5):1014–1028. https://doi.org/10.1007/s10637-019-00733-3

Penso J, Beitner R (2002) Clotrimazole decreases glycolysis and the viability of lung carcinoma and colon adenocarcinoma cells. Eur J Pharmacol 451(3):227–235. https://doi.org/10.1016/s0014-2999(02)02103-9

Pushpakom S, Iorio F, Eyers PA, Escott KJ, Hopper S, Wells A, Doig A, Guilliams T, Latimer J, McNamee C, Norris A, Sanseau P, Cavalla D, Pirmohamed M (2019) Drug repurposing: progress, challenges and recommendations. Nat Rev Drug Discov 18(1):41–58. https://doi.org/10.1038/nrd.2018.168

Qiao S, Tao S, Rojo de la Vega M, Park SL, Vonderfecht AA, Jacobs SL, Zhang DD, Wondrak GT (2013) The antimalarial amodiaquine causes autophagic-lysosomal and proliferative blockade sensitizing human melanoma cells to starvation- and chemotherapy-induced cell death. Autophagy 9(12):2087–2102. https://doi.org/10.4161/auto.26506

Raber I, McCarthy CP, Vaduganathan M, Bhatt DL, Wood DA, Cleland JGF, Blumenthal RS, McEvoy JW (2019) The rise and fall of aspirin in the primary prevention of cardiovascular disease. Lancet 393(10186):2155–2167. https://doi.org/10.1016/S0140-6736(19)30541-0

Rai S, Tanaka H, Suzuki M, Espinoza JL, Kumode T, Tanimura A, Yokota T, Oritani K, Watanabe T, Kanakura Y, Matsumura I (2020) Chlorpromazine eliminates acute myeloid leukemia cells by perturbing subcellular localization of FLT3-ITD and KIT-D816V. Nat Commun 11(1):4147. https://doi.org/10.1038/s41467-020-17666-8

Rajamanickam S, Panneerdoss S, Gorthi A, Timilsina S, Onyeagucha B, Kovalskyy D, Ivanov D, Hanes MA, Vadlamudi RK, Chen Y, Bishop AJ, Arbiser JL, Rao MK (2016) Inhibition of FoxM1-mediated DNA repair by imipramine blue suppresses breast cancer growth and metastasis. Clin Cancer Res 22(14):3524–3536. https://doi.org/10.1158/1078-0432.CCR-15-2535

Ren A, Fu G, Qiu Y, Cui H (2017) Leflunomide inhibits proliferation and tumorigenesis of oral squamous cell carcinoma. Mol Med Rep 16(6):9125–9130. https://doi.org/10.3892/mmr.2017.7755

Rios Perez MV, Roife D, Dai B, Pratt M, Dobrowolski R, Kang Y, Li X, Augustine JJ, Zielinski R, Priebe W, Fleming JB (2019) Antineoplastic effects of auranofin in human pancreatic adenocarcinoma preclinical models. Surg Open Sci 1(2):56–63. https://doi.org/10.1016/j.sopen.2019.05.004

Salako KS (2021) Screening of amodiaquine for its in vitro anti-cancer activity on breast cancer cell lines—a case study for drug reprofiling. Pan Afr J Life Sci 5(2):263–273. https://doi.org/10.36108/pajols/1202.50.0240

Sarvi S, Crispin R, Lu Y, Zeng L, Hurley TD, Houston DR, von Kriegsheim A, Chen CH, Mochly-Rosen D, Ranzani M, Mathers ME, Xu X, Xu W, Adams DJ, Carragher NO, Fujita M, Schuchter L, Unciti-Broceta A, Brunton VG, Patton EE (2018) ALDH1 bio-activates nifuroxazide to eradicate ALDH high melanoma-initiating cells. Cell Chem Biol 25(12): 1456–1469.e6. https://doi.org/10.1016/j.chembiol.2018.09.005

Sasaki JI, Ramesh R, Chada S, Gomyo Y, Roth JA, Mukhopadhyay T (2002) The anthelmintic drug mebendazole induces mitotic arrest and apoptosis by depolymerizing tubulin in non-small cell lung cancer cells. Mol Cancer Ther 1(13):1201–1209

Shi C, Zhang N, Feng Y, Cao J, Chen X, Liu B (2017) Aspirin inhibits IKK-β-mediated prostate cancer cell invasion by targeting matrix metalloproteinase-9 and urokinase-type plasminogen activator. Cell Physiol Biochem 41(4):1313–1324. https://doi.org/10.1159/000464434

Shi T, Fujita K, Gong J, Nakahara M, Iwama H, Liu S, Yoneyama H, Morishita A, Nomura T, Tani J, Takuma K, Tadokoro T, Himoto T, Oura K, Tsutsui K, Kobara H, Masaki T (2020) Aspirin inhibits hepatocellular carcinoma cell proliferation in vitro and in vivo via inducing cell cycle arrest and apoptosis. Oncol Rep 44(2):457–468. https://doi.org/10.3892/or.2020.7630

Shim JS, Rao R, Beebe K, Neckers L, Han I, Nahta R, Liu JO (2012) Selective inhibition of HER2-positive breast cancer cells by the HIV protease inhibitor nelfinavir. J Natl Cancer Inst 104(20): 1576–1590. https://doi.org/10.1093/jnci/djs396

Shin DH, Kim OH, Jun HS, Kang MK (2008) Inhibitory effect of capsaicin on B16-F10 melanoma cell migration via the phosphatidylinositol 3-kinase/Akt/Rac1 signal pathway. Exp Mol Med 40(5):486–494. https://doi.org/10.3858/emm.2008.40.5.486

Singh SK, Banerjee S, Acosta EP, Lillard JW, Singh R (2017) Resveratrol induces cell cycle arrest and apoptosis with docetaxel in prostate cancer cells via a p53/p21WAF1/CIP1 and p27KIP1 pathway. Oncotarget 8(10):17216–17228. https://doi.org/10.18632/oncotarget.15303

Sleire L, Førde HE, Netland IA, Leiss L, Skeie BS, Enger PØ (2017) Drug repurposing in cancer. Pharmacol Res 124:74–91. https://doi.org/10.1016/j.phrs.2017.07.013

Soprano M, Sorriento D, Rusciano MR, Maione AS, Limite G, Forestieri P, D'Angelo D, D'Alessio M, Campiglia P, Formisano P, Iaccarino G, Bianco R, Illario M (2016) Oxidative stress mediates the antiproliferative effects of nelfinavir in breast cancer cells. PLoS One 11(6): e0155970. https://doi.org/10.1371/journal.pone.0155970

Srirangam A, Mitra R, Wang M, Gorski JC, Badve S, Baldridge L, Hamilton J, Kishimoto H, Hawes J, Li L, Orschell CM, Srour EF, Blum JS, Donner D, Sledge GW, Nakshatri H, Potter DA (2006) Effects of HIV protease inhibitor ritonavir on Akt-regulated cell proliferation in breast cancer. Clin Cancer Res 12(6):1883–1896. https://doi.org/10.1158/1078-0432.CCR-05-1167

Steinberg GR, Dandapani M, Hardie DG (2013) AMPK: mediating the metabolic effects of salicylate-based drugs? Trends Endocrinol Metab 24(10):481–487. https://doi.org/10.1016/j.tem.2013.06.002

Sun L, Niu L, Zhu X, Hao J, Wang P, Wang H (2012) Antitumour effects of a protease inhibitor, nelfinavir, in hepatocellular carcinoma cancer cells. J Chemother 24(3):161–166. https://doi.org/10.1179/1973947812Y.0000000011

Sun X, Song J, Li E, Geng H, Li Y, Yu D, Zhong C (2019) (−)-Epigallocatechin-3-gallate inhibits bladder cancer stem cells via suppression of sonic hedgehog pathway. Oncol Rep 42(1): 425–435. https://doi.org/10.3892/or.2019.7170

Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F (2021) Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71(3):209–249. https://doi.org/10.3322/caac.21660

Talevi A, Bellera CL (2020) Challenges and opportunities with drug repurposing: finding strategies to find alternative uses of therapeutics. Expert Opin Drug Discov 15(4):397–401. https://doi.org/10.1080/17460441.2020.1704729

Thongchot S, Loilome W, Yongvanit P, Dokduang H, Thanan R, Techasen A, Namwat N (2015) Chloroquine exerts anti-metastatic activities under hypoxic conditions in cholangiocarcinoma cells. Asian Pac J Cancer Prev 16(5):2031–2035. https://doi.org/10.7314/apjcp.2015.16.5.2031

Tomic T, Botton T, Cerezo M, Robert G, Luciano F, Puissant A, Gounon P, Allegra M, Bertolotto C, Bereder JM, Tartare-Deckert S, Bahadoran P, Auberger P, Ballotti R, Rocchi S (2011) Metformin inhibits melanoma development through autophagy and apoptosis mechanisms. Cell Death Dis 2(9):e199. https://doi.org/10.1038/cddis.2011.86

Ward NC, Watts GF, Eckel RH (2019) Statin toxicity. Circ Res 14(2):328–350. https://doi.org/10.1161/CIRCRESAHA.118.312782

Wei G, Xu Y, Peng T, Yan J, Wang Z, Sun Z (2017) Sanguinarine exhibits antitumor activity via up-regulation of Fas-associated factor 1 in non-small cell lung cancer. J Biochem Mol Toxicol 31(8). https://doi.org/10.1002/jbt.21914

Wu JY, Lin SS, Hsu FT, Chung JG (2018) Fluoxetine inhibits DNA repair and NF-κB-modulated metastatic potential in non-small cell lung cancer. Anticancer Res 38(9):5201–5210. https://doi.org/10.21873/anticanres.12843

Xia Y, Xu F, Xiong M, Yang H, Lin W, Xie Y, Xi H, Xue Q, Ye T, Yu L (2021) Repurposing of antipsychotic trifluoperazine for treating brain metastasis, lung metastasis and bone metastasis of melanoma by disrupting autophagy flux. Pharmacol Res 163:105295. https://doi.org/10.1016/j.phrs.2020.105295

Yadav V, Varshney P, Sultana S, Yadav J, Saini N (2015) Moxifloxacin and ciprofloxacin induces S-phase arrest and augments apoptotic effects of cisplatin in human pancreatic cancer cells via ERK activation. BMC Cancer 15(1):581. https://doi.org/10.1186/s12885-015-1560-y

Yang Y, Zhang K, Wang Y, Li M, Sun X, Liang Z, Wang L, Chen L, Yang H, Zhu L (2016) Disulfiram chelated with copper promotes apoptosis in human breast cancer cells by impairing the mitochondria functions: disulfiram-Cu and atomic force microscope and cytoskeleton. Scanning 38(6):825–836. https://doi.org/10.1002/sca.21332

Yang CE, Lee WY, Cheng HW, Chung CH, Mi FL, Lin CW (2019) The antipsychotic chlorpromazine suppresses YAP signaling, stemness properties, and drug resistance in breast cancer cells. Chem Biol Interact 302:28–35. https://doi.org/10.1016/j.cbi.2019.01.033

Yang MH, Ha IJ, Um JY, Ahn KS (2021) Albendazole exhibits anti-neoplastic actions against gastric cancer cells by affecting STAT3 and STAT5 activation by pleiotropic mechanism(s). Biomedicine 9(4):362. https://doi.org/10.3390/biomedicines9040362

Yuan P, Ito K, Perez-Lorenzo R, Del Guzzo C, Lee JH, Shen CH, Bosenberg MW, McMahon M, Cantley LC, Zheng B (2013) Phenformin enhances the therapeutic benefit of BRAF(V600E) inhibition in melanoma. PNAS 110(45):18226–18231. https://doi.org/10.1073/pnas.1317577110

Zeng N, Hongbo T, Xu Y, Wu M, Wu Y (2018) Anticancer activity of caffeic acid n-butyl ester against A431 skin carcinoma cell line occurs via induction of apoptosis and inhibition of the mTOR/PI3K/AKT signaling pathway. Mol Med Rep 17:5652. https://doi.org/10.3892/mmr.2018.8599

Zhang Y, Duan W, Owusu L, Wu D, Xin Y (2015) Epigallocatechin-3-gallate induces the apoptosis of hepatocellular carcinoma LM6 cells but not non-cancerous liver cells. Int J Mol Med 35(1):117–124. https://doi.org/10.3892/ijmm.2014.1988

Zhang GF, Liu X, Zhang S, Pan B, Liu ML (2018) Ciprofloxacin derivatives and their antibacterial activities. Eur J Med Chem 146:599–612. https://doi.org/10.1016/j.ejmech.2018.01.078

Zhang L, Bochkur Dratver M, Yazal T, Dong K, Nguyen A, Yu G, Dao A, Bochkur Dratver M, Duhachek-Muggy S, Bhat K, Alli C, Pajonk F, Vlashi E (2019) Mebendazole potentiates radiation therapy in triple-negative breast cancer. Int J Radiat Oncol Biol Phys 103(1):195–207. https://doi.org/10.1016/j.ijrobp.2018.08.046

Zhang X, Du R, Luo N, Xiang R, Shen W (2020a) Aspirin mediates histone methylation that inhibits inflammation-related stemness gene expression to diminish cancer stemness via COX-independent manner. Stem Cell Res Ther 11(1):370. https://doi.org/10.1186/s13287-020-01884-4

Zhang Z, Zhou L, Xie N, Nice EC, Zhang T, Cui Y, Huang C (2020b) Overcoming cancer therapeutic bottleneck by drug repurposing. Signal Transduct Target Ther 5(1):113. https://doi.org/10.1038/s41392-020-00213-8

Zhao J, He Q, Gong Z, Chen S, Cui L (2016) Niclosamide suppresses renal cell carcinoma by inhibiting Wnt/β-catenin and inducing mitochondrial dysfunctions. Springerplus 5(1):1436. https://doi.org/10.1186/s40064-016-3153-x

Zheng ZY, Li J, Li F, Zhu Y, Cui K, Wong ST, Chang EC, Liao YH (2018) Induction of N-Ras degradation by flunarizine-mediated autophagy. Sci Rep 8(1):16932. https://doi.org/10.1038/s41598-018-35237-2

Zhou W, Wu Y, Pan M, Liu D, Liu B (2019) Proliferation and migration of lung cancer could be inhibited by Oxymatrine through the regulation for miR-520/VEGF. Am J Chin Med 47(4):865–878. https://doi.org/10.1142/S0192415X19500459

Zhu Y, Zuo W, Chen L, Bian S, Jing J, Gan C, Wu X, Liu H, Su X, Hu W, Guo Y, Wang Y, Ye T (2019) Repurposing of the anti-helminthic drug niclosamide to treat melanoma and pulmonary metastasis via the STAT3 signaling pathway. Biochem Pharmacol 169:113610. https://doi.org/10.1016/j.bcp.2019.08.012

Chapter 6
Repurposing of Flavonoids as Promising Phytochemicals for the Treatment of Lung Carcinoma

Vivek Yadav, Lovika Mittal, Swati Paliwal, Shailendra Asthana, and Rajiv K. Tonk

Abstract Flavonoids are the polyphenolic secondary metabolites that were obtained from the plants. In recent years, the study and research of flavonoids have become important due to new findings about their diverse pharmacological actions. Here in this chapter, we described about the repurposed use of flavonoids to treat different types of lung cancers such as small-cell lung cancer and non–small-cell lung cancer. The flavonoids covered in this chapter are vital in terms of their role and mechanism of action to hit many targets linked with lung cancer. Further this chapter includes repurposing, structural modification, as well as an in-depth understanding of the various functional groups present in flavonoids and their impact on the biological mechanism to develop some improved therapeutic strategies for the prevention and treatment of lung carcinomas. The repurposing of flavonoids provides a fruitful alternative against the toxic chemotherapy treatments for the various types of cancer and diseases.

Keywords Flavonoids · Repurposing · Secondary metabolite · Lung cancer

6.1 Introduction

Flavonoids are a family of polyphenolic secondary metabolites that are found in plants and are therefore often ingested in human diets. Flavonoids are known as the anthocyanin pigments of plant tissues that are characteristically red, blue, and purple in color. Flavonoids are extensively distributed secondary metabolites with diverse metabolic activities in plants. Flavonoids generally accumulate in the epidermis of

V. Yadav · L. Mittal · S. Paliwal · R. K. Tonk (✉)
Delhi Pharmaceutical Sciences and Research University, New Delhi, India
e-mail: rajiv.tonk@dpsru.edu.in

S. Asthana
Translational Health Science and Technology Institute (THSTI), Faridabad−Gurugram Expressway, Faridabad, India

R. C. Sobti et al. (eds.), *Drug Repurposing for Emerging Infectious Diseases and Cancer*, https://doi.org/10.1007/978-981-19-5399-6_6

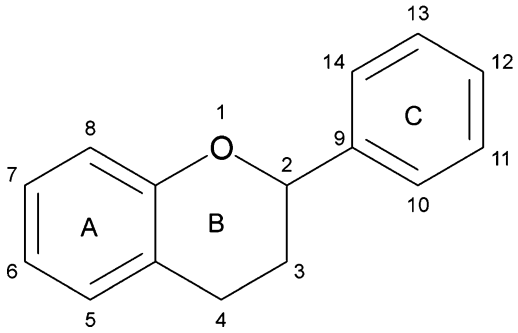


Fig. 6.1 Basic chemical structure of flavonoid

leaves and skin of fruits. They are involved in pigmentation, immunity development, UV protection, and stimulation of nitrogen-fixing in plants. Flavonoids are derived from chalcones with an arrangement of 3 aromatic rings (A, B, C) with 15 carbons and a C6–C3–C6 skeleton. Ring A is a benzene ring fused with a 6-membered ring (C) carrying ring B, phenyl benzene at position 2 as a substituent as shown in Fig. 6.1. In flavonoids, mixed biosynthesis is observed and these are products of both the shikimate and acetate pathways. The glycosidic sugars are joined with flavonoids, and along with hydroxyl groups, they increase water solubility and the substituents like methyl and isopentenyl groups increase lipophilicity (Lin and Weng 2006).

It has been shown that eating foods rich in flavonoids reduces the risk of coronary heart disease, myocardial infarction, cancer, and other chronic diseases such as neurodegenerative psychological diseases and other chronic diseases. Flavonoids may act as antioxidants to promote health by reducing oxidative stress, which is thought to be a factor in the development of several diseases. Along with their antioxidant capabilities, flavonoids have been shown to have a variety of additional biological effects, including antiviral, antibacterial, anti-inflammatory, vasodilatory, anticancer, and anti-ischemic qualities. There is significant and persistent scientific evidence that plant-based diets, particularly those high in vegetables and fruits, protect against a wide range of malignancies. Whether this is due to the diet's low energy content or its specific ingredients, it has been shown to have an effect. Although the nutritional profiles of plant-derived meals vary, they are generally good providers of essential nutrients (e.g., fiber, carotenoids, vitamin C, folate, minerals) and a variety of less well-characterized bioactive substances (phytochemicals). Drugs like aspirin and biguanides (metformin and phenformin), which were originally generated from phytochemical backbones (triterpenoids, flavonoids, retinoids), are multitarget medicines with antiangiogenic/anti-anti-inflammatory properties that were originally derived from phytochemical backbones. These compounds could be repurposed in cancer and other chronic complex diseases as part of combinatorial chemoprevention and interception techniques, or as part of chemoprevention/therapy regimens, for example. In this chapter, we have shed light on the repurposing of different flavonoids in the treatment of lung carcinoma.

6.1.1 Classifications of Flavonoids

The flavonoids, ubiquitous in plants, are the largest class of polyphenols, with a common structure of diphenylpropanes (C6–C3–C6), consisting of two aromatic rings linked through three carbons. The classifications of six subclasses of flavonoids are tabulated in Table 6.1 and the structures of some flavonoids are showed in Fig. 6.2 below.

They are found in fruits, vegetables, tea, cocoa, and wine among other plant-based foods. Within the flavonols and flavones subgroups, the flavonol quercetin is the most abundant component in foods. Additionally, kaempferol, myricetin, and the flavonoids apigenin and luteolin are prevalent. The primary dietary sources of flavonols and flavones are tea and onions (Marchand 2002).

Table 6.1 The six major subclasses of flavonoids

S. no.	Subclass	Flavonoids	Sources
1	Flavones	Apigenin, luteolin	Apple skin, celery
2	Flavonols	Quercetin, kaempferol, myricetin	Onions, apples, tea
3	Flavanones	Naringenin, hesperetin	Citrus fruits, grapefruit
4	Flavanols	Catechins, epicatechin, gallocatechin	Tea
5	Anthocyanidins	Cyaniding, pelargonidin	Berries
6	Isoflavones	Genistein, daidzein	Soya

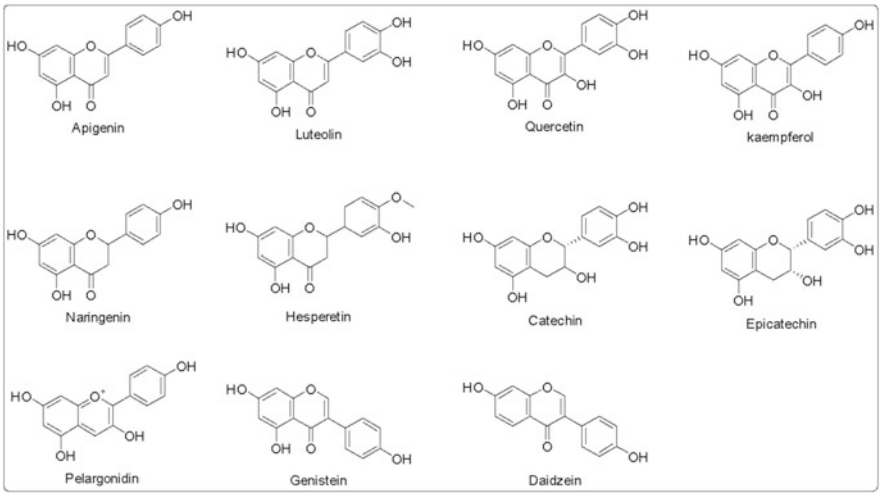


Fig. 6.2 Structure of some flavonoids

6.2 Reported Flavonoids Against Lung Cancer

Lung cancer is the leading cause of cancer-related death globally. Lung cancer has caused ~1.76 million fatalities by 2021, accounting for over one-fifth (18.4%) of all cancer-related deaths. Lung cancer is divided into two distinct subtypes: non–small-cell lung cancer (NSCLC) and small-cell lung cancer (SCLC). NSCLC accounts for around 85% of all lung malignancies. Lung cancer has a high mortality/incidence rate (more than 80%) and a low 5-year survival rate (10–20%). This dismal prognosis is a result of lung cancer cells' propensity for metastatic spread. The majority of individuals are diagnosed only after developing clinical symptoms associated with extensive metastases. Additionally, lung cancer cells have a proclivity for developing resistance to a wide array of anticancer treatments (Zanoaga et al. 2019a).

Therapeutic strategies for lung cancer include chemotherapy, radiotherapy, surgery, immunotherapy, and targeted therapy. Chemotherapy is the primary treatment option, and it is used as a first-line option or as an adjuvant/neoadjuvant in combination therapies. Among the conventional chemotherapy agents used to treat NSCLC are etoposide, docetaxel, paclitaxel, cisplatin, irinotecan, and doxorubicin, which are typically given in paired combinations. Phytochemicals emerge as natural, plentiful, low-cost, and nontoxic compounds with a variety of pharmacological properties, including anticancer potential. Numerous studies have demonstrated that flavonoids have anticancer action in cancer cells both in vitro and in vivo by causing cell cycle arrest, apoptosis, autophagy, and/or senescence.

6.2.1 Anticarcinogenic Properties and Targets of Flavonoids

Various studies have shown that flavonoids have the ability to scavenge free radical and stop oxidative stress-related diseases like cancer, diabetes, Alzheimer's, and asthma. There are a lot of flavonoids accessible which show anticancer activity but still, the mechanism liable for anticancer effects have not been elucidated yet. There are two crucial causes of cancer in which internal factors include oxidative stress and genetic mutations, while external factors are smoking, U-V rays and exposure to radiation. The change in metabolism, induction of angiogenesis, metastasis formation, impaired cell cycle and resistance to immune reaction are major characteristics of the cancer cells (Kopustinskiene et al. 2020). Figure 6.3, represents the mechanisms of action and targets of flavonoids involved in cancer prevention and treatment therapy (Chabot et al. 2010).

Numerous flavonoids have been shown to be antimutagenic, such as quercetin, which can decrease the mutagenic effect of benzopyrene, a potent carcinogen in bacterial mutagenesis assays. The flavonoids 3,5,7-trihydroxyflavone, commonly known as Galangin, have been demonstrated to have anticlastogenic properties in vitro and in vivo using bleomycin or benzopyrene models. Mutagenesis produced

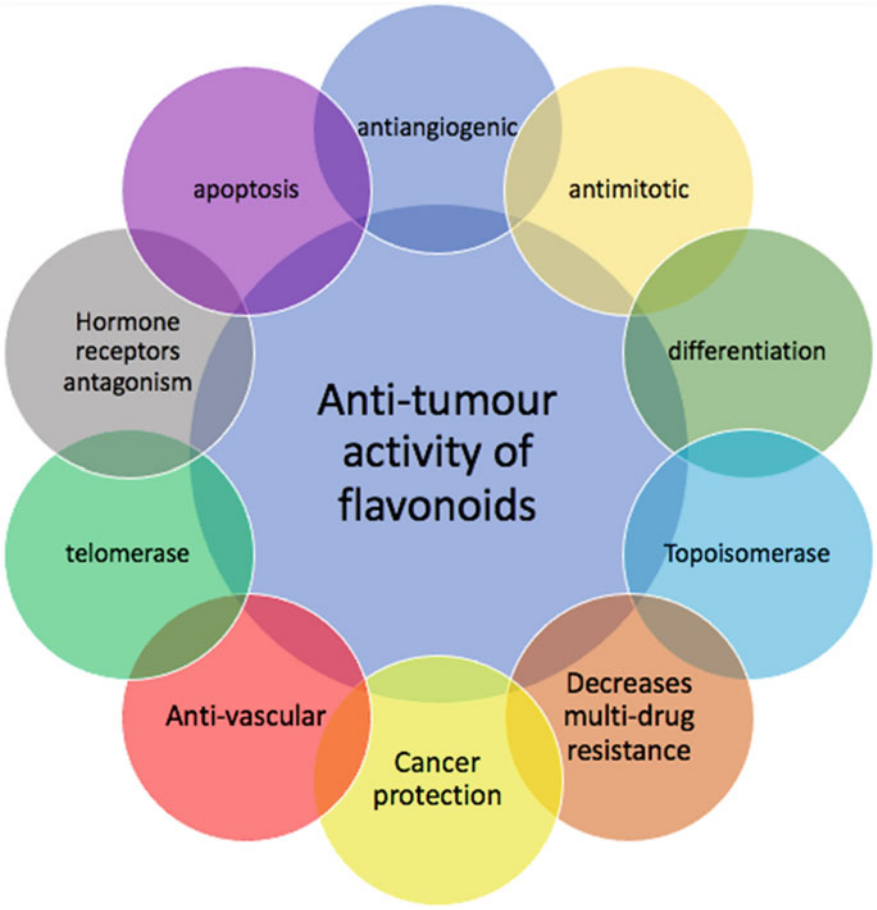


Fig. 6.3 Flavonoids anticancer activity and their targets

by benzopyrene diol-epoxide can also be prevented by hydroxylated flavones, and numerous synthesized flavones have been demonstrated to be antimutagenic in the Ames test. Flavonoids are thought to hinder a chemical interaction between a reactive metabolite and DNA, which is why they are thought to prevent carcinogenesis. To prevent the DNA–carcinogen covalent bond, polyphenols have been proven to inhibit the enzymes that activate them, notably cytochrome P450 1A1 and 1B1 cytochrome P450s. In addition, flavonoids can inhibit the cytochromes' P450s protein expressions, inhibiting the synthesis of DNA-reactive mutagens.

6.2.2 *Molecular Mechanism of Action of Flavonoids*

Despite the fact, the information supporting cancer prevention remains contentious in humans, probably thanks to the inherent difficulties to conduct this type of epidemiological study, which has shown that diet rich in polyphenol is found to be very fruitful in preventing certain kinds of cancer and significantly lower the danger of dying from this disease.

Studies have shown that those who consume large amounts of flavonoids have a lower risk of developing lung cancer. Tea and flavonoids consumption has been linked to a reduced incidence of cancer in epidemiological studies. The pictorial representation of the flavonoids mechanism on the cell is shown in Fig. 6.4.

According to in vivo and in vitro investigations, macromolecular activities include antioxidant effects, electrophile binding, stimulation of protective enzymes (phase 2 with conjugating activities), enhancement of caspase-mediated necrobiosis, suppression of cellular reproduction, inhibition of lipid peroxidation, inhibition of angiogenesis, inhibition of H-donation, and inhibition of DNA oxidation (Patil and Masand 2018).

6.2.2.1 Carcinogenic Metabolic Activation Pathway Targeting by Flavonoids

Flavonoids have shown antileukemic effects by changing the uptake and temperament or disposition of carcinogens. Mechanism of action of flavonoids can be shown in two ways either: (a) interacting with cytochrome P450 includes (CYP1A1 and CYP1A2) which is metabolizing enzyme belongs from phase 1 clinical trial and then activating the pro-carcinogens which are converted into reactive species or either

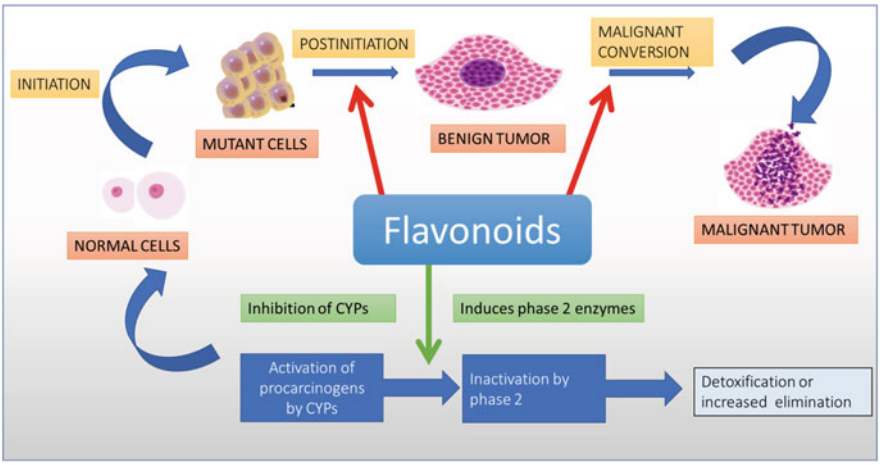


Fig. 6.4 Molecular mechanism of action of flavonoids

(b) interact with cellular nucleophiles resulting activated carcinogenesis and show antitumor effect. Flavonoids interact with the metabolizing enzymes (phase II clinical trials) like glutathione-S-transferase, quinone reductase, and UDP-glucuronyl transferase and show their involvement in the detoxification of carcinogens for their expeditious elimination.

6.2.2.2 Antiproliferative Activity

By inhibiting the pro-oxidant process and polyamine biosynthesis which causes tumor promotion, flavonoids show their antiproliferative effect example—flavopiridol and quercetin. Flavonoids also stop the enzymes which express their involvement in cell proliferation like Kinase enzymes (protein kinase enzyme, protein kinase C, phosphoinositide 3-kinase), xanthine oxidase.

6.2.2.3 Cell Signaling

Cell cycle signaling is associated with cyclin-dependent kinase (CDKs); thus, variation in the activity of CDK is of interest to progress innovative anticancer agents. There are some examples of flavonoids like silymarin, genistein, quercetin, daidzein, luteolin, kaempferol, apigenin, and epigallocatechin 3-gallate which have shown alteration in the cell cycle.

Flavonoids act on various kinase signaling pathways like the MAP kinase (mitogen-activated protein kinase), the phosphoinositide 3-kinase (PI 3-kinase), the Akt/protein kinase B (Akt/PKB), the tyrosine kinases, and the protein kinase C (PKC) signaling pathways and inhibit these pathways which affect cellular functions.

6.2.2.4 Apoptotic Effect

Induction of apoptosis through flavonoids has been reported by a various mechanisms like changing of DNA topoisomerase I/II activity, ROS, HSP expression, signaling pathways, caspase-9, caspase-3, Bcl-X(L) expression, Bax, as well as Bak expression, nuclear transcription factor kappaB, Bcl-2 family protein (Mcl-1).

Quercetin-induced apoptosis occurs because of cell cycle arrest in the S phase and the inhibition of thymidylate synthase. Catechin, a natural phenol and antioxidant, is found in green tea, and induces apoptosis and chunk cell cycle in tumor cells.

6.2.2.5 Differentiation

For cell differentiation, flavones and isoflavones, both are the well-reported inducers. Through rebalance of normal cellular homeostasis and elimination of tumor genic cells, new anticancer therapies can also be developed.

Reports have shown that the flavonoids found to be more active which consist these substituents like 3-OH; 5,6,7,8,30,40-OMe > 5,7,30,40-OH > 5,6,7,8,40-OMe. Genistein as well as quercetin and luteolin were also found to introduce HL60 cells differentiation. The foremost promising condition for differentiation is covalent bond needed at the C2–C3 and an unopened C ring because chalcones are inactive.

6.2.2.6 Antiangiogenic Effect

The VEGF (vascular endothelial growth factor) and HIF-1 (hypoxia-inducible factor-1) inhibition occurred via angiogenesis inhibitors of flavonoids. Also, these inhibitors act by interfering with the basement destruction of blood cells, and the rapid increase and migration of endothelial cells. Along with this, it has also been reported that EGCG could decrease the vascular endothelial growth factor mRNA and crucially decrease the growth of gastric tumors. Quercetin also shows inhibition of NO synthase in angiogenesis inhibition in vivo as well as in vitro.

6.2.2.7 Multidrug Resistance

Flavonoids show their anticancer property through multidrug resistance-related protein (MRP) or Pgp (P-glycoprotein). Recent work has been done on molecular mechanisms for multidrug resistance involving the direct interaction of EGCG (epigallocatechin gallate flavonoid) on ATP binding site on GRP78 (glucose-regulated protein) of a chaperone protein. There are some other mechanisms involved in multidrug resistance like blocking ATPase activity, inhibiting the overexpression of multidrug resistance gene-1, hydrolysis of nucleotide, etc. Flavonoids also help in multidrug resistance by targeting Pgp to build up doxorubicin.

6.2.2.8 Antioxidant Activity

The flavonoids have been reported as a natural antioxidant (e.g., catechin, anthocyanins, flavonols, and flavones) that focus on the modulation of ROS as well as on proliferation pathways and cell cycle signaling. They show their anticancer mechanism which is based on antioxidant activity by direct removal of free radicals. Flavonoid acts directly with the free radical and creates less reactive as well as more stable phenolic radicals resulting in inhibition of LDL oxidation. Flavonoid

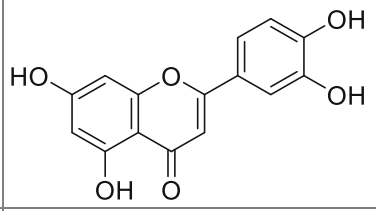
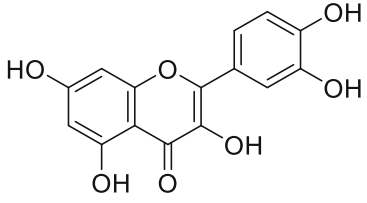
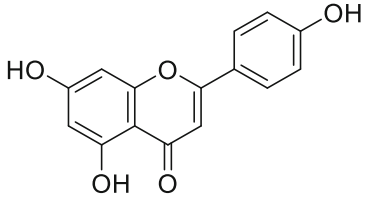
interferes with NO-synthase activity to remove reactive oxygen species (ROS). Flavonoids stop NO reaction along free radicals, resulting in prevention of peroxynitrites which directly oxidizes ROS. In colon and stomach cancer, flavonoid's antioxidant activity plays a vital role in the treatment of the disease.

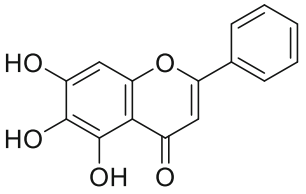
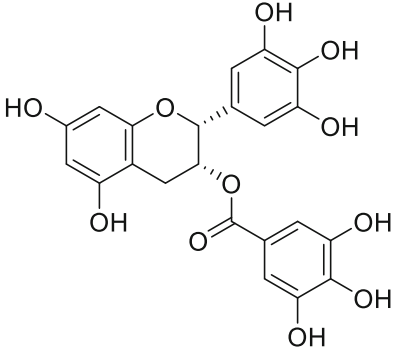
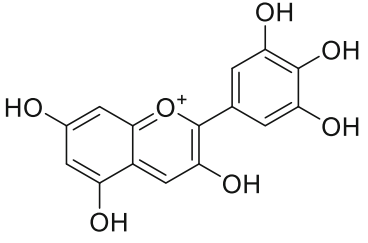
6.3 Flavonoids and Their Reported Biological Activities

The several enzymatic inhibitions were reported from the flavonoids for their different pharmacological activities. Some of the reported flavonoids are tabulated in Table 6.2 with the anticancer activities. We briefly mentioned below about the different types of flavonoids with their biological activities toward the lung cancer-related diseases.

1. **Luteolin**: Extracts of *Blumea balsemifera* leaves, luteolin-7-methyl ether, demonstrated significant cytotoxicity against human lung cancer cell lines (NCI-H187) and moderate toxicity against oral cavity cancer cell lines (KB) with IC_{50} values of 1.29 and 17.83 μg/mL, respectively (Saewan et al. 2011). Studies have demonstrated that it lowers Sirt-1 expression levels and has an anticancer effect by inducing apoptosis in cancer cells through the Sirt-1 pathway (Ma et al. 2015). According to the findings, luteolin also functions as a radiosensitizer by increasing apoptotic cell death by activating a p38/ROS/caspase cascade in the presence of γ-ionizing radiation (Cho et al. 2015).
2. **Kaempferol**: Claudin-2 is highly expressed in human lung adenocarcinoma tissues, and its knockdown reduces cell proliferation, suggesting that it could be a new target for cancer chemotherapy. In lung adenocarcinoma, A549 cells, flavonoids like kaempferol, chrysin, and luteolin reduced claudin-2 expression in a concentration-dependent manner. Mitogen-activated protein kinase (MEK)/ERK/c-Fos and phosphoinositide 3-kinases (PI3K)/Akt and nuclear factor-B (NF-B) pathways upregulate claudin-2 expression, but these activities were not inhibited by the kaempferol, chrysin, and luteolin compounds studied (Sonoki et al. 2017). The luciferase reporter vector-based promoter deletion assay revealed that kaempferol and luteolin inhibit the transcriptional factor's ability to bind to the region of the claudin-2 promoter between codons 395 and 144. The decrease in promoter activity was prevented by a mutation in the STAT-binding site, which is located between 395 and 144. Although the phosphorylation level of STAT3 was not decreased, kaempferol and luteolin in chromatin immunoprecipitation assays suppressed STAT3 binding to the promoter region. Ectopic expression of claudin-2 partially reversed the effects of kaempferol and luteolin on cell proliferation. The inhibition of STAT3 binding to the claudin-2 promoter region was the mechanism by which kaempferol and luteolin reduced claudin-2 expression and proliferation in A549 cells. Lung adenocarcinoma development may be prevented by consuming foods and nutrients rich in these flavonoids. The PI3K/AKT pathway may be inhibited by kaempferol as

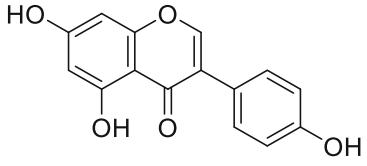
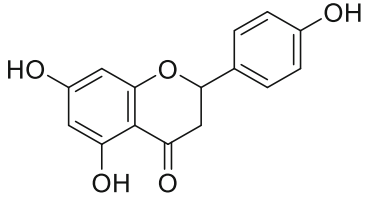
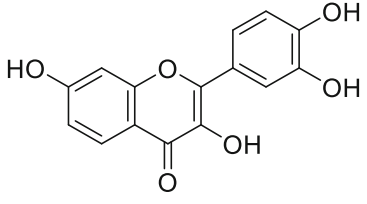
Table 6.2 List of flavonoids with structure and biological activity

Compounds	Chemical structure	Biological activity
Luteolin		IC50: 1.29 μg/mL
Quercetin		Kd: 25.6 ± 3.5 μmol/L
Apigenin		IC50: 93.7 ± 3.7 μM

Baicalein		IC50: 80 ± 6 μM
Epigallocatechin gallate		Kd = 3.47 μM
Delphinidin		Significant inhibition activity at 55 μM dose

(continued)

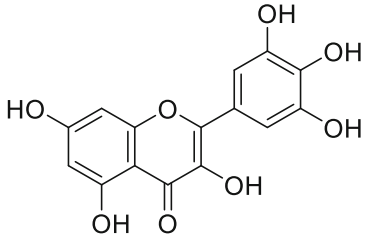
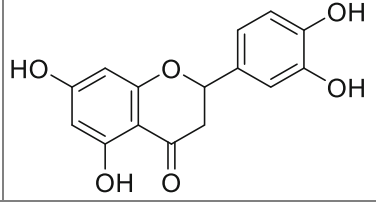
Table 6.2 (continued)

Compounds	Chemical structure	Biological activity
Genistein		IC50: 72 µM
Naringenin		IC50: 19.28 ± 3.21 µg/mL and 37.63 ± 7.27 µg/mL
Fisetin		IC50: 214.47 µM and 320.42 µM

Hesperidin		Significant inhibition activity at 12.5 and 25 μM dose
Delicaflavone		IC50: 16 μM

(continued)

Table 6.2 (continued)

Compounds	Chemical structure	Biological activity
Myricetin		Inhibition activity at 20 μM dose
Eriodictyol		IC50: 50 μM

well as by increasing the expression of miR-340, PTEN, and PTEN-related factors (Han et al. 2018).

3. **Broccolini leaf flavonoids** *(BLF)*: They have been shown to have an antiproliferative effect on the A549 cell line (adenocarcinomic human alveolar basal epithelial cells) in a dosage-dependent manner (Wang and Zhang 2012). BLF has an IC50 value, 79.77 μg/mL in an A549 cell line study.
4. **Quercetin**: A number of in vitro and in vivo investigations have indicated that the Quercetin target aurora B kinase has a direct effect on lung cancer cells by inhibiting their growth (Xingyu et al. 2016). The lowest equilibrium dissociation constant (K_d) was found to be 25.6 ± 3.5 μmol/L, indicating the strongest binding between quercetin and aurora B. Quercitrin (QR; quercetin-3-O-rhamnoside) has been used in the past as an antibacterial agent and has been found to block the oxidation of low-density lipoproteins and avoid an allergic reaction. Furthermore, it has been proven that QR protects lung fibroblast cells from H_2O_2-induced dysfunction (Cincin et al. 2014). Anticarcinogenic and antiproliferative properties of QR on lung cancer cells have been demonstrated via regulating the immune response. Cell adhesion, phosphatidylinositol signaling, and leukocyte trans-endothelial migration were found to be the most statistically significant pathways in NCI-H358 and A549 cells, respectively. This shows that it could be a novel and potent anticancer drug for NSCLC. It is noticed that Quercetin reduces the stability of claudin-2 mRNA in A549 cells, which reduces its expression. Claudin-2 levels were restored after quercetin boosted miR-16 expression, and an inhibitor of miR-16 reversed the effects of quercetin on claudin-2 levels. Quercetin may reduce claudin-2 expression in lung cancer cells by increasing miR-16 expression (Sonoki et al. 2015).
5. **Apigenin**: As a nonmutagenic flavonoid found in fruits and vegetables, Apigenin (APG) has been shown to have many potential targets. APG inhibited A549 human lung cancer cell proliferation, migration, and invasion by targeting the PI3K/Akt signaling pathway, according to the findings (Zhou et al. 2017). When APG inhibits the PI3K/Akt signaling pathway, the migration and invasion of A549 cells is significantly reduced, indicating that APG can inhibit lung cancer cell migration (IC_{50} value of 93.7 ± 3.7 μM for 48 h) and invasion by modulating this pathway. Additionally, the results indicated that APG not only inhibited Akt phosphorylation, thereby inhibiting its activation, but also inhibited matrix metalloproteinases-9, glycogen synthase kinase-3, and HEF1 expression downstream of Akt. Snail/Slug-mediated EMT was also shown to be suppressed by APG, preventing NSCLC cells with varying EGFR statuses from migrating or invading (Chang et al. 2018). A549 xenograft growth and metastasis were drastically reduced in vitro by APG, which targeted CD26. The ability of lung cancer cells to invade and the poor prognosis of patients with this type of cancer were positively correlated with CD26 expression. With $CD26^{high}/Akt^{high}$ tumors, patients had the shortest recurrence-free survival times, according to the results. Overall, APG is a new Akt inhibitor in lung cancer and a possible natural compound for cancer chemoprevention.

6. **Baicalein**: Chinese herbal medicine, Baicalein, has a wide range of pharmacological effects. Baicaleins were found to have a significant impact on the proliferation of A549 and H1299 cell lines (Su et al. 2018). In addition, CyclinD1 and CDK1 mRNA expression was decreased, as well as cell invasion and E-cadherin expression. It has an IC_{50} value of 80 ± 6 μM determined from the MTT assay. Studies have shown that it may be a potential flavonoid for the treatment of NSCLC.
7. **Epigallocatechin gallate** *(*EGCG*):* Chemopreventive properties of green tea's main biologically active compound, EGCG, are well established. Proliferation and apoptosis of H1299 lung cancer cells can be inhibited by EGCG, which has an effect on the PI3K/Akt signaling pathway by preventing its activation (Gu et al. 2018). EGCG's antitumor properties were primarily due to the inhibition of the EGFR signaling pathway (Ma et al. 2014). It was also found that long-term EGCG treatment reduced EGFR expression in both the nucleus and membranous tissues, as well as in the downstream target gene cyclin D1, suggesting that it inhibited the process of EGFR transactivation. EGCG also appears to inhibit TGF-induced EMT in NSCLC cells by reducing phosphorylation of Smad2 and Erk1/2 (Liu et al. 2012). In human lung cancer cells, EGCG inhibited p300/CBP activity (Ko et al. 2013). TGF-1 induces p300/CBP activity, which is inhibited by EGCG. It has been shown to influence hypoxia-induced factor (HIF) activity but also suppresses HIF-1 production and protein accumulation in the body (Wang et al. 2011). The role of miRNA in lung development has been established. Different miRNA groups are expressed in growing and mature lung tissues, implying that these miRNAs play distinct roles in controlling lung tissue cell proliferation and differentiation as well as maintaining proper lung functioning. EGCG has been shown to prevent lung carcinogenesis and slow the growth of lung cancer research. HIF-1, an activator of miR-210 expression, was found to accumulate in cells treated with EGCG. EGCG regulates cellular activity in a unique manner by targeting the HIF protein and miR-210. Lung cancer cells' proliferation and anchorage-independent growth are slowed when miR-210 is overexpressed. When cancer cells are exposed to EGCG, the overexpression of miR-210 indicates an earlier response and may contribute to the anticancer effect of EGCG. Affinity binding assays demonstrated that EGCG selectively binds HIF-1 with a $K_d = 3.47$ μM.
8. **Delphinidin**: Delphinidin, an anthocyanidin-containing polyphenol found in a variety of pigmented fruits and vegetables, possesses significant antioxidant, anti-inflammatory, antimutagenic, and anticancer properties. It was found that delphinidin had significant antiangiogenic effects on A549 human lung cancer cells by reducing the expression of vascular endothelial growth factor (VEGF) (Kim et al. 2017). Delphinidin also decreased the CoCl2- and EGF-stimulated expression of hypoxia-inducible factor (HIF-1), a transcription factor for VEGF. HIF-1 binding to the hypoxia response element (HRE) promoter was reduced by delphinidin, suggesting that the inhibition of VEGF expression is due to the suppression of HIF-1 binding to HRE promoters. It was also discovered that delphinidin inhibited the CoCl2 and EGF-induced HIF-1 protein expression

specifically by blocking the ERK and PI3K/Akt/mTOR/p70S6K signaling pathways, but not the p38-mediated pathways. Thus, the results suggest that delphinidin may have a novel role in antiangiogenic activity by inhibiting the expression of HIF-1 and VEGF. Cell proliferation and apoptosis in non–small-cell lung cancer (NSCLC) are reduced by delphinidin, which targets EGFR/VEGFR2 signaling pathways (55 μM on A549 cells) (Pal et al. 2013).

9. **Genistein**: Soybean genistein, a major isoflavone constituent, has been shown to have multiple antitumor effects, including cell cycle arrest, apoptosis, and inactivation of critical signaling pathways in a small number of human cancer cells. H446 cells' proliferation and migration were significantly slowed by genistein, which was also found to cause apoptosis and a halt in the G2/M phase of the cell cycle (IC_{50} value of 72 μM) (Tian et al. 2014). The FoxM1 protein was weakened by genistein, and a number of FoxM1 target genes involved in the cell cycle and apoptosis were downregulated, including Cdc25B, cyclin B1, and survivin. Genistein exerted multiple antitumor effects in H446 SCLC cells for the first time, and these were at least partially mediated by FoxM1 downregulation. A lot more research is needed on FoxM1 because it has the potential to be a new treatment for SCLC. It also has the ability to stop the growth of A549 lung cancer cells in vitro and cause them to die (Zhang et al. 2018). Its antitumor activity is mediated by a decrease in Bcl-2 expression and an increase in Bax expression. Lung cancer patients could benefit from its use in the clinical setting. It is also reported that the miR-27a and MET signaling pathways are both inhibited by genistein in A549 human lung cancer cells (Yang et al. 2016).
10. **Daidzein**: By inducing apoptosis, daidzein inhibited NSCLC cell proliferation in a concentration-dependent manner (Chen et al. 2017). Its treatment significantly restored STK4 expression and increased YAP1 phosphorylation, resulting in cell apoptosis, as evidenced by increased cleaved caspase3 expression levels. Daidzein's apoptosis-inducing effects on NSCLC cells were reduced when STK4 was silenced rather than STK3.
11. **Naringenin**: There were numerous ways by which naringenin prevented the migration of lung cancer A549 cells, including the inhibition of AKT activities as well as the lowering of MMP-2 and MMP-9 activities (Chang et al. 2017). Human lung cancer A549 cells were treated with naringenin, which increased the expression of death receptor 5 and increased apoptosis (Jin et al. 2011). By binding to its death-inducing receptors, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) has been shown to selectively trigger apoptotic cell death in a variety of tumor cells (Dai et al. 2015). The MTT assay demonstrated a concentration-dependent decrease in cell viability for naringenin-NE (nanoemulsion) and free naringenin, with IC_{50} values of 19.28 ± 3.21 and 37.63 ± 7.27 μg/mL, respectively.
12. **Fisetin**: Many different kinds of tumors have responded favorably to the anticancer effects of the naturally occurring flavonoid fisetin (Wang and Huang 2018). Analysis of cell viability by flow cytometry showed that the A549 cell line was apoptotic because fisetin decreased c-myc, CDKN1A/B,

and CDKN2D expression, increased CDKN1A/B and CDKN2D expression, and increased the activity of caspase-3/9 by targeting the extracellular signal-regulated kinase signaling pathway. Fisetin showed IC_{50} values for A549 and A549-CR cells were 214.47 μM and 320.42 μM, respectively. Fisetin's antitumor effects in NSCLC were demonstrated in vitro, which could lead to a new treatment strategy in the clinic.

13. **Hesperidin**: Hesperidin induced apoptosis in human NSCLC A549 cells via the mitochondrial apoptotic pathway and G0/G1 arrest (Xia et al. 2018). Hence, the development of hesperidin as a therapeutic drug for NSCLC is possible.

6.4 Combination of Strategies and Futuristic Approaches

It has been demonstrated that EGCG acts as an adjuvant in the treatment of NSCLC cell lines, primarily through the CTR1 mechanism activated by EGCG and the crosstalk between NEAT1/mir-98-5p (Zanoaga et al. 2019b). It has been observed that metformin sensitized NSCLC cells to EGCG treatment after treatment with a combination of metformin and EGCG. This was observed by suppressing the Nrf2/HO-1 signaling pathway in NSCLC cells. Quercetin enhances the sensitivity to gemcitabine treatment in lung adenocarcinoma by increasing cell apoptosis via inhibiting HSP70 expression. Simultaneous treatment of resveratrol and clofarabine induced apoptosis in H-2452 cells by reducing the Mcl-1 protein level. In chemoresistant lung cancer cells, EGCG induces the reversion of cisplatin resistance mediated by the downregulation of AXK and TYRO3 receptor tyrosine kinases. After combination treatment with honokiol and cetuximab in non–small-cell lung cancer, H226 cell line has been observed downregulation of HER family and their signaling pathways. Since radiotherapy is one of the prime treatment measures for lung cancer, the need to enhance radiotherapy efficacy and protect normal tissues has appeared. A combination treatment of quercetin and gemcitabine had significant antiproliferative and pro-apoptotic activities by the downregulation of the HSP70 expression. The combined treatment of diosmetin and paclitaxel synergistically suppressed lung cancer cells via ROS accumulation through the PI3K/Akt/GSK-3β/Nrf2 pathway disruption. A549 non–small-lung cancer cell line has shown that fisetin synergizes with paclitaxel at in vivo doses, as well (Klimaszewska-Wisniewska et al. 2016). This synergism's processes include the following: the enhancement of multipolar spindle formation induces mitotic catastrophe. Autophagy is used to remove cells that have suffered a mitotic catastrophe and have been the prospective target in lung carcinoma (Sarmah et al. 2021a; Sarmah et al. 2021b). A significant increase in autophagy is responsible for the switch from cytoprotective autophagy to autophagic cell death. Since cancer treatment is hampered by the resistance to apoptosis, new medicines that trigger nonapoptotic cell death pathways are now being considered as an alternative strategy for overcoming this impediment. All three flavonoids, apigenin, luteolin, and quercetin, were shown

to induce NK-92 cell-mediated cytotoxicity against lung cancer cells (Oo et al. 2021). Apigenin and luteolin treatment of natural killer cells (NK cells) increases the release of cytotoxic granules, which in turn increases the NK-cell-mediated cytotoxicity against lung cancer. Quercetin, on the other hand, had no influence on this process. While NK cells are being activated against lung cancer, it is possible that quercetin will activate other intracellular processes. It is reported that apigenin, luteolin, and quercetin may be useful in the treatment of cancer using natural killer cells (NK cells). Further research into these three flavonoids, as well as their effects on immunomodulation in vivo, is warranted and encouraged.

Combining antioxidant flavonoids with chemotherapy and radiotherapy treatment in lung cancer has been shown to significantly increase anticancer activity in nearly all cases. Normal cells are protected from the side effects of chemotherapy while at the same time, flavonoids modulate the suppression of important multiple signaling pathways that are activated in cancer cells. This has both advantages and disadvantages. It has been noticed that there is a favorable effect in some instances. However, in certain instances, this can be attributed to a decrease in the efficiency of cytotoxic therapy. It is critical to have a thorough understanding of the interactions between the various functional groups found in flavonoid's structures, as well as their impact on the molecular mechanism, in order to make further advancements and modifications to the basic structure. With this knowledge, researchers will be able to develop better therapeutic strategies for the prevention and treatment of solid tumors, such as lung cancer. It would be more reasonable to use the various combinations of therapeutic agents and flavonoids in order to achieve a successful therapy while also reducing the dose of chemotherapeutics.

6.5 Conclusions

Flavonoids have garnered a great deal of attention in recent years, owing to the wide diversity of biological functions that they have, particularly their ability to inhibit the growth of cancerous cells. The flavonoids covered in this chapter are important because of their role and mechanism of action in the treatment of many targets linked with lung cancer. However, there are numerous restrictions to their usage in clinical trials, which explains why there is a dearth of data from such studies. A combination of these drugs, as opposed to a single agent, would be more beneficial for use in future clinical studies. Using alternative combinations of therapeutic agents and flavonoids, while also reducing the number of chemotherapeutics, would be more realistic for successful therapy. This would result in reduced toxicities while simultaneously delivering the greatest possible effectiveness through the activation of several different signaling pathways. In-depth knowledge of the various functional groups present in flavonoids and their impact on the biological mechanism, in addition to additional knowledge or structural modification of these flavonoids, may aid in the development of improved therapeutic strategies for the prevention and treatment of solid tumors such as lung cancer and other carcinomas.

References

Chabot GG, Touil YS, Pham MH, Dauznne D (2010) Flavonoids in cancer prevention and therapy: chemistry, pharmacology, mechanisms of action, and perspectives for cancer drug discovery. In: Alternative and complementary therapies for cancer, pp 583–612. https://doi.org/10.1007/978-1-4419-0020-3-23

Chang HL, Chang YM, Lai SC, Chen KM, Wang KC, Chiu TT, Chang FH, Hsu LS (2017) Naringenin inhibits migration of lung cancer cells via the inhibition of matrix metalloproteinases-2 and -9. Exp Ther Med 13:739–744. https://doi.org/10.3892/etm.2016.3994

Chang JH, Cheng CW, Yang YC, Chen WS, Hung WY, Chow JM, Chen PS, Hsiao M, Lee WJ, Chien MH (2018) Downregulating cd26/dppiv by apigenin modulates the interplay between akt and snail/slug signaling to restrain metastasis of lung cancer with multiple egfr statuses. J Exp Clin Cancer Res 37:199. https://doi.org/10.1186/s13046-018-0869-1

Chen Z, Miao H, Zhu Z, Zhang H, Huang H (2017) Daidzein induces apoptosis of non-small cell lung cancer cells by restoring STK 4/YAP 1 signaling. Int J Clin Exp Med 10:15205–15212

Cho HJ, Ahn KC, Choi JY, Hwang SG, Kim WJ, Um HD, Park JK (2015) Luteolin acts as a radiosensitizer in nonsmall cell lung cancer cells by enhancing apoptotic cell death through activation of a p38/ros/caspase cascade. Int J Oncol 46:1149–1158. https://doi.org/10.3892/ijo.2015.2831

Cincin ZB, Unlu M, Kiran B, Bireller ES, Baran Y, Cakmakoglu B (2014) Molecular mechanisms of quercitrin-induced apoptosis in non-small cell lung cancer. Arch Med Res 45:445–454. https://doi.org/10.1016/j.arcmed.2014.08.002

Dai X, Zhang J, Arfuso F, Chinnathambi A, Zayed ME, Alharbi SA (2015) Targeting TNF-related apoptosis-inducing ligand (TRAIL) receptor by natural products as a potential therapeutic approach for cancer therapy. Exp Biol Med 240:760–773

Gu J-J, Qiao K-S, Sun P, Chen P, Li Q (2018) Study of EGCG induced apoptosis in lung cancer cells by inhibiting PI3K/Akt signaling pathway. Eur Rev Med Pharmacol Sci 22:4557–4563

Han X, Liu CF, Gao N, Zhao J, Xu J (2018) Kaempferol suppresses proliferation but increases apoptosis and autophagy by up-regulating microRNA-340 in human lung cancer cells. Biomed Pharmacother 108:809–816. https://doi.org/10.1016/j.biopha.2018.09.087

Jin CY, Park C, Hwang HJ, Kim GY, Choi BT, Kim WJ, Choi YH (2011) Naringenin up-regulates the expression of death receptor 5 and enhances trail-induced apoptosis in human lung cancer A549 cells. Mol Nutr Food Res 55:300–309. https://doi.org/10.1002/mnfr.201000024

Kim M-H, Jeong Y-J, Cho H-J, Hoe H-S, Park K-K, Park Y-Y (2017) Delphinidin inhibits angiogenesis through the suppression of HIF-1α and VEGF expression in A549 lung cancer cells. Oncol Rep 37:777–784. https://doi.org/10.3892/or.2016.5296

Klimaszewska-Wisniewska A, Halas-Wisniewska M, Tadrowski T, Gagat M, Grzanka D, Grzanka A (2016) Paclitaxel and the dietary flavonoid fisetin: a synergistic combination that induces mitotic catastrophe and autophagic cell death in A549 non-small cell lung cancer cells. Cancer Cell Int 16:10

Ko H, So Y, Jeon H, Jeong M-H, Choi H-K, Ryu S-H (2013) TGF-β1-induced epithelial-mesenchymal transition and acetylation of Smad2 and Smad3 are negatively regulated by EGCG in human A549 lung cancer cells. Cancer Lett 335:205–213. https://doi.org/10.1016/j.canlet.2013.02.018

Kopustinskiene DM, Jakstas V, Savickas A, Bernatoniene J (2020) Flavonoids as anticancer agents. Nutrients 12:457. https://doi.org/10.3390/nu12020457

Lin JK, Weng MS (2006) Flavonoids as nutraceuticals. In: Grotewold E (ed) The science of flavonoids. Springer, New York. https://doi.org/10.1007/978-0-387-28822-2-8

Liu L-C, Tsao TC-Y, Hsu S-R, Wang H-C, Tsai T-C, Kao J-Y (2012) EGCG inhibits transforming growth factor-β-mediated epithelial-to-mesenchymal transition via the inhibition of Smad2 and Erk1/2 signaling pathways in nonsmall cell lung cancer cells. J Agric Food Chem 60:9863–9873. https://doi.org/10.1021/jf303690x

Ma Y-C, Li C, Gao F, Xu Y, Jiang Z-B, Liu J-X (2014) Epigallocatechin gallate inhibits the growth of human lung cancer by directly targeting the EGFR signaling pathway. Oncol Rep 31:1343–1349

Ma L, Peng H, Li K, Zhao R, Li L, Yu Y, Wang X, Han Z (2015) Luteolin exerts an anticancer effect on nci-h460 human non-small cell lung cancer cells through the induction of sirt1-mediated apoptosis. Mol Med Rep 12:4196–4202. https://doi.org/10.3892/mmr.2015.3956

Marchand LL (2002) Cancer preventive effects of flavonoids—a review. Biomed Pharmacother 56: 296–301

Oo AM, Mohd Adnan LH, Nor NM, Simbak N, Ahmad NZ, Lwin OM (2021) Immunomodulatory effects of flavonoids: an experimental study on natural-killer-cell-mediated cytotoxicity against lung cancer and cytotoxic granule secretion profile. Proceedings of Singapore Healthcare, pp 279–285. https://doi.org/10.1177/2010105820979006

Pal HC, Sharma S, Strickland LR, Agarwal J, Athar M, Elmets CA, Afaq F (2013) Delphinidin reduces cell proliferation and induces apoptosis of non-small-cell lung cancer cells by targeting EGFR/VEGFR2 signaling pathways. PLoS One 8:e77270. https://doi.org/10.1371/journal.pone.0077270

Patil VM, Masand N (2018) Anticancer potential of flavonoids: chemistry, biological activities, and future perspectives. Stud Nat Prod Chem 59:401–430. https://doi.org/10.1016/B978-0-444-64179-3.00012-8

Saewan N, Koysomboon S, Chantrapromma K (2011) Anti-tyrosinase and anticancer activities of flavonoids from Blumea balsamifera DC. J Med Plants Res 5(6):1018–1025

Sarmah DT, Bairagi N, Chatterjee S (2021a) Tracing the footsteps of autophagy in computational biology. Brief Bioinform 22(4):286. https://doi.org/10.1093/bib/bbaa286

Sarmah DT, Bairagi N, Chatterjee S (2021b) The interplay between DNA damage and autophagy in lung cancer: a mathematical study. Biosystems 206:104443. https://doi.org/10.1016/j.biosystems.2021.104443

Sonoki H, Sato T, Endo S, Matsunaga T, Yamaguchi M, Yamazaki Y, Sugatani J, Ikari A (2015) Quercetin decreases claudin-2 expression mediated by up-regulation of microrna mir-16 in lung adenocarcinoma A549 cells. Nutrients 7:4578–4592. https://doi.org/10.3390/nu7064578

Sonoki H, Tanimae A, Endo S, Matsunaga T, Furuta T, Ichihara K (2017) Kaempherol and Luteolin decrease Claudin-2 expression mediated by inhibition of STAT3 in lung adenocarcinoma A549 cells. Nutrients 9:597. https://doi.org/10.3390/nu9060597

Su G, Chen H, Sun X (2018) Baicalein suppresses non small cell lung cancer cell proliferation, invasion and notch signaling pathway. Cancer Biomark 22:13–18. https://doi.org/10.3233/CBM-170673

Tian T, Li J, Li B, Wang Y, Li M, Ma D, Wang X (2014) Genistein exhibits anti-cancer effects via down-regulating foxm1 in H446 small-cell lung cancer cells. Tumour Biol 35:4137–4145. https://doi.org/10.1007/s13277-013-1542-0

Wang J, Huang S (2018) Fisetin inhibits the growth and migration in the a549 human lung cancer cell line via the ERK1/2 pathway. Exp Ther Med 15:2667–2673. https://doi.org/10.3892/etm.2017.5666

Wang B, Zhang X (2012) Inhibitory effects of Broccolini leaf flavonoids on human cancer cells. Scanning 34:1–5

Wang H, Bian S, Yang CS (2011) Green tea polyphenol egcg suppresses lung cancer cell growth through upregulating mir-210 expression caused by stabilizing hif-1alpha. Carcinogenesis 32: 1881–1889. https://doi.org/10.1093/carcin/bgr218

Xia R, Sheng X, Xu X, Yu C, Lu H (2018) Hesperidin induces apoptosis and G0/G1 arrest in human non-small cell lung cancer A549 cells. Int J Mol Med 41:464–472. https://doi.org/10.3892/ijmm.2017.3250

Xingyu Z, Peijie M, Dan P, Youg W, Daojun W, Xinzheng C, Xijun Z, Yangrong S (2016) Quercetin suppresses lung cancer growth by targeting aurora b kinase. Cancer Med 5:3156–3165. https://doi.org/10.1002/cam4.891

Yang Y, Zang A, Jia Y, Shang Y, Zhang Z, Ge K, Zhang J, Fan W, Wang B (2016) Genistein inhibits A549 human lung cancer cell proliferation via MIR-27A and met signaling. Oncol Lett 12:2189–2193. https://doi.org/10.3892/ol.2016.4817

Zanoaga O, Braicu C, Jurj A, Rusu A, Buiga R, Berindan-NeagoeInt I (2019a) Progress in research on the role of flavonoids in lung cancer. J Mol Sci 20:4291. https://doi.org/10.3390/ijms20174291

Zanoaga O, Braicu C, Jurj A, Rusu A, Buiga R, Berindan-Neagoe I (2019b) Progress in research on the role of flavonoids in lung cancer. Int J Mol Sci 20:4291. https://doi.org/10.3390/ijms2017429

Zhang L, Ma X, Dong Y (2018) Effect of genistein on apoptosis of lung adenocarcinoma A549 cells and expression of apoptosis factors. J BUON 23:641–646

Zhou Z, Tang M, Liu Y, Zhang Z, Lu R, Lu J (2017) Apigenin inhibits cell proliferation, migration, and invasion by targeting akt in the a549 human lung cancer cell line. Anticancer Drugs 28:446–456. https://doi.org/10.1097/CAD.0000000000000479

Chapter 7
Targeted Therapies Used in the Treatment of Non–Small-Cell Lung Cancer: An Overview

Tanuja Yadav, Velisha Mehta, Nilam Bhusare, Meena Chintamaneni, and Y. C. Mayur

Abstract Non-small cell lung cancer (NSCLC) is one of the highest killer disease in the world among all cancers. It is found that the survival rate after the treatment for NSCLC is lesser compared to other types of prevailing cancer. As patients are diagnosed in their late stage of NSCLC, chemotherapeutic drugs and targeted drugs have been used in recent therapies. This cancer cannot be cured but the lifespan of a patient suffering from NSCLC can be improved upto a certain level. Increased understanding of disease biology and mechanism of tumour progression with advanced early detection is found helpful in achieving significant advancements in the treatment of NSCLC. This review highlights the advance and current treatment regimen of drugs used for the treatment of NSCLC. Authors have discussed in detail the numerous drugs targeting various receptors with their mechanism of action and adverse effects. The use of these targeted therapies has managed to provide clinical benefits to selected patients. But complete cure and survival rates for NSCLC remain elusive, especially in metastatic disease. Hence, there is a continuous search for targeted drugs and combination therapies to enhance the clinical benefits and outcomes in NSCLC. So the research is progressing towards increasing the potential of existing drugs by studying drug resistance mechanisms for the enhancement of cure rate in patients with NSCLC to live a comfortable life.

Keywords Non–small-cell lung cancer (NSCLC) · advanced therapy · targeted drugs · chemo drugs · combination therapy

T. Yadav · V. Mehta · N. Bhusare · M. Chintamaneni
Department of Pharmaceutical Chemistry, SPP School of Pharmacy and Technology Management, SVKM's NMIMS, Mumbai, India

Y. C. Mayur (✉)
Department of Pharmaceutical Chemistry, SPP School of Pharmacy and Technology Management, SVKM's NMIMS, Mumbai, India

Somaiya Institute for Research & Consultancy, Somaiya Vidyavihar University, Mumbai, India

R. C. Sobti et al. (eds.), *Drug Repurposing for Emerging Infectious Diseases and Cancer*, https://doi.org/10.1007/978-981-19-5399-6_7

Highlights

- Advancement in knowledge and understanding of various molecular processes involved in NSCLC has led to identifying specific targets.
- Those targeted therapies found to target BRAF, ALK, and EGFR gene mutations were found to be of utmost benefit.
- A combination of chemo and target specific drugs exhibited a synergistic effect.
- Recent advancements in targeted therapy with an understanding of molecular biomarkers would be the best therapeutic option for NSCLC.

7.1 Introduction

The World Health Organization (WHO) report shows that lung cancer is a major disease that causes one of the highest rates of mortality. The death rate of lung cancer is more than any other type of cancer, such as breast, colorectal, and pancreatic cancers (Dela Cruz et al. 2008). The American Cancer Society's 2022 data showed that in the United States alone, there are 236,740 cases of lung cancer and out of these 130,180 mortalities occur every year (68,820 in men and 61,360 in women). The data predicted that men are more prone to lung cancer than females. Generally, lung cancer is observed mostly in older people (American Cancer Society 2017). There are fewer chances of survival of a patient suffering from lung cancer compared to any other type of cancer concerning the survival rate. Respiratory epithelium normal cells present in the lung are the ones that undergo the process of preneoplasia of cells to malignant cells which finally results in change in genes, epigenetics, DNA, and progression of the unyielded proliferation of cells and damage to the local tissues that depict metastases finally.

Around the world, around 84% of cases of lung cancer are found to be non–small-cell lung cancer (NSCLC). The multiplication rate is slower in case of NSCLC than in small-cell lung cancer (SCLC). The signs of NSCLC are not identified during the initial stages but are observed in advanced stage, till proper treatment is provided, the patient is not benefited from treatment. The symptoms in an advanced stage include a change in constant croup, the narrowness of breathing, expectorate sputum with signs of blood, pain when grasping, anorexia, weakness, and weight loss. The cause of NSCLC is mostly seen due to smoking. Approximately in 13% of cases, lung cancer spreads around the body during an early phase of disease progression (WHO 2018).

7.1.1 Classification of Lung Cancer

Lung cancer is classified based on its histopathology type. They are broadly classified into three types of lung cancer. They are NSCLC, SCLC, and other types of lung cancer are adenosquamous carcinoma and sarcomatoid carcinoma. The NSCLC is

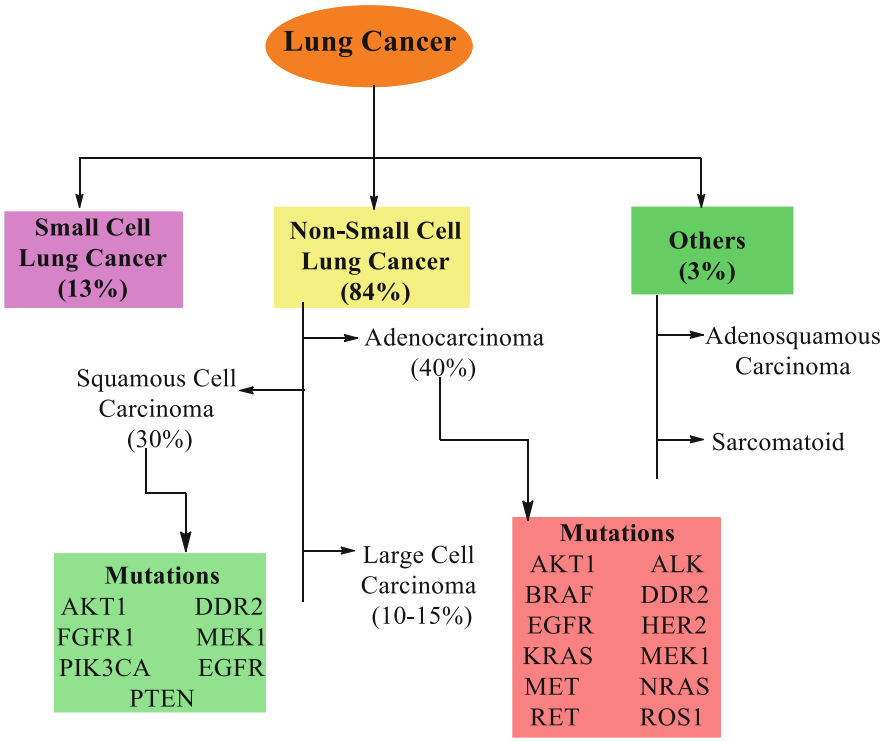


Fig. 7.1 Classification of lung cancer types

further categorized into three types such as adenocarcinoma, squamous cell carcinoma, and large-cell carcinoma which are shown in (Fig. 7.1). The most common mutations for adenocarcinoma are serine threonine protein kinase 1 (AKT1), B-Raf proto-oncogene (BRAF), anaplastic lymphoma receptor tyrosine kinase (ALK), discoidin domain receptor tyrosine kinase 2 (DDR2), epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), KRAS proto-oncogene (KRAS), mitogen-activated protein kinase MEK1 (MEK1), MET protooncogene, neuroblastoma RAS viral oncogene homolog (NRAS), and RET proto-oncogene and ROS proto-oncogene 1. In the squamous cell carcinoma, mutations are shown as AKT1, DDR2, fibroblast growth factor receptor 1(FGFR1), MEK1, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA), EGFR, and phosphatase and tensin homolog (PTEN) (Ruiz-Ceja and Chirino 2017).

Adenocarcinoma is observed in 40% of the cases and mostly arises from small airway epithelial type II alveolar cells, which secrete mucus and other substances. They are mostly found in smokers and nonsmokers in men and women regardless of their age. It occurs in the periphery of the lung. Squamous cell carcinoma is observed in 25–30% of cases and they originate from airway epithelial cells in the bronchial

tubes in the center of the lungs. They are mostly associated with smokers. Large-cell carcinoma is observed in 10–15% and they are initiated from the central part of the lung, lymph nodes, and chest wall and they are largely correlated with smoking (Tsao et al. 2016).

7.1.2 Risk Factors in Lung Cancer

The major risk factors responsible for the cause of lung cancer are mostly smoking cigarettes. Almost 80% of the cases are found in populations who smoke. Chances of lung cancer are observed in the population of smokers as compared to nonsmokers who are prone to lung cancer. Other than a smoker, nonsmokers are also prone to cancer due to ionizing radiation, risk of occupation where radioactive agents are used, exposure to polluted air indoors and outdoors, and diseases associated with NSCLC (Molina et al. 2008). Radon gas also causes lung cancer and deep-fried food, cured meat, cured pork, and chili nutritional supplement like β-carotenoids shows an increased risk of lung cancer (https://www.epa.gov/sites/production/files/2016-02/documents/2012_a_citizens_guide_to_radon.pdf; Krewski et al. 2005). Diseases such as pulmonary tuberculosis, chronic bronchitis, and emphysema are associated with NSCLC. A higher risk of cancer is observed among people consuming alcohol at least 30 g/day (Brenner et al. 2011; Darby et al. 2005). The background of a family suffering from any ailment is a liability factor associated with lung cancer. The connection of genes and chromosomes leads to the risk of lung cancer.

The other theory that is put forward for the cause of lung cancer is the human papillomavirus (HPV) which belongs to the papillomaviridae which belongs to the large family of epitheliotropic DNA viruses. It has been seen that the viral life cycle is dependent on the HOV gene expression and controlled by epithelial cell differentiation. It has been observed that the virus infection is seen due to the infection of the undifferentiated cells in the basal layers of the stratified squamous epithelium which is due to the scratching of the epithelial cells. It is also observed that the HPV has a very high degree of affinity to the squamous epithelium which is connected to the bronchus and lung and that is how it is postulated that HPV has a link with the lung neoplasms. A whole lot of research work on the meta-analysis data is in progress to find a correlation on this data (Ghittoni et al. 2015).

7.1.3 Diagnosis of NSCLC and Its Various Stages

The NSCLC is accurately diagnosed by observing the histopathology of the tissue slides. There are three major methods of diagnosis, where first is the imaging test like chest X-ray and computed tomography (CT) scan for examination of internal lung parts and detection of abnormal mass or nodule, second, sputum cytology for testing

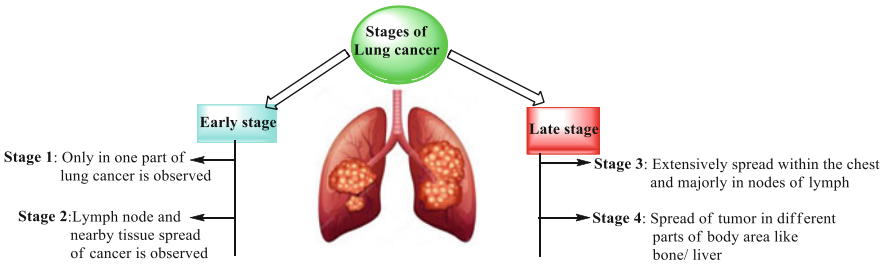


Fig. 7.2 Stages of lung cancer

of mucus samples from lungs, and third, biopsy for observing cells by histopathology. Biopsy such as CT-guided lung biopsy, bronchoscopy, endobronchial ultrasound, mediastinoscopy, or anthracoscopy gives a complete insight into the lung tissue and stage of the lung cancer and its spread (Goebel et al. 2019).

Different types of staging levels are observed which indicates the spread of lung cancer in the body parts. This helps in identifying the treatment modes needed for the patient. To identify the stage at which stage the patient is suffering from cancer, several tests are performed. The tests such as radiological/nuclear medicine X-rays, CT scans, magnetic resonance imaging (MRI), bone scans, and abnormal blood chemistry tests are performed. Lung cancer detected in an initial stage can be successfully treated by the surgery but in the late stage, the condition of the cancer patients gets worse and treatment becomes difficult (Fig. 7.2).

Different therapies are used according to the different stages of lung cancer as depicted in the table below (Ruiz-Ceja and Chirino 2017; Zappa and Mousa 2016; Tanoue 2008) (Table 7.1).

7.2 Treatment for Non–Small-Cell Lung Cancer

The method for treating and curing NSCLC confides mainly on the types and stages of lung cancer, the location of the cancerous cell, the spread of cancerous cell in the lung or different body parts and directly affects the patient's health.

The figure depicts the options for the therapy of curing lung cancer are classified as follows (Fig. 7.3) (Ruiz-Ceja and Chirino 2017).

7.2.1 Surgery

In the initial stage of lung cancer, surgery is performed to remove the tissues affected with cancer from the lung. This treatment procedure works only if lung cancer is in the initial stage. It is a complicated process to be performed and is carried out by well-trained surgeons. Before surgery, the pulmonary function test is carried out so

Table 7.1 Therapies to treat different stages of lung cancer

Stage	Size of tumor	Treatment
Occult stage		
Occult stage	Cancer cells are found in sputum	It depends on the patient's health and sometimes the tumor is removed by surgery.
Stage 0		
Stage 0	Abnormal cells in the lining of airways	Surgery
Stage I		
Stage IA	Tumor <3 cm not spread to lymph node	Surgery, radiation therapy Platinum-based adjuvant chemo drugs
Stage IB	Tumor>3 cm but <5 cm. Spread is up to the main bronchus	
Stage II		
Stage IIA	Tumor >3 cm but <5 cm, the spread is up to lymph node	Surgery, Radiation therapy Chemo drugs Postoperative adjuvant chemotherapy with six courses of cisplatin
Stage IIB	Tumor >5 cm but <7 cm, spreads of cells in airways	
Stage III		
Stage IIIA	Tumor >7 cm, spreads up to chest wall & diaphragm	Surgery, radiation therapy, Chemo drugs & targeted drugs
Stage IIIB	Any size of the tumor can be seen & spreads in form of nodules in the lobes of the lung	Targeted drugs (Gefitinib, Erlotinib, Gemcitabine)
Stage IV		
Stage IV	Spreads in another part of the lungs & cells found in the fluid of the heart &/or lung	Targeted drugs (Gefitinib, Gemcitabine, Docetaxel, Vinorelbine)

that physicians can preserve the nutritious tissue after surgery, as well as other biological tests are performed to confirm the functioning of other organs. In the treatment of NSCLC, different methods are adopted such as pneumonectomy, lobectomy, segmentectomy or wedge resection, sleeve resection, and video-assisted thoracic surgery (VATS). Among them, VATS surgery is less painful than any other type of surgery. The recovery rate is similar to the other types of operation.

Due to the surgery, the chances of several adverse effects may result from a response to the chemicals used such as anesthesia, too much loss of blood, coagulation in a lung, injury, and pneumonia. Suffocation may occur at the different stages of surgery (https://www.cancer.org/cancer/non-small-cell-lung-cancer/treating/surgery.html).

7.2.2 *Radiotherapy*

It is a therapy that is used along with other treatment options to cure lung cancer and is also used in the initial phase of lung cancer only. It utilizes high-power of radiation which affects the DNA inside the tumor cells, thus killing the tumor cells. This

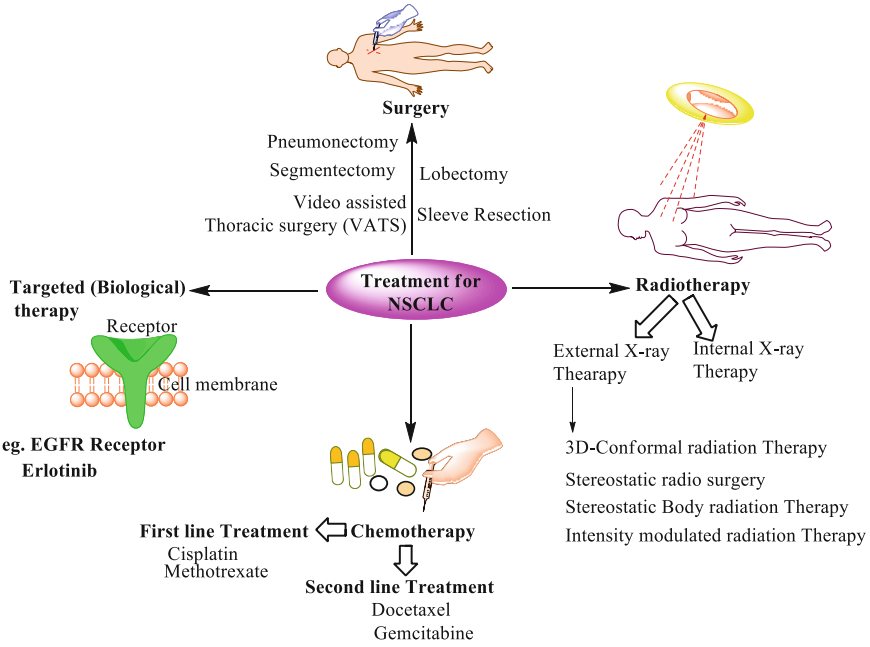


Fig. 7.3 Classification of different therapies used for treating NSCLC

treatment can be used to stop or to remove the tumors at particular locales present in the lung. Intimates suffering from NSCLC restricted to the upper part of the body and for surgical incision would gain an advantage by X-ray therapy. Radiation therapy additionally is a piece of palliative care that enhances the strength in the life of NSCLC intimate and doesn't respond to chemotherapy. A procedure termed stereotactic body radiation treatment (SBRT) is utilized in the beginning of NSCLC having a miniature nodule in the lung with no metastases in nearby lymph nodes. This strategy utilizes a higher technology system to exactly find the tumor and guarantee the exact situation of the recording device. This empowers the conveyance of robust and highly focused X-ray therapy. It was discovered that SBRT gives 2-year growth survival, brings down expenses and more important patient comfort. Radiotherapy is also classified into two types mainly such as external-beam X-ray therapy and internal X-ray therapy (Qiao et al. 2003).

In the external beam X-ray therapy, the technique used for the treatment of NSCLC and the cancer cells are hit by the painless radiation technique and less time consuming. The x-rays focused on the affected part of the lungs are cited and fixed to impart the predetermined dose of rays on the cancer cells. With the advancement of technology, the newer treatment techniques that are adopted are the three-dimensional conformal radiation therapy (3D-CRT), intensity-modulated radiation therapy (IMRT), stereotactic body radiation therapy (SBRT), and

stereotactic radiosurgery (SRS). Among them, IMRT is a more developed and advanced 3D technique, which is known as volumetric-modulated arc therapy (VMAT).

In an internal X-ray therapy, the technique used is the radiation to target the cancer cells in the airways to reduce the clinical symptoms. Bronchoscopy is performed through surgery also. Radiations are used and they travel to a minimal area and do not harm the nearby tissues (Fournel et al. 2005).

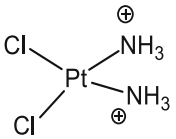
The adverse effects of X-ray therapy are fatigue, nausea and vomiting, anorexia, weight loss, treated part of skin showing reddening, blister, and peeling, and hair fall due to radiation which is observed till the therapy is continued. Radiation also affects the esophageal track exposed to rays and results in sore throat, improper swallowing problems, when the radiation reaches the cerebrum symptoms like amnesia, head pain, decrease in sexual desire, less ability to think are observed (Sause 1999).

7.2.3 Chemotherapeutic Drugs

Chemotherapy is also used in the later stages of the tumor of the lung and also in the treatment of curing NSCLC. Generally, a patient suffering from lung cancer is already in stage 4, and chemotherapeutic treatment is usually started at this stage. This treatment process aims to cure the patients of cancer and enhance their endurance and decrease adverse effects. The American Society of Clinical Oncology report states that the drugs are used in curing NSCLC. The usual drugs used are cisplatin, carboplatin, paclitaxel, albumin-bound paclitaxel, docetaxel, gemcitabine, vinorelbine, irinotecan, etoposide, vinblastine, and pemetrexed. Here is a regimen that is followed in tandem with the first line of treatment of drugs for stage 4 treatment of NSCLC. The combined drugs are used to treat the patients. Many combinations of drugs are used such as gemcitabine plus vinorelbine or paclitaxel and with cisplatin or carboplatin. Generally, chemo drugs are given within a specified period (usually 1–3 days) and given in a specific cycle of treatment. Normally four to six chemo cycles are given to a patient with a chemo combination in advanced stages of cancer. When first-line treatment is ineffective to the progression of the cancer stage, then second-line treatment is given to the patient. The patient is also given maintenance therapy along with chemo drugs. Single chemo drugs are also utilized in second-line treatment using docetaxel or pemetrexed, or therapy of targeted area or drug immunotherapy. Few adverse reactions include fall of hair, injuries in the oral cavity, anorexia, nausea and vomiting, diarrhea, and stoppage of chemotherapy increased the chances of disease leading to easy loss of blood, and weakness. After the treatment is completed, many adverse reactions are observed. The above reactions are reduced over a regular period. A few medications have a particular adverse reaction. For instance, cisplatin, vinorelbine, docetaxel, or paclitaxel can cause nerve damage. This shows that the side effects are mostly observed in the hands and feet, and also can lead to a situation like an agony, shivering, loss of sensations, which affect the ability to cool or warm and such effects are seen in these aspects (Cortés et al. 2015; Schiller et al. 2002).

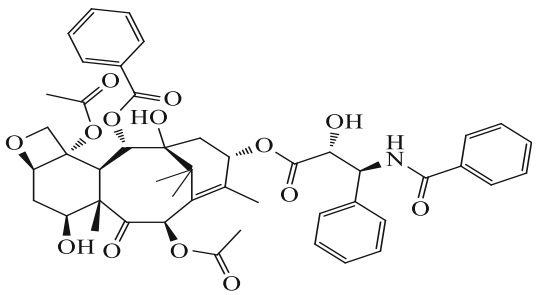
7.2.3.1 First-Line Treatment for NSCLC

7.2.3.1.1 Cisplatin (1978)



Cis-diamminedichloroplatinum (Cisplatin). Though, it shows nephrotoxicity, ototoxicity, and neurotoxicity with fewer symptoms which leads to the development of another analogue with fewer side effects. Cyclobutane-1,1-dicarboxylato platinum (II) (Carboplatin) came into the picture by the FDA in 1989. These platinum drugs utilize natural cation transporter 2 (OCT-2) as a take-up component and forms adduct with DNA activating oxidative stress, DNA, and apoptosis, which unfortunately have similar impacts in tissues that communicates OCT transporters (David and Johnson 2000). The overexpression of these transporters is associated with various tumors such as breast, gastric, and hepatic, among others; it has not been revealed in lung carcinoma. Substantial mtDNA (mitochondrial) transformations, for example, mitochondrially encoded NADH (MTND4), which encodes the subunit of NADH ubiquinone oxidoreductase, were observed in a patient with ovarian carcinoma and treated with cisplatin which suggested that resistance to cisplatin therapy observed was due to MTND4 mutation. There are alterations observed in miRNA, i.e., MiR-192 which is identified as a crucial modulator against cisplatin therapy. However, these alterations have not been described in NSCLC but they might be involved in resistance against cisplatin treatment (David and Johnson 2000; Einhorn 2008).

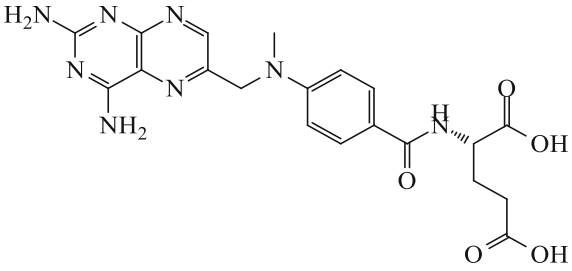
7.2.3.1.2 Paclitaxel (2012)



Chemical name: (2*R*, 3*S*)-*N*-benzoyl-3-phenylisoserine (Paclitaxel). It acts as a microtubule blocker that binds to β-tubulin and induces the development of stable groups of microtubules thereby de-polymerization is prevented. It meddles with the working of cell microtubules and prompts mitotic capture and cell mortality. The tumor cell protection is associated with overexpression of the efflux transporter

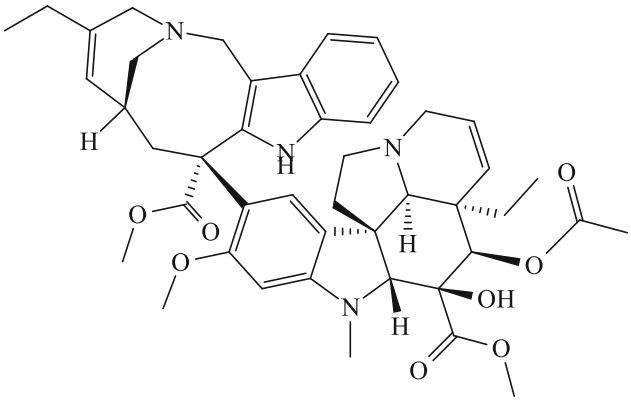
P-glycoprotein, which is encoded by the MDR1 gene. Resistance to the paclitaxel is due to beta-tubulin gene. According to the American Cancer Society, when there is a failure of combination chemotherapy or relapse observed after 6 months of adjuvant chemo-drug treatment, then this drug is utilized as first-line therapy in patients suffering from squamous carcinoma (Ramalingam and Belani 2004; Socinski 1999; Rosell et al. 2019).

7.2.3.1.3 Methotrexate (2014)



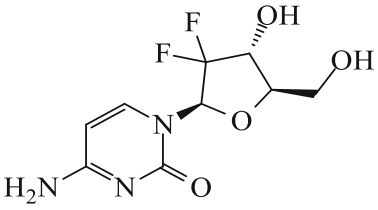
Chemical name: *N*-[4-[[(2,4-diamino-6-pteridinyl) methyl] methylamino] ben-zoyl]-L glutamic acid (Methotrexate). It acts as a dihydrofolate reductase (DHFR) inhibitor, which causes a reduction in the synthesis of purine and pyrimidine and hinders the multiplication of cells in the late G1 stage. Rhee et al.; explained five different resistance mechanisms involved in methotrexate treatment such as an increase in DHFR, reduced accumulation because of hindered transport, diminished retention due to no polyglutamate development, binding of mutated DHFR with methotrexate than the normal one and an expanded level of γ-glutamyl hydrolase that hydrolyses methotrexate polyglutamates (Rhee et al. 1993). Especially in the treatment of squamous cells and small cell cancer, it is used alone or in a mixture with anticancer drugs (Bonomi 1986).

7.2.3.1.4 Vinorelbine (1994)



Chemical name: 3′,4′-didehydro-40-deoxy-C′-norvincaleukoblastine [R-(R*,R*)-2,3-dihydroxybutanedioate (1:2)] (vinorelbine). It is an alkaloid that disturbs the development of microtubule assemblies during mitosis. It causes mitotic arrest and cell death by binding with the β-tubulin subunit. It likewise upsets the digestion of amino corrosive, cyclic AMP, glutathione, nucleic acids, and lipid biosynthesis. The expression of MDR1, P-gp, MRP-1, RAF1, RLIP76 genes, and the activation of AKT/ERK proteins have been involved in sensitivity of cancer cells towards this drug (Piccirillo et al. 2010). The drug is used for the resistance reversal of A549/DDP cells of human lung cancer. The drug is used alone or with cisplatin for the treatment of NSCLC in the third stage and as a single therapy for earlier treated patients (Faller and Pandit 2011; Julien et al. 2000; Venorelbone n.d.).

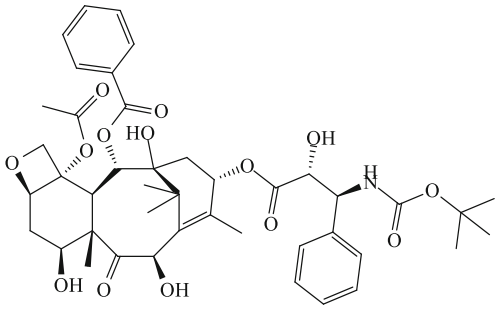
7.2.3.1.5 Gemcitabine (1996)



Chemical name: 2′-deoxy-2′,2′-difluorocytidine monohydrochloride (β-isomer; Gemcitabine) It is mainly involved in the procedure required for DNA synthesis. It is administered intravenously in presence of enzyme deoxycytidine kinase and gets converted to active metabolite difluoro deoxycytidine diphosphate (dFdCDP) and difluoro deoxycytidine triphosphate (dFdCTP). The dFdCTP competes with deoxycytidine triphosphate and is introduced into DNA. During DNA replication, the DNA polymerase chain is blocked and results in the termination of the process causing cell death. The dFdCDP blocks ribonucleotide reductase (RNR) that reduces the deoxynucleotide pool available for the synthesis of DNA. The intracellular concentration of dCTP is reduced which will lead to the incorporation of dFdCTP into DNA (Toschi et al. 2005). Reduction of dCDA activity, and activation of NF-kB following overexpression of Bfl-1 leads to resistance of cancer cells to Gemcitabine. It is used in combination with cisplatin as a first-line treatment for patients with stage IIIA, IIIB, or IV (Hayashi et al. 2011) (Sandler and Ettinger 1999).

7.2.3.2 Second-Line Treatment for NSCLC

7.2.3.2.1 Docetaxel (1999)



Chemical name: (2*R*,3*S*)-*N*-carboxy-3-phenylisoserine, *N*-tert-butyl ester, 13- ester with 5β-20-epoxy-1,2α,4,7β,10β,13α-hexahydroxytax-11- en-9-one 4-acetate 2-benzoate, trihydrate (Docetaxel). It is an antineoplastic drug that belongs to the taxane family and hinders microtubule depolymerization which causes transition arrest of the metaphase to anaphase and results in apoptosis (Gubens and Wakelee 2010). Resistance is observed to drugs due to variations in docetaxel metabolism, and deregulation of the cell cycle and apoptosis pathways. Various mutations and alterations in microtubule gene expression have been reported. It is utilized as second-line therapy for phase IIIA, IIIB, or IV after the disappointment of platinum therapy and can be used as a part or in combination with cisplatin (Neal et al. 2015).

7.2.4 Targeted Therapy Used for Curing of NSCLC

In the later stages of NSCLC, targeted therapy is used. The functioning of the drugs which are used in chemotherapy is different from that of targeted drugs. Targeting drugs function more efficiently when chemotherapy treatment does not work or shows lesser adverse effects (Simon and Somaiah 2014). These are targeted to that area of the lung cells where mutations of cells occur. Due to mutations in cells, the growth of cancer cells occurs and to stop the growth of the mutation cells, drugs are targeted to that specific location to inhibit that cell's functioning. Drugs used for targeted therapy are as below (Kumar et al. 2015; https://www.cancer.gov/about-cancer/treatment/types/targeted-therapies/targeted-therapies-fact-sheet). They are also used during chemotherapy treatment and in combinations. Several of them are used in combination to give a better output of results with low adverse effects (Chan and Hughes 2015) (Table 7.2).

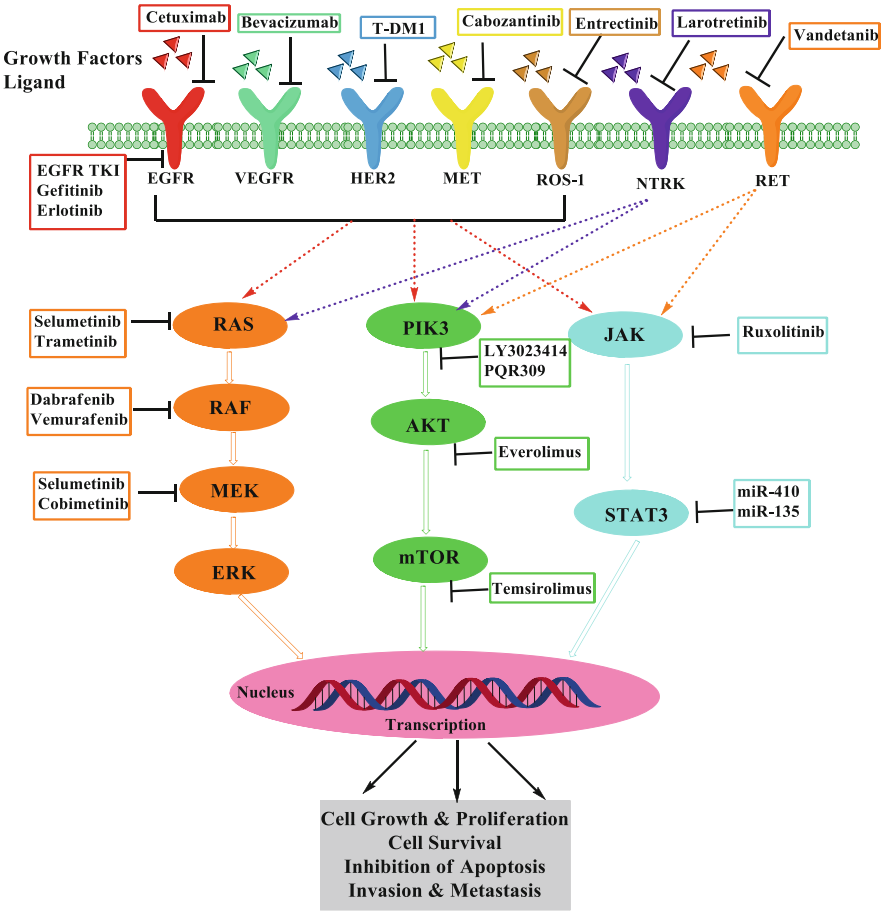
Table 7.2 Classification of targeted drugs used for the treatment of NSCLC and their action on different targets

Receptor	Drugs used	Status
VEGF blockers	Bevacizumab	2006
	Ramucirumab	2014
MMP blockers	Batimastat	Phase III
	Marimastat	Phase III
	Prinomastat	Phase III
	Tanomastat	Phase III
	ONO-4817	Phase I
Vascular targeting agents	Z D6126	Phase I
EGFR inhibitors	Erlotinib	2004
	Afatinib	2013
	Gefitinib	2003
Inhibitors target cells with T790M mutation	Osimertinib	2015
Inhibitors used for squamous cells	Necitumumab	2015
Others	Rociletinib	Phase II
	EGF816	Phase III
	ASP8273	Phase III
	HM61713	Phase II
ALK receptor inhibitors	Crizotinib	2011
	Ceritinib	2014
	Alectinib	2015
	Brigatinib	2017
	Lorlatinib	2018
BRAF receptor inhibitors	Dabrafenib	2013
	Trametinib	2017
	Vemurafenib	2011
	Selumetinib	Phase II
MET receptor inhibitors	Cabozantinib	2011
HER-2 receptor	Trastuzumab Emtansine	1998
	Afatinib	2013
	Dacomitinib	2018
ROS-1 receptor inhibitors	Cabozantinib	2011
	Ceritinib	2014
	Lorlatinib	2018
	Entrectinib	2019
RET receptor inhibitors	Cabozantinib	2011
	Alectinib	2015
	Vandetanib	Phase III
	Lenvatinib	Phase II
	Ponatinib	Phase II
NTRK1 receptor inhibitors	Entrectinib	2019
	Cabozantinib	2011
PIK3CA receptor inhibitors	LY3023414	Phase II

(continued)

Table 7.2 (continued)

Receptor	Drugs used	Status
	PQR309	Phase II
MAP2K1 pathway inhibitors	Selumetinib	Phase II
	Trametinib	2017
	Ccobimetinib	Phase II



7.2.4.1 Drugs Targeting Angiogenesis

The growth of tumor in NSCLC is dependent on the angiogenesis process which provides oxygen and nourishment. The expansion of the tumor cells can be stopped by blocking it at the primary site and metastatic site. Angiogenesis is classified into two types: sprouting angiogenesis and nonsprouting angiogenesis. In the first one,

branching of the blood capillaries from the predate blood vessel in the lung, whereas in the latter one, the occurrence of size enlargement, division, and fusion of anticipated vessels form during the expansion of cells on the surface of the vessel. Bridging is observed in the vessels by this type of angiogenesis. Both types of angiogenesis are observed to occur concurrently (Barzi and Pennell 2010).

The vascular endothelial growth factor (VEGF)/vascular permeability factor (VPF) is the angiogenic molecule that controls both angiogenesis and vascular permeability which leads to the development of a tumor and pleural effusion in NSCLC (Yano et al. 2003). The VEGF family comprises VEGF-A, VEGF-B, VEGF-C, VEGF-D, and VEGF-E, which were proposed to be the most intense growth factor for endothelial cells that play a prominent role in the process of angiogenesis. The VEGF comprises no less than four isoforms (VEGF121, VEGF165, VEGF189, and VEGF 206) which are controlled by splicing at the mRNA level where VEGF165 is the most abundant isoform. The VEGF ties a high affinity to two tyrosine kinase receptors, VEGFR-1 (Flt-1) and VEGFR-2 (Flk-1/KDR). In NSCLC, VEGF-C articulation is accounted to connect with lymph-hub metastasis and lymphatic attack. Thus, it provides information regarding the VEGF family and its receptors which is an essential target area for the blockade of lymphangiogenesis and angiogenesis in NSCLC (Piperdi et al. 2015; Jain et al. 2011) (Fig. 7.4).

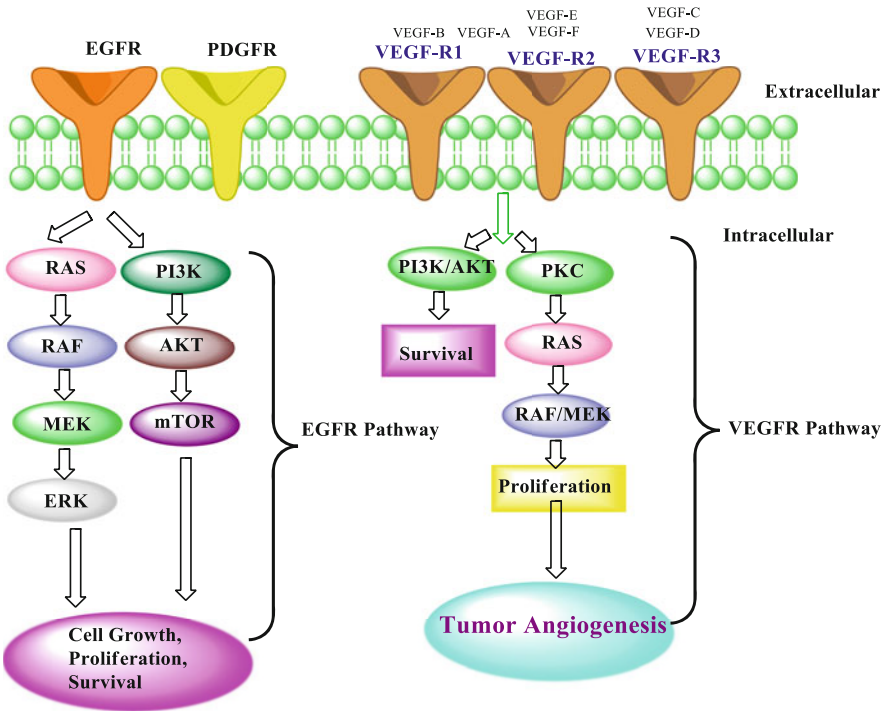


Fig. 7.4 Mechanism of action for drugs targeting angiogenesis and EGFR pathway

- **Antitumor Vasculature Therapy**
  Targeting the vascular region in the lungs was introduced almost three decades ago and now the same therapy is introduced because of the development of new anticancer vasculature drugs. Benefits gained by this therapy against the chemo drugs include physical availability and stability of genes of targeted molecules (Korpanty et al. 2011; Bar and Goss 2012). It focuses not only on angiogenesis (neovascularization) which forms little tumors in the lungs (antiangiogenic agents) but also on the vessel linked with a tumor that is larger (vascular targeting agents) and even focuses on the tubulin present in the endothelial cells. It utilizes longer duration molecules to destroy the vessels containing cancerous cells, anticancer drugs and EGFR blockers (Yano et al. 2003; Pallis and Syrigos 2013).

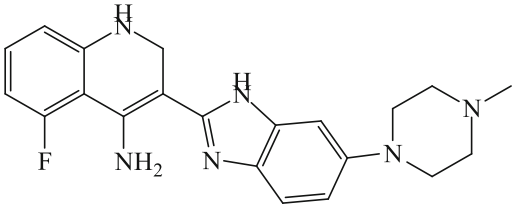
7.2.4.1.1 Antiangiogenic Agents

Two types of agents are used for blocking the tumor blood vessels. They are VEGF blockers and Matrix metalloproteinase (MMP) blockers (Korpanty et al. 2011).

VEGF Blockers

The drugs that block the VEGF receptors: are bevacizumab and ramucirumab.

Bevacizumab (2006)



It is a humanized endothelial monoclonal antibody against VEGF. The drug attaches with the VEGF inhibitors (Flt-1 and KDR) to stop the process of endothelial cell multiplication and angiogenesis. Its mechanism of resistance is not comprehended but, estrogen induced marked resistance to bevacizumab that is by enhancing myeloid recruitment and pericyclic coverage. With this drug, different types of cancer studies have been carried out such as colorectal cancer treatment, lung cancer, and breast cancer which showed a positive result in both increased period of improvement and survival time when the drug was supplemented in the combined form (Sandler 2007). The drug is combined with carboplatin plus weekly paclitaxel which is used as a first-line therapy regimen for patients with advanced NSCLC (Yamasaki et al. 2017). Sometimes the combination of erlotinib and bevacizumab is also administered. This drug indulges in increased incidence or severity of typical chemotherapy, toxicity such as hypertension, thrombosis, albuminuria, and

hemorrhage are more repeatedly found in a patient treated with these drugs (Di Costanzo et al. 2008; Zeng et al. 2020).

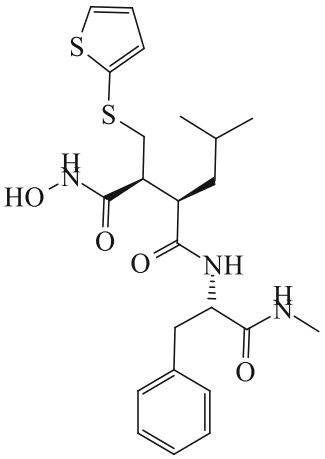
Ramucirumab (2014)

This is a human recombinant IgG1 monoclonal antibody and used as a third-generation antiangiogenic agent in a phase III trial of advanced NSCLC patients. The drug binds to VEGFR-2 and stops the binding of VEGF ligand to VEGFR-2, which controls the movement of cell and cell expansion. Resistance to this drug is not reported. Platinum-based chemo drugs are used in combination with the drug initially, next, docetaxel is used in combination therapy with the drug at a later stage IV for treatment of NSCLC (Camidge et al. 2014). It was initially used for curing gastric cancer. It is used even for treating NSCLC, colorectal, and breast cancer. The side effects of the drug are leukopenia, asthenia, increase in blood pressure, epistaxis, loose stool, stomatitis, etc. (Das and Wakelee 2014).

MMP Blockers

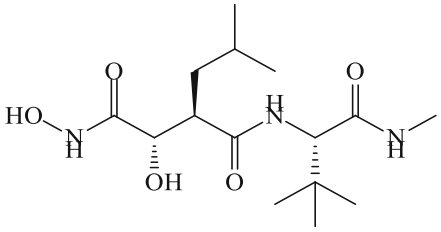
MMP plays a crucial role in angiogenesis, metastasis, and tumor invasion. These are extracellular protease that causes tissue degradation in various disease conditions. MMP blockers used for curing cancer of NSCLC are categorized according to different types of generation, which are such as first-generation drug: Batimastat; second-generation drugs: Marimastat, MM1270, and Prinomastat; and third-generation drugs: BAY12-95666 (Tanomastat) and ONO-4817 (Winer et al. 2018; Merchant et al. 2017).

Batimastat



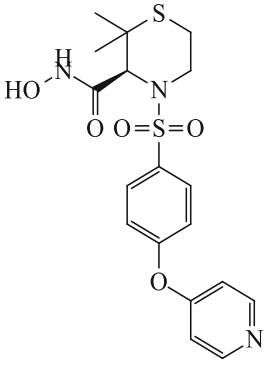
This drug is a first-generation, broad-spectrum, and potent matrix metalloproteinase inhibitor (MMPI), which is under clinical investigation. It is the first to enter the clinical cancer trials but its formation resulted in poor bioavailability. The drug is not given through the oral route. It was introduced after the desire of the effusions and fundamentally diminished the quantity of pleural goal required. Adverse effects seen were mellow and dyspnea scores of patients fundamentally enhanced following 1 month of treatment with the drug (Bin et al. 2013; Merchant et al. 2009).

Marimastat



Marimastat (BB-2516), a second-generation drug, is in the advanced phase of a clinical trial. The principle behind this medicine is its lethality in a trademark disorder comprising of musculoskeletal agony and solidness, regularly beginning in the joints of the hands, and spreading to arms and shoulders. These musculoskeletal adverse reactions are dosage related. The drug was in phase III of a clinical trial for the treatment of patients with third stage NSCLC (Goffin et al. 2005; Marimastat 2020).

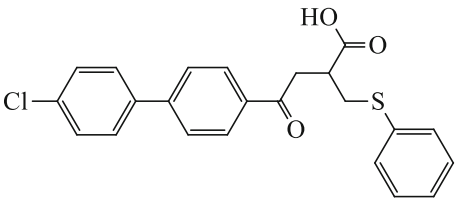
Prinomastat



It is a broad spectrum and second-generation drug used for the treatment of NSCLC and administered through the oral route. Phase I clinical trials of the drug have been

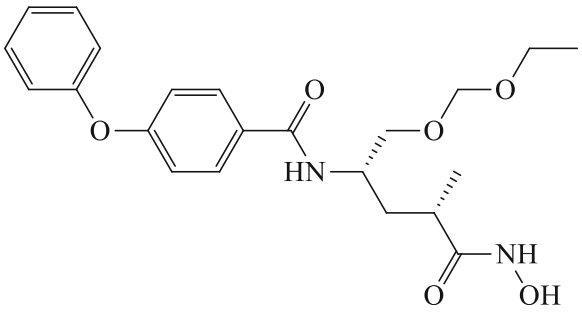
completed in the patient suffering from a late stage of cancer and the individuals suffering from prostate malignancies and phase III clinical trials were carried out in patients suffering from cancer of NSCLC. But the drug is not effective for the treatment of advanced stage of cancer. In human lung disease xenograft, it reduces the mass of primary tumor and mediastinal lymph nodes and reduces the general metastasis 2 weeks after the implantation. Another trial was performed with the two drugs, cisplatin and gemcitabine, during the stage IV or ongoing NSCLC (Bissett et al. 2005).

BAY12-95666 (Tanomastat)



It is a third-generation drug that inhibits MMP-2, MMP-3, and MMP-9. It causes blockade of extracellular matrix deterioration and even inhibits angiogenesis, cancer cell expansion, influx, and metamorphosis. It was in phase III human clinical trial but gave a negative result for SCLC and pancreatic cancer (Lavanya et al. 2014).

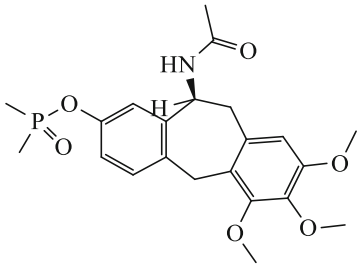
ONO-4817



It is a synthetic hydroxamic acid-based nonpeptide molecule that is given through the oral route. As it acts as a selective inhibitor and blocks MMP-8, MMP-9, MMP-12, MMP-2, and MMP-13, but it doesn't inhibit MMP-1 and MMP-7. The studies prove that ONO-4817 is more effective than tanomastat at MMP-2 and MMP-9 sites. The drug shows less effect of inhibition on MMP-1 suppression (Yamamoto et al. 2003).

7.2.4.1.2 Vascular Targeting Agent

The agent that is used for vascular treatment is **Z D6126**.



The drug has tubulin-binding activity and has the potential to go into vascular tissue. It blocks the microtubule polymerization as it is a prodrug containing phosphate, i.e., N-acetylcolchinol. When the drug reaches the site of action, it destructs the cytoskeleton of cancerous endothelial cells. This causes the blockage of the cancer blood vessels, stopping the blood loss from the site and mortality of cancerous cells due to a lack of supply of oxygen and nutrition in a small rodent xenograft model (Raben et al. 2004; Davis et al. 2002).

7.2.4.2 Drugs Targeting EGFR

EGFR is a transmembrane glycoprotein having two major domains, one extracellular EGF binding domain and other intracellular tyrosine kinase domain. It regulates signaling pathways that control the proliferation of cells. Targeting EGFR is used as the most selected therapy for curing cancer of NSCLC. The activation channels of the cell progression and development occur by the receptor known as cell surface tyrosine receptor (Johnston et al. 2006; Zukin 2012). The EGFR is a cell surface tyrosine kinase protein located on the seventh chromosome and belongs to the family of ErbB a 170 k dalton. The family includes HER1 (EGFR), HER2, HER3, and HER4. During malignancy, a metamorphosis of cell surface receptors delivers unlimited cell division through consistent enactment (Ogunleye et al. 2009). The receptor gets activated by the binding ligand to the extracellular part. Without binding at the receptor site no dimerization possible, there is no activity at the enzymatic site of the extracellular part (Pirker et al. 2010). The EGFR plays a part in the other body parts such as the head and neck, cervix, lungs, etc. The overexpression of genes observed in NSCLC is in the range of 40–89% and is usually found in the squamous cancer cell and 41% is found in adenocarcinoma types of cells. The drugs gefitinib and erlotinib are preferred in the later stage of cancer of NSCLC (Shien et al. 2015).

There are parts in which the EGFR antagonistic action is observed. (1) anti-EGFR monoclonal antibody, (2) EGFR TKIS inhibitors, (3) inhibitors target cells with T790M mutation, and (4) inhibitors used for squamous cells.

7.2.4.2.1 Anti-EGFR Monoclonal Antibodies

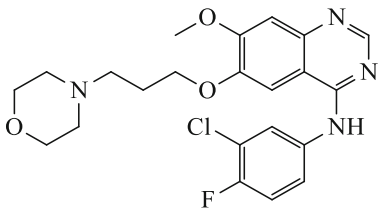
Cetuximab

It is the first-line treatment drug used for advanced curing of cancer of NSCLC, which is in phase III clinical trials. It is a murine monoclonal immunoglobulin G1 antibody that stops the working of the receptor, the activity of the receptor is altered, moderates the cell-mediated antibody-dependent cytotoxicity and receptor downregulation. Before giving treatment with the drug, an antiallergic drug is given to the patient. The treatment is continued till the disease is in progress. The adverse reactions are skin rash and loose motion. The drug is supplemented in combination with other chemo treatments in clinical trial II and III studies. Cetuximab inhibits T790M-mediated resistance in EGFR. When a drug is given alone for treatment, it is observed that 4.5% in recurrent EGFR- expressing NSCLC cancer, but it attains 25–35% when the drug is given in combination with the platinum drugs (Sgambato et al. 2014; Govindan 2004). Also, these clinical studies indicted better therapeutic benefits from the EGFR-directed monoclonal antibodies in the patients with NSCLC including squamous cell lung cancer (https://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm279174.htm; Martin et al. 2016).

7.2.4.2.2 EGFR Tyrosine Kinase Inhibitors (TKIs)

These inhibitors inhibit the growth of tumor cells, tumor cell adhesion-invasion, and receptor phosphorylation. The examples of this group members are—gefitinib, erlotinib, afatinib, and dacomitinib.

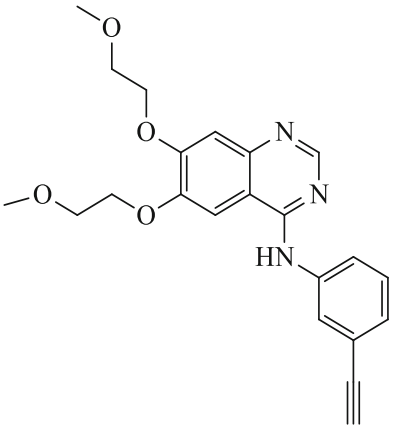
Gefitinib (2003)



Chemical name: N-(3-chloro-4-fluorophenyl)-7-methoxy-6-[3–4-morpholin) propoxy]. It is a selective EGFR-TKI that blocks EGFR activation with mutations

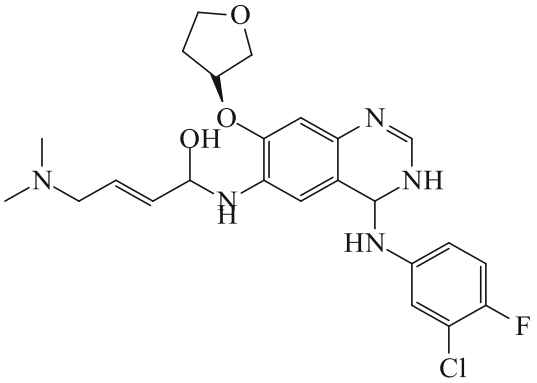
in EGFR such as L858R and exon 19 deletions. It is a monotherapy for curing patients with a late stage of NSCLC followed by platinum-based and docetaxel treatment. The drug was approved as a first-line treatment for curing the NSCLC having tumor EGFR exon 19 deletions of exon 21 substitution (Pao et al. 2005). The drug blocks the EGFR activation when competing at ATP binding site and inhibits receptor phosphorylation, tumor cell adhesion, and cancer cell growth. T790M mutation is one of the commonly obtained resistance to gefitinib (Ma et al. 2011). It is the main substrate of CYP2D6 and CYP3A4, which can cause many drug interactions. CYP2C19 and CYP2D6 are weak blockers and cause harm to drug metabolism (http://0-online.lexi.com.library.touro.edu/action/home).

Erlotinib (2004)



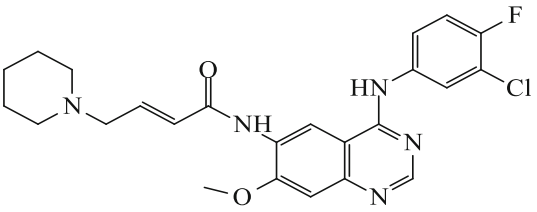
Chemical name: N-(3-ethynylphenyl)-6,7 bis(2-methoxyethoxy)-4-quinazolin-amine. The drug was endorsed by US FDA as a second-line agent in curing NSCLC. It blocks the ATP-binding site by binding with the intracellular TK domain of EGFR. This drug is used as first-line treatment for patients in the third and fourth stages of NSCLC where mutations exon 19 deletions or exon 21 substitutions are seen. Many randomized trials have contemplated the adequacy of the drug versus the blend of carboplatin or cisplatin with gemcitabine. Resistance toward drugs related to T790 mutation, methylation of death-associated protein kinase (DAPK), and MET activation (Wu et al. 2015). The National Comprehensive Cancer Network (NCCN) now prescribes that patients with cancer cells found to have EGFR changes after the beginning of chemo treatment might be changed to the drug alone (www.NCCN.org/professionals/physician_gls/pdf/nscl.pdf n.d.). The most widely recognized unfavorable effect stated with its utilization is skin eruption. Extreme unfavorable effects of interstitial lung infection, gastrointestinal aperture, and hepatotoxicity were uncommon.

Afatinib (2013)



Chemical name: 2-butenamide, N-[4-[(3-chloro-4-fluorophenyl)amino] 7- [[(3S)-tetrahydro-3-furanyl]oxy]-6-quinazolinyl]-4-(dimethylamino)-,(2E)-,(2Z)-2 butenedioate (1:2). It is an irreversible EGFR TKI, for curing individuals having metastatic NSCLC, which have EGFR exon 19 deletions or L858R substitutions (Ho et al. 2019). It showed a controllable, predicted, and bearable profile. Though several in-vitro studies indicated that activation of EGFR-dependent downstream pathways could develop drug resistance, it is used as therapy for overcoming resistance to first-generation EGFR-TKI. This drug is also utilized as first-line treatment for stage IV NSCLC patients with EGFR mutant (Neal et al. 2015). Loose motion, rash, skin break out, nail impacts, and stomatitis were among the most as often as possible revealed unfriendly occasions in its group. Extreme skin inflammation and skin eruption are normal and may show as rankling, exfoliative sores (https://gilotrifhcp.com/sites/default/files/pdfs/PC-GF-0426-PROF_MVA_7_(Digital_Version)_091916_Digital_Proof.pdf).

Dacomitinib (2018)

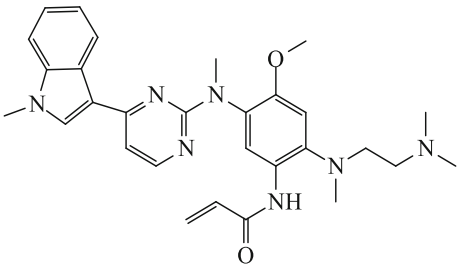


Chemical name: (E)-N-(4-((3-chloro-4-fluorophenyl)amino)-7-methoxyquinazolin-6-yl)-4-(piperidin-1-yl)but-2-enamide. It is a small molecule, second-generation critical ATP competitive, highly selective, and potent EGFR-TKI. It also inhibits the signaling from members of the HER receptor family. Dacomitinib controls osimertinib L718Q mutation to the patients that are resistance to osimertinib. FDA

approved the drug as first-line therapy for patients having metastatic EGFR-mutated NSCLC (Lau et al. 2019; Shen et al. 2021).

7.2.4.2.3 Inhibitors Target Cells with T790M Mutation

Osimertinib (2015)



Chemical name: N-(2-((2-(dimethylamino)ethyl)(methyl)amino)-4-methoxy-5-(methyl(4-(1-methyl-1*H*-indol-3-yl)pyrimidin-2-yl)amino)phenyl)acrylamide. It is a third-era EGFR TKI, which inhibits proliferation and DNA synthesis of cancer cells. This drug binds with EGFR having mutations such as L858R, T790 M, and exon 19 deletions (Cross et al. 2014). It demonstrates powerful clinical viability in patients with T790 M-changed lung disease. For patients with illness against first-line EGFR TKIs, the drug might be an alternative. The most widely recognized system against EGFR TKIs is the existence of T790M mutation, which happens in over half of NSCLC tumors that are re biopsied. Resistance in osimertinib is heterogeneous that follows EGFR-dependent and EGFR-independent mechanisms. Basic symptoms were ill-advised, looseness of the bowels, sickness, and diminished craving (Gil-Bazo and Rolfo 2016).

It is a noteworthy substrate of CYP3A4 and has the potential for some medical associations. The treatment should be observed in such a manner to cure side effects related to interactions and drug malignancies (Pirker et al. 2017; osimertinib resistance.pdf n.d.).

7.2.4.2.4 Inhibitors Used for Squamous Cells

Necitumumab (2015)

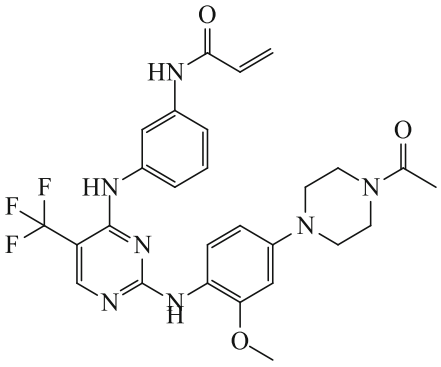
Second-era human IG1 EGFR monoclonal counter acting agent (Necitumumab). It is also known as IMC-11F8, recombinant IgG1 anti-EGFR immune response mab. It prevents activation of the EGFR receptor and downstream signaling by inhibiting the activity of EGFR. And the activation of EGFR and MAP kinases is prevented by blocking the domain III binding site of EGFR (Thakur and Wozniak 2017; Greillier et al. 2015). No procured resistance data has been accounted for yet. It is preferred to

give a mixture of gemcitabine and cisplatin chemotherapy as the first-line agent for patients with advanced squamous NSCLC (Thatcher et al. 2015; Li et al. 2008).

As compared to cetuximab, it showed few hypersensitivity responses because of its fully humanized nature. It showed a high affinity for EGFR in A431 epidermoid cell lines in human tumor xenografts. It also showed inhibitory activity against the MAPK pathway as well as EGFR phosphorylation by binding with EGFR in various cancer cell lines. It also demonstrated that the ligand-binding site was blocked through the attachment of the drug to the domain III of EGFR. Due to which it stops dimerization and ultimately inhibits downstream signaling of the EGFR receptor (Thakur and Wozniak 2017; Pillai and Ramalingam 2014).

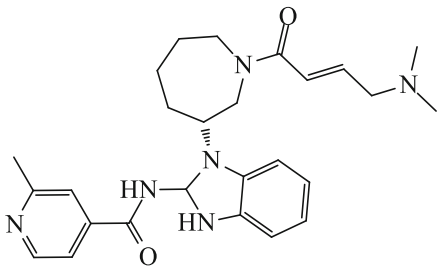
7.2.4.2.5 Others

Rociletinib



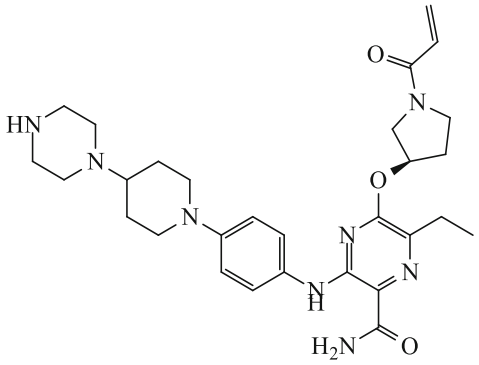
Chemical name: *N*-(3-((2-((4-(4-acetylpiperazin-1-yl)-2-methoxyphenyl)amino)-5-(trifluoromethyl)pyrimidin-4-yl)amino)phenyl)acrylamide. It is an advanced EGFR inhibitor which is administered orally and is an irreversible mutant selective blocker of generally changed types of surface cell receptor (exon 19 deletions, L858R, and T790M). Drug before clinical examination exhibited that it has irrelevant action towards wild-sort EGFR. Shrinkage of tumor cells shrinks observed in xenograft and transgenic models of NSCLC with EGFR. During the clinical trial stage, I/II study, the drug was administered to the patient who had to develop resistance against the first and second eras of EGFR inhibitors (Tran and Klempner 2016). During the II stage of clinical trials, the objective response rate (ORR) was 59% in a patient with positive NSCLC and ORR was 29% in a patient with negative NSCLC disease. Thus, it showed that the drug showed activity in a patient with NSCLC. Phase II trial is going on and the drug is compared with the first-generation drug erlotinib for the activity. In the phase III trial, the drug vs chemo drug was studied (Sequist et al. 2015; Yang et al. 2017).

EGF816 (Nazartinib)



Chemical name: N-(1-((R)-1-((E)-4-(dimethylamino)but-2-enoyl)azepan-3-yl)-2,3-dihydro-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide. It is a third-generation EGFR blocker which has strong blocking ability towards EGFR mutants such as L858R, del19 and T790M. In mouse xenograft models, EGF816 is superior to earlier generation EGFR blockers. The drug profile of targeting proposes that it addresses the option and superior treatment choice against changes observed in T790M. Loose bowels, inflammation in the stomach, rash, and itching sensation are the most well-known side effects. It is in phase III of a clinical trial with erlotinib or gefitinib in patients with locally advanced or metastatic NSCLC with EGFR activating mutations (L858R or ex19 del). Nazartinib overcomes the acquired resistance of T790M (Jia et al. 2016; Kasibhatla et al. 2014).

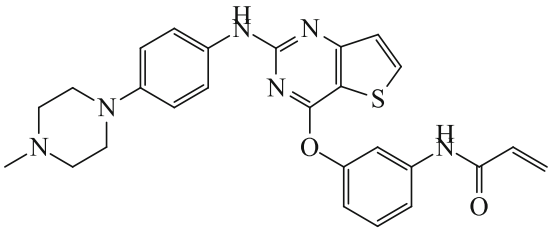
ASP8273



This is another drug entity that is an irreversible TKI blocker that especially inhibits EGFR with activating mutations. During the in vitro enzymatic study and cell-based tests, a drug was assessed across EGFR mutants (L858R, exon 19 del, L858R/T790M, and del19/T790M) and WT EGFR. It was identified that it covalently attaches to a mutant EGFR (L858R/T790M) in the kinase domain of EGFR (Sakagami et al. 2014; Konagai et al. 2015). The drug demonstrated activity against EGFR-mutated cell lines which are resistant to other EGFR TKIs such as AZD9291

and CO-1686. The most widely recognized side effects are GI poisonous quality and blood disorder (Goto et al. 2015; Yu et al. 2015). The phase III clinical study of drug vs erlotinib or gefitinib was carried out in patients and in advanced stage of IIIB/IV of NSCLC which recommended discontinuation of study due to toxicity and limited predicted efficacy drug vs other EGFR inhibitor (ClinicalTrials.gov 2020a).

HM61713 (Olmutinib)



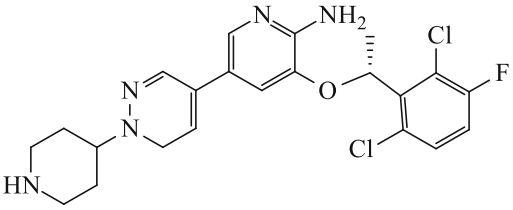
Chemical name: N-(3-((2-((4-(4-methylpiperazin-1-yl)phenyl)amino)thieno[3,2-d] pyrimidin-4-yl)oxy)phenyl)acrylamide. It is an irreversible, third-generation kinase inhibitor and covalently binds to a cysteine residue close to the kinase domain of mutant EGFR. Drug increases clinical benefits over other earlier generation EGFR TKI due to inhibitory activity activated mutations (e.g., 19 Del and L858R) and overcoming of T790 M mutation resistance, with good selectivity over wild type EGFR. Currently, it is in the clinical phase of testing for NSCLC in various countries but it was approved in South Korea for the management of patients having advanced or metastatic EGFR T790 mutation positive NSCLC which was previously treated with EGFR-TKI (Kim 2016). The shelf life of the drug is more than 24 h for the hindrance of EFGR. It causes hindrance in cell lines H1975 (L858R and T790M) and HCC827 (ex 19 Del). In vivo investigations of the drug on xenograft models with unions of H1975 and HCC827 showed dynamic action toward cancer, that doesn't show adverse symptoms. The ongoing clinical trial study stage I/II investigation of the drug in an intimate suffering NSLC in their later stage doesn't give a positive result in past EGFR TKIs. It shows favorable treatment for an intimate with T790M positive NSCLC (Lee et al. 2014; Park et al. 2020).

7.2.4.3 Drugs Targeting ALK Receptor

About 5% of NSCLC have a rearrangement in the gene called *ALK*. These changes were observed in nonsmokers who are younger and who have the adenocarcinoma subtype of NSCLC. Changes in the ALK gene produces an abnormal ALK protein causing uncontrolled cell growth and proliferation. The echinoderm microtubule-associated protein like 4 (EML-4) gene was found to be fused with ALK, which resulted in mutant fusion gene EML-4-ALK that initiates tumorigenesis in patients with NSCLC (Chidambaram and Adjei 2015).

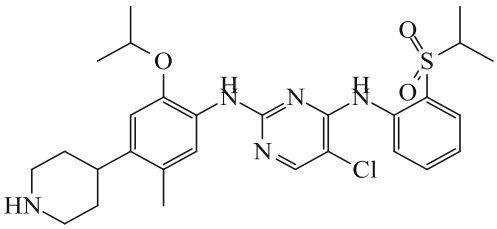
The ALK changes are noncovering with other oncogenic transformations related to NSCLC, for example, EGFR or RAS. Other transformations of ALK that do not include EML-4 have been identified with KIF5B-ALK and TFG-ALK. Regarding therapy, an intimate having EML4-ALK combinations or modifications on ALK cells don't gain benefit from EGFR-particular tyrosine kinase inhibitor treatment (Wu et al. 2016; Sullivan and Planchard 2016). The drugs which are used for the treatment by inhibiting ALK protein are crizotinib, ceritinib, alectinib, and brigatinib.

7.2.4.3.1 Crizotinib (2011)



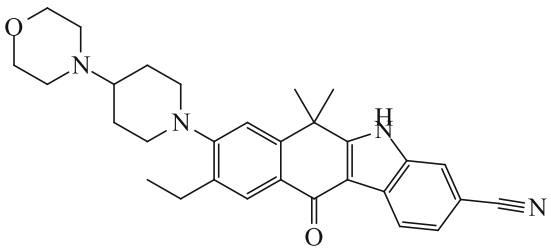
Chemical name: (*R*)-3-[1-(2,6-Dichloro-3-fluorophenyl) ethoxy]-5-[1-(piperi- din-4-yl)-1*H*-pyrazol-4-yl]pyridin-2-amine. It is an oral, tyrosine kinase inhibitor targeting ALK, MET, and ROS1 tyrosine kinases. The ALK is a receptor tyrosine kinase typically expressed in discrete regions of the nervous system. Resistance can be seen when changes in the TK domain diminishes the activity of this. The mutations can initiate another signaling pathway, in this way taking out the signal section through the stage initially repressed by the drug. According to ACS, the drug is utilized as a first-line treatment of ALK-rearranges at stage IV of NSCLC. There are reports of many side effects and not mentioned conductively during trials. These include erythema multiforme, intense interstitial lung disease, renal polycytosis, contact esophagitis, diminish GFR, and hypersensitivity responses (Kuribayashi and Tabata 2016).

7.2.4.3.2 Ceritinib (2014)



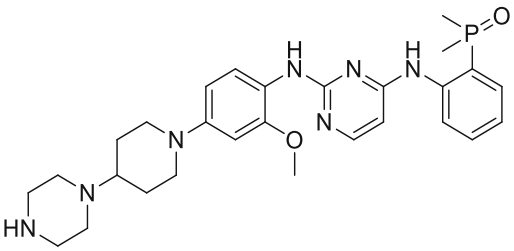
Chemical name: 5-Chloro-*N*-4- [2-[(1 methylethyl) sulfonyl] phenyl] –*N*-2- [5-methyl- 2-(1-methylethoxy)- 4-(4 piperidinyl) phenyl] -2,4- pyrimidinediamine. It is a second-generation ALK inhibitor that was allowed for quick endorsement by FDA for a patient having ALK mutation-positive metastatic NSCLC. It is an ATP-competitive TKI of ALK which inhibits the insulin receptor and insulin-like growth factor-1 (IGF-1) receptor. This drug is more potent than crizotinib against ALK but not towards MET. It is used as a second-line treatment for the patient who is crizotinib intolerant and for ALK-rearranged at stage IV of NSCLC. This drug can overcome crizotinib-resistant mutations (Rothschild 2014). The most widely recognized dosage related unfavorable impacts were loose bowels, spewing, sickness, drying out, lifted alanine aminotransferase, and hypophosphatemia. It is a noteworthy substrate and solid inhibitor of CYP3A4 with a high potential for drug relations; it is likewise a direct inhibitor of CYP2C9 (Shaw et al. 2014).

7.2.4.3.3 Alectinib (2015)



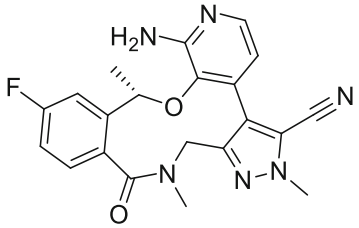
Chemical name: 9-ethyl-6, 6-dimethyl-8-[4-(morpholin-4-yl)piperidin-1-yl]-11-oxo-6,11-dihydro-5H-benzo[b]carbazole-3-carbonitrile. This is a second-generation and selective inhibitor of ALK and RET. It can cause apoptosis by inhibiting ALK-induced tumor regression. It is also effective against EML4-ALK tumors which is the fusion of EML4 and ALK in NSCLC. Resistance against alectinib is not reported yet but treatment with heat shock protein 90 (Hsp 90) inhibitors. It is used as first- or second-line treatment for curing a patient having ALK-positive or metastatic NSCLC and where crizotinib has failed. Few cases of neutropenia and cerebral pain were observed with this drug. Others are raised in creatinine phosphokinase, dyspnea, and stomach torment. It is a minor substrate of CYP3A4, with negligible to-direct potential for associations (Shaw et al. 2016a). It is under clinical trial II with cobimetinib for the treatment of patients with ALK-rearranged advanced NSCLC (ClinicalTrials.gov 2020b).

7.2.4.3.4 Brigatinib (2017)



Chemical name: (2-((2-((2-methoxy-4-(4-(piperazine-1-yl)piperidine-1yl)phenyl) amino) pyrimidin-4-yl)amino)phenyl)dimethylphosphine oxide. It is a second-generation ALK inhibitor. The drug inhibits the two types of receptors ALK receptor and EGFR receptor. It is a TKI having in vitro action toward numerous kinases including ALK, ROS1, and IGF-1. It also inhibits ALK phosphorylation and activation of the downstream signaling pathway. The FDA titled this drug an orphan drug. The drug was better than osimertinib, the EGFR inhibitor if they are combined with anti-EFGR antibodies like cetuximab or panitumumab. It is more potent than crizotinib and ceritinib. It is used as a second-line treatment for patients with ELK4-ALK-rearranged NSCLC. Resistance to the brigatinib is due to the G1202R mutations. Side effects reported with the drug are an increase in blood pressure, cardiac arrhythmia, visual side effects, and other vital unfriendly responses such as pneumonitis, pancreatitis, and rhabdomyolysis (McCall and Lu 2017; brigatinib resistance.pdf n.d.).

7.2.4.3.5 Lorlatinib (2018)



Chemical name: (*R*)-1-amino-13-fluoro-6,9,15-trimethyl-10-oxo-8,9,10,15-tetrahydro-6H-benzo[c]pyrazolo[3,4-h]pyrido[4,3-j][1,6]oxaazacycloundecine-5-carbonitrile. It is a third generation inhibitor receptor tyrosine kinase, ALK and C-ros oncogene-1 (ROS 1). It is a unique drug and successful in all known safe mutants. In investigations during the preclinical phase, it showed activity in a crizotinib-restrain tumor in vitro and xenograft models. To beat ALK changes and ALK blocker protection, lorlatinib joined as a PI3K pathway blocker, like others gedatolisib/PF-05212384 (PI3K/mTOR), pictilisib/GDC0941 (pan PI3K), and taselisib/GDC0032 (beta-sparing PI3K). It is a TKI specially designed to overcome TKI resistant

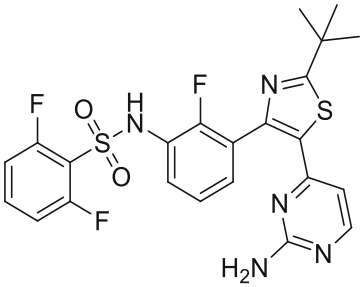
mutations and penetrate the blood–brain barrier. It is used as a second- or third-line treatment for patients with ALK-positive metastatic NSCLC (Shaw et al. 2016b; Wangari-Talbot and Hopper-Borge 2012).

7.2.4.4 Drugs Targeting BRAF Receptor

BRAF is a proto-oncogene, which plays a crucial role in both normal and cancer cells. This gene leads to the production of BRAF protein, a controlled signal transduction serine/threonine protein kinase that is involved in cell multiplication and survival. In all NSCLC 1–4% BRAF, physical changes have been found, most regularly in individuals suffering from adenocarcinomas. These transformations have been linked with past/present smokers. Mutated BRAF causes activation of the MAPK pathway that stimulates cell survival and proliferation. In a few cases, it is reported that BRAF mutation is one of the resistant to EGFR-TKI. G469A and D594G BRAF are also there but $BREF^{V600E}$ mutation is the most commonly observed. An extraordinary greater part of BRAF transformations observed the nonoverlapping with other changes in oncogenic of NSCLC (EGFR changes, ALK adjustments, and so forth. In addition to EGFR, ALK and ROS1, BRAF was found to be the therapeutic target for the treatment of advanced NSCLC (Sánchez-Torres et al. 2013; Baik et al. 2017).

The drugs which act as an inhibitor of BRAF cells are such as dabrafenib, trametinib, vemurafenib, and selumetinib which are described as follows.

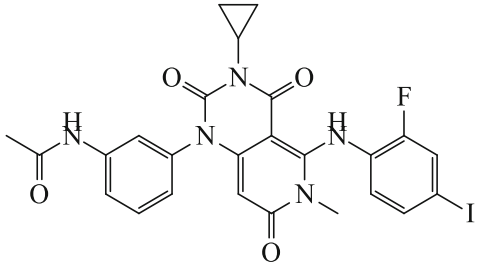
7.2.4.4.1 Dabrafenib (2013)



Chemical name: *N*-(3-(5-(2-aminopyrimidin-4-yl)-2-(tert-butyl)thiazol-4-yl)-2-fluorophenyl -2,6-difluorobenzenesulfonamide. The drug acts as a blocker of the enzyme-linked to BRAF which participate in controlling the growth of the cells. It is used for curing the tumor linked to the version of the gene BRAF which is mutated. The clinical activity shows the safety effects of the drug during stages I and II of clinical trials with the BRAF-mutated metastatic cancer. Initially, a drug was approved alone by FDA, but the clinical trial data do not show any significant effect and developed a resistance to the drug. The combination of the drug with trametinib (MEK inhibitor) which overcomes the resistance developed by the drug alone and

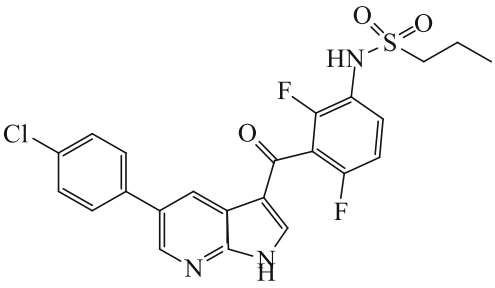
showed a good outcome. Hence, this combination is indicated for the treatment of patients with metastatic NSCLC with patients suffering from NSCLC having mutation of BRAF ^{V600E} mutations (Khunger et al. 2018). The drug does not show a promising effect for EGFR mutation positive or ALK TKI for ALK-rearranged NSCLC. But the combination treatment is the best because it gave a great result and the disease has not increased and the life-threatening squamous cell tumor has decreased when compared with the drug alone (Kawamura and Murakami 2016).

7.2.4.4.2 Trametinib (2017)



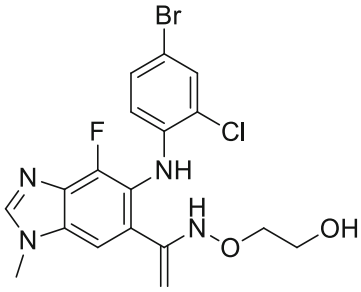
Chemical name: *N*-(3-(3-cyclopropyl-5-((2-fluoro-4-iodophenyl)amino)-6-methyl-2,4,7-trioxo-3,4,6,7-tetrahydropyrido[4,3-d]pyrimidin-1(2H)-yl)phenyl)acetamide. It is MEK inhibitor which showed antineoplastic activity by targeting MEK-1 and MEK-2 protein kinase. The drug showed better results for the treatment of cancer with BRAF V600E mutation in stage III clinical trials studies. In the xenograft cancer model, blocking of ERK phosphorylation and suppression of Ki67 was observed with the drug. Due to this, the spreading of cancer cells is reduced and leads to the arrest of the cell cycle ultimately causing cell death. The drug is administered alone through oral as well as intravenous routes. The drug is given in the combination with other BRAF agents such as dabrafenib for the treatment of metastatic NSCLC with BRAF positive mutation (Khunger et al. 2018; Lugowska et al. 2015).

7.2.4.4.3 Vemurafenib (2011)



Chemical name: *N*-(3-(5-(4-chlorophenyl)-1H-pyrrolo[2,3-b]pyridine-3-carbonyl)-2,4-difluorophenyl)propane-1-sulfonamide. It exhibited activity against BRAF kinase with V600E mutations. It exerts action by binding with the ATP domain of mutant BRAF. The drug showed a mechanism causing programmed cell mortality in the cancerous cell line. This drug alone is effective alone for BRAF ^{V600} mutated NSCLC but not for BRAF ^{V600} nonmutated NSCLC. When a combination treatment of dabrafenib and trametinib is not feasible for BRAF ^{V600} mutated, then vemurafenib monotherapy is used. Resistance develops when the cell surface protein increases, stromal cell secretion of hepatocyte growth factor (Rolfo and Caparica 2016).

7.2.4.4.4 Selumetinib (AZD6244)



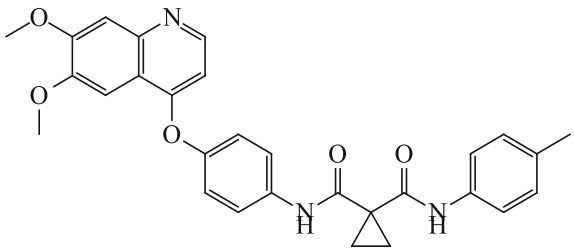
Chemical name: 2-(((1-(5-((4-bromo-2-chlorophenyl)amino)-4-fluoro-1-methyl-1*H*-benzo[d] imidazol-6-yl)vinyl)amino)oxy)ethanol. The drug activity on MAPK/ERK pathway is decreased which is inhibited through the MAP kinase enzyme. It inhibits another type of enzyme MEK1 and MEK2 enzyme. During the clinical phase II study, the drug plus docetaxel showed good efficacy than docetaxel alone in previously treated patients with advanced KRAS-mutant NSCLC. But in the case of clinical phase III of the same combination, the study failed because no significant effect was observed in KRAS mutant NSCLC (Bernabé et al. 2016; Casaluce et al. 2017).

7.2.4.5 Drugs Targeting MET Receptor

MET is considered the second largest family of membrane receptors. It is considered a potential target in various types of cancer including NSCLC. MET is a proto-oncogene located on chromosome 7, encodes a receptor tyrosine kinase which shows multiple mechanisms for activation of cells including autocrine and paracrine stimulatory mechanism, protein expression, somatic mutation or gene amplification (Rehman and Dy 2019). The MET/HGF signaling pathway plays a crucial role in cell survival, cell proliferation, migration, and tumor invasion. Targeting MET has been shown to re-sensitize tumors resistant to EGFR TKIs in preclinical models. The

investigation is going on MET inhibitors in combination with other tyrosine kinase inhibitors. For example, MET inhibitors such as savolitinib, cabozantinib, and capmatinib are in phase II clinical studies of NSCLC. Tivantinib plus erlotinib in phase II and foretinib plus erlotinib in clinical phase I of NSCLC. Crizotinib is approved as an ALK inhibitor and it also inhibits MET, ALK, and ROS1. Tepotinib is under phase II of clinical study with MET-altered NSCLC (Janku et al. 2010; Wang et al. 2019).

7.2.4.5.1 Cabozantinib



Chemical name: *N*-(4-((6,7-dimethoxyquinolin-4-yl)oxy)phenyl)-N-(p-tolyl)cyclopropane-1,1-dicarboxamide. The drug is a multiple inhibitor of TKI which involves MET, RET, and VEGF receptors. It is titled an orphan drug and endorsed for curing thyroid cancer, and treatment of renal cancer after treatment with antiangiogenic therapy. This drug is in market from 2011 used in NSCLC patients with MET exon 14 skipping mutation and MET amplification pretreated or not MET inhibitors. The drug is combined with gefitinib which doesn't show any beneficial effect on sensitive NSCLC cell lines. The addition of a drug to erlotinib showed a better anticancer effect than a drug administered to block the EGFR site (Wakelee et al. 2017; Drilon et al. 2016).

7.2.4.6 Drug Targeting HER2

HER2 is a member of the ERBB family of tyrosine kinase receptors. Activation of HER2 occurred through homodimerization or heterodimerization with other receptors of the ERBB family. HER2 gene was found to be important in various types of cancers. In NSCLC, approximately 4% has been found to cause HER2 mutations. A combination of different drugs has been tried to treat NSCLC. Drugs such trastuzumab, pertuzumab, lapatinib, Afatinib, dacomitinib, and trastuzumab emtansine (T-DM1) (Garrido-Castro and Felip 2013).

Trastuzumab: It is humanized monoclonal antibody which blocks the HER2 receptor. It is approved for advanced breast cancer where overexpression of HER2 is observed. In 20% of NSCLC, overexpression of HER2 is reported. Initially, the drug

was in combination with docetaxel in the case of advanced NSCLC, where platinum therapy got failed, but limited clinical activity was observed (Lara et al. 2004).

Pertuzumab: It is a monoclonal antibody that obstructs the dimerization of HER2. This drug was in phase II of a clinical trial with erlotinib in relapsed NSCLC but clinical applicability was limited (Hughes et al. 2014).

Lapatinib: It is a reversible and dual kinase inhibitor due to activity against both kinases (EGFR and HER2). It reduces downstream extracellular signaling and AKT signaling also. It was in the second phase of clinical testing as first- or second-line treatment in patients with metastatic or advanced NSCLC. But it did not show beneficial clinical activity (Ross et al. 2010).

Trastuzumab emtansine: It is also known as T-DM1 is a conjugate of monoclonal antibody trastuzumab with highly potent chemo drug DM1. The drug blocks the HER2 receptor. It is the first targeted therapy for the management of breast cancer. The drug is even used for curing cancer of the stomach and NSCLC. The drug shows a good survival rate when compared with other drugs like lapatinib and capecitabine in an individual at the HER2 receptor site. The drug was alone in phase II of clinical studies with HER2 positive in advanced or metastatic NSCLC. Various case reports and ongoing studies have reported that the drug is capable of targeting HER2 mutations or amplification (Peters and Zimmermann 2014; Peddi and Hurvitz 2013).

Afatinib: This drug is the first-line treatment option for patients with advanced NSCLC with EGFR mutations. It was in clinical trial II for previously treated patients having advanced NSCLC with HER2 mutations. But the drug did not cope with the desired potential for disease control (Ding et al. 2016).

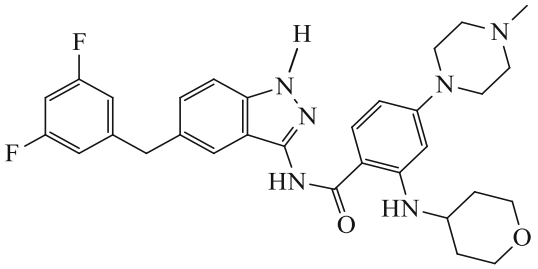
Dacomitinib: It is a small molecule that is extremely critically ATP competitive, highly selective and potent EGFR-TKI. It irreversibly blocks receptors of TKI such as HER-1, HER-4, and HER-2 receptors of TKIs. The phase I and II studies of the drug suggested that it has activity against HER2-mutated NSCLC (Mok et al. 2014).

7.2.4.7 Drugs Targeting ROS-1 Receptor

ROS-1 is a receptor tyrosine kinase of the insulin receptor family. The rearrangement of the chromosome is the primary mechanism observed in lung cancer and other cancers also, even in non-squamous lung cancer. Its fusion shows downstream signaling by activating the cellular pathway which is involved in cell growth and proliferation. Fifty percent sequence homology is observed between ROS-1 and ALK receptors. Genomic alterations are observed in NSCLC patients and studies show ROS-1 translocation in approximately 1–2% of patients with NSCLC.

Crizotinib showed clinical benefits for patients suffering from advanced NSCLC with ROS-1 rearrangement. It is the first targeted monotherapy for ROS-1 positive tumors (Bebb et al. 2019; Joshi et al. 2019). Other drugs which act on this receptor are cabazontinib, ceritinib, lorlatinib, and entrectinib.

7.2.4.7.1 Entrectinib (2019)

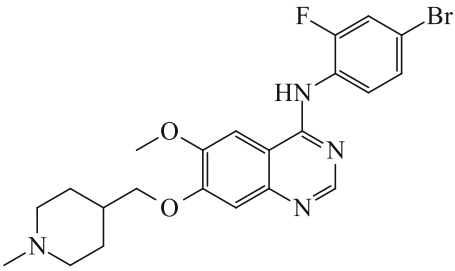


Chemical name: N-(5-(3,5-difluorobenzyl)-1H-indazol-3-yl)-4-(4-methylpiperazin-1-yl)-2-((tetrahydro-2H-pyran-4-yl)amino)benzamide. It is strong inhibitor of tropomyosin receptor kinase (TRK) family, i.e., TRKA, TRKB, TRKC, and also blocks ROS-1 and ALK. The drug showed in-vitro and in-vivo antitumor activity against TRK, ROS-1, and ALK xenograft models for different types of cancer. FDA approved drug for the treatment of patients having metastatic NSCLC with ROS-1 positive tumors. This drug also displayed good clinical responses in patients with advanced or metastatic NSCLC whose tumors are NTRK-fusion positive. The most serious adverse reactions to the drug are hepatotoxicity, hyperuricemia, congestive heart failure, skeletal fractures, and CNS effects (Bubendorf et al. 2016; Facchinetti and Friboulet 2019).

7.2.4.8 Drugs Targeting RET Receptor

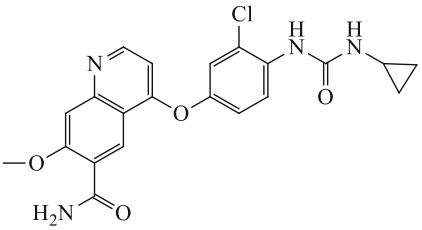
The RET is a proto-oncogene, considered a therapeutic target in NSCLC. RET receptor tyrosine kinase is 170 kDa which is composed of the intracellular, extracellular kinase domain and a transmembrane region. The pathway gets activated to result in auto-phosphorylation on intracellular tyrosine residue that begins Ras/Map kinase, PI3K/AKT, and phospholipase C pathway that signals cell proliferation, migration, and differentiation. The studies showed that RET helps in the development of an enteric nervous system, kidney morphogenesis and spermatogenesis. In NSCLC, it is coupled with eight different types of genres such as KIF5B, CCDC6, NCOA, TRIMM33, COX1, KIAA1468, KIAA1217, and FRMD4A. KIF5B is normally fused with RET in NSCLC. A patient with RET-positive cancer is observed with molecular and genetic changes and is detected by clinico-pathologic characteristics. RET is observed in 1–2% of lung adenocarcinoma and one who does not smoke. The RET inhibitors which are in their clinical phase studies are cabozantinib, alectinib, vandetanib, and lenvatinib (Chao et al. 2019).

7.2.4.8.1 Vandetanib



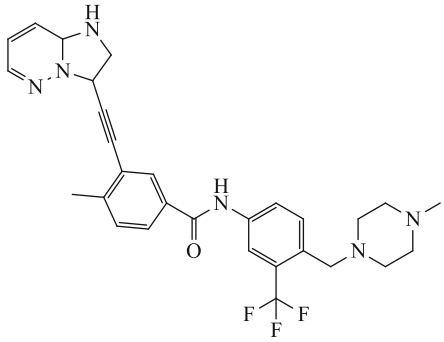
Chemical name: *N*-(4-bromo-2-fluorophenyl)-6-methoxy-7-((1-methylpiperidin-4-yl) methoxyquinazolin-4-amine. The drug is selective towards tyrosine kinases especially EGFR, VEGFR-2 and RET. It is evaluated for clinical safety and efficacy and is administered through an oral route in a patient with advanced RET rearranged NSCLC. The third phase of the clinical trial showed that the drug is moderately active in pretreated patients with advanced NSCLC having RET rearrangements. The drug demonstrated clinical antitumor activity and controllable safety in treating NSCLC patients (Yoh et al. 2017).

7.2.4.8.2 Lenvatinib



Chemical name: 4-(3-chloro-4-(3-cyclopropylureido)phenoxy)-7-methoxyquinoline-6-carboxamide. It is multitargeted as it inhibits VEGFR, FGFR, RET, and other tyrosine receptors. In the second phase of the clinical trial, the drug showed activity in patients suffering from RET-fusion positive lung adenocarcinoma. But ORR was observed to be very less. The side effects that the drug showed during a clinical trial in the treatment of NSCLC are hypertension, nausea, vomiting, reduced hunger, looseness of the bowels, and albuminuria (Hida et al. 2019).

7.2.4.8.3 Ponatinib



Chemical name: 4-methyl-*N*-(4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl-3-((1,2,3,8a-tetrahydroimidazo[1,2-b]pyridazin-3-yl)ethynyl)benzamide. It is a multi-TKI. Phase II clinical testing of the drug has been effective in patients with advanced RET-rearranged NSCLC (Tan et al. 2019).

7.2.4.9 Drugs Targeting NTRK1 Receptor

Neurotrophin receptor kinase 1 (NTRK1) is a member of the tyrosine kinase receptor. The genes are located on chromosome 1q21-22 and encode high-affinity nerve growth factor receptors. About 3.3% of cases of lung adenocarcinoma are found. On the NTRK1 receptor, the two fusions have been described: MPRIP-NTRK1 and CO74-NTRK1. The fusion study in the clinical trial shows that TRKA auto-phosphorylation leads to the oncogene process (Morgensztern et al. 2015). NTRK1 fusion is rare in lung adenocarcinoma but not observed in squamous cell carcinoma. NTRK1 rearrangement is identified by fluorescence in situ hybridization technique. The technique does not identify particular NTRK1 fusion. The drugs used for the treatment of NTRK1 fusion in NSCLC are larotretinib, entrectinib, and cabozantinib (Pao and Girard 2011; Vaishnavi et al. 2013).

7.2.4.10 Drugs Targeting PIK3CA

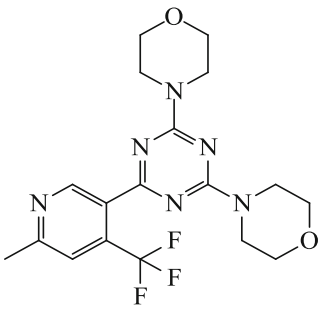
Phosphatidylinositol-3-kinase plays a crucial role in cell proliferation and metabolism. PI3K/mTOR/AKT is regularly activated pathway in NSCLC. The PIK3CA mutation is the main oncogenic driver for tumor formation in NSCLC. This type of mutation is observed in 1–3% of an individual having NSCLC. The alterations of genes are observed in exon 9 and exon 20. The alteration of genes is more commonly found in the squamous cell than in adenocarcinoma. PIK3CA mutation occurs

simultaneously with EGFR mutation. The drugs that act as a PIK3CA blocker are LY3023414 and PQR309.

7.2.4.10.1 LY3023414

It is a dual inhibitor as it blocks both PI3K and mTOR. The phase II clinical study of the drug in combination with necitumumab (monoclonal antibody) demonstrated that the combination of both was found to be nontoxic and acceptable in patients with squamous NSCLC.

7.2.4.10.2 PQR309 (Bimiralisib)



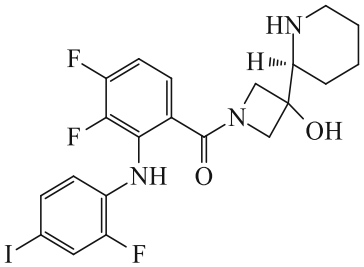
Chemical name: 4,4′-(6-(6-methyl-4-(trifluoromethyl)pyridin-3-yl)-1,3,5-triazine-2,4-diyl) dimorpholine. It is a dual inhibitor (PI3K/mTOR). It is in phase II of clinical testing in patents with squamous NSCLC (Scheffler et al. 2015).

7.2.4.11 Drugs Targeting MEK-1 Receptor

It is also known as MAPK2-1. It follows the MAPK/ERK pathway, which is involved in the regulation of various cellular processes. It is a serine-threonine kinase that shows a mutation in 1% of NSCLC. The drugs used for the treatment of MAP2K1 mutation are selumetinib, trametinib, and cobimetinib. Several clinical studies of these MEK inhibitors are in process.

Various clinical data suggested that the MEK inhibitors should be developed in combination with chemotherapy rather than as a single agent (Casaluce et al. 2017; Stinchcombe and Johnson 2014).

7.2.4.11.1 Cobimetinib



Chemical name: (*S*)-(3,4-difluoro-2-((2-fluoro-4-iodophenyl)amino)phenyl) (3-hydroxy-3-(piperidin-2-yl)azetidin-1-yl)methanone. It is a highly selective molecule which blocks MEK-1. It was approved in combination with vemurafenib (BRAF inhibitor) for the treatment of patients with metastatic melanoma with BRAF V600 mutations. Currently, it is under clinical trial II with atezolizumab for the treatment of patients with metastatic NSCLC (Rothschild 2015; Arcila et al. 2016).

7.3 Conclusion

The therapeutic area of NSCLC treatment has tremendously changed after the discovery of EGFR mutation and the development of EGFR TKI. Even though the discovery of EGFR TKI for treating NSCLC, other therapies are still growing in the field of genomic aberrations as potential biomarkers. The genotype derived treatment showed a 3-year survival rate. Even though, treatment in a highly selected patient during the last decades demonstrated the advancement in molecular understanding and novel treatment has essentially enhanced the result for the subgroup of NSCLC patients.

NSCLC is a disease that cannot be cured. Currently able to recognize the mechanism of amidoquinolines at the molecular level is an advantage, usage of high-throughput advances including upcoming generation sequences in clinical and disclosure of targeted therapeutics have enhanced the prediction and personal satisfaction for patients in the group with advanced NSCLC.

Currently, the rules prescribe investigation of the accompanying seven genes for the treatment of lung adenocarcinoma: KRAS, EGFR, ALK, ROS-1, BRAF, RET, and HER-2. Other genes like NTRK1, MAP2K1, and PIK3CA also play an important role in the treatment of NSCLC. EGFR and ALK therapies are easily available and are used for the treatment of advanced NSCLC nowadays. The drugs which target the specific receptor are combined with other chemo drugs and improve the safety and efficacy of the drug which shows a synergistic effect for the treatment of NSCLC. But due to the development of resistance, the combination of chemotherapy

and targeted therapy became a challenge in curing NSCLC. But the futuristic approach of a combination of either targeted therapy or immunotherapy with better knowledge of biomarkers could lead to the best option for curing NSCLC. Thus, to improve the tolerability of these approaches, investigation are going on.

References

American Cancer Society (2017) Key statistics for lung cancer. American Cancer Society, p 1–3

Arcila ME, Drilon A, Sylvester BE, Lovly CM, Borsu L, Reva B, Kris MG, Solit DB, Ladanyi M (2016) MAP2K1 (MEK1) mutations define a distinct subset of lung adenocarcinoma associated with smoking. Clin Cancer Res 21(8):1935–1943

Baik CS, Myall NJ, Wakelee HA (2017) Targeting *BRAF* -mutant non-small cell lung cancer: from molecular profiling to rationally designed therapy. Oncologist 22(7):786–796

Bar J, Goss GD (2012) Tumor vasculature as a therapeutic target in non-small cell lung cancer. J Thorac Oncol 7(3):609–620

Barzi A, Pennell NA (2010) Targeting angiogenesis in non-small cell lung cancer: agents in practice and clinical development article. Eur J Clin Med Oncol 2(1):31–42

Bebb DG, Agulnik J, Albadine R, Banerji S, Bigras G, Butts C et al (2019) Crizotinib inhibition of ROS1-positive tumours in advanced non-small-cell lung cancer: a Canadian perspective. Curr Oncol 26(4):e551–e557

Bernabé R, Patrao A, Carter L, Blackhall F, Dean E (2016) Selumetinib in the treatment of non-small-cell lung cancer. Future Oncol 12:2545

Bin WY, Hu Y, Li Z, Wang P, Xue YX, Yao YL et al (2013) Artemether combined with shRNA interference of vascular cell adhesion molecule-1 significantly inhibited the malignant biological behavior of human glioma cells. PLoS One 8(4):1–12

Bissett D, O'Byrne KJ, Von Pawel J, Gatzemeier U, Price A, Nicolson M et al (2005) Phase III study of matrix metalloproteinase inhibitor prinomastat in non-small-cell lung cancer. J Clin Oncol 23(4):842–849

Bonomi P (1986) Brief overview of combination chemotherapy in non-small-cell lung cancer. Semin Oncol 13(3)

Brenner DR, McLaughlin JR, Hung RJ (2011) Previous lung diseases and lung cancer risk: a systematic review and meta-analysis. PLoS One 6(3):e17479

brigatinib resistance.pdf

Bubendorf L, Büttner R, Al-Dayel F, Dietel M, Elmberger G, Kerr K et al (2016) Testing for ROS1 in non-small cell lung cancer: a review with recommendations. Virchows Arch 469(5):489–503

Camidge DR, Berge EM, Doebele RC, Ballas MS, Jahan T, Haigentz M et al (2014) A phase II, open-label study of ramucirumab in combination with paclitaxel and carboplatin as first-line therapy in patients with stage IIIB/IV non-small-cell lung cancer. J Thorac Oncol 9(10): 1532–1539

Casaluce F, Sgambato A, Maione P, Sacco PC, Santabarbara G, Gridelli C (2017) Selumetinib for the treatment of non-small cell lung cancer. Expert Opin Investig Drugs 26(8):973–984

Chan BA, Hughes BGM (2015) Targeted therapy for non-small cell lung cancer: current standards and the promise of the future. Transl Lung Cancer Res 4(1):36–54

Chao BH, Ohio T, Cancer GJ, Solove RJ, Briesewitz R, James AG et al (2019) RET fusion genes in non-small-cell. Lung Cancer 30(35):4439–4441

Chidambaram A, Adjei AA (2015) Second-generation ALK inhibitors for therapy of ALK-rearranged non-small cell lung cancer. J Target Ther Cancer 4:1–14

A study of ASP8273 vs. erlotinib or gefitinib in first-line treatment of patients with stage IIIB/IV non-small cell lung cancer tumors with EGFR activating mutations—Full Text View—ClinicalTrials.gov [Internet]. [cited 2020a Jun 1]. https://clinicaltrials.gov/ct2/show/NCT02588261

A phase IB/II study of alectinib combined with cobimetinib in advanced ALK-rearranged (ALK+) NSCLC—Full Text View—ClinicalTrials.gov [Internet]. [cited 2020b Jun 1]. https://clinicaltrials.gov/ct2/show/NCT03202940

Cortés ÁA, Urquizu LC, Cubero JH (2015) Adjuvant chemotherapy in non-small cell lung cancer: state-of-the-art. Transl Lung Cancer Res 4(2):191–197

Cross DAE, Ashton SE, Ghiorghiu S, Eberlein C, Nebhan CA, Spitzler PJ et al (2014) AZD9291, an irreversible EGFR TKI, overcomes T790M-mediated resistance to EGFR inhibitors in lung cancer. Cancer Discov 4(9):1046–1061

Darby S, Hill D, Auvinen A, Barros-Dios JM, Baysson H, Bochicchio F et al (2005) Radon in homes and risk of lung cancer: collaborative analysis of individual data from 13 European case-control studies. Br Med J 330(7485):223–226

Das M, Wakelee H (2014) Angiogenesis and lung cancer: ramucirumab prolongs survival in 2(nd)-line metastatic NSCLC. Transl Lung Cancer Res 3(6):397–399

David H, Johnson M (2000) Evolution of cisplatin-based chemotherapy in non-small cell lung cancer* A historical perspective and the Eastern Cooperative Oncology Group experience. Chest 117(4 Suppl. 1):138S–143S

Davis PD, Dougherty GJ, Blakey DC, Galbraith SM, Tozer GM, Holder AL et al (2002) ZD6126: A novel vascular-targeting agent that causes selective destruction of tumor vasculature. Cancer Res 62(24):7247–7253

Dela Cruz CS, Tanoue LT, Richard AM (2008) [The necessity for opening a specific clinical pathology from the aspect of advancement made in nervous pathology in China]. Zhonghua Bing Li Xue Za Zhi 37(12):861–862

Di Costanzo F, Mazzoni F, Mela MM, Antonuzzo L, Checcacci D, Saggese M et al (2008) Bevacizumab in non-small cell lung cancer. Drugs 68(6):737–746

Ding L, Li QJ, You KY, Jiang ZM, Yao HR (2016) The use of Apatinib in treating nonsmall-cell lung cancer. Medicine (Baltimore) 95(20):1–4

Drilon A, Rekhtman N, Arcila M, Wang L, Ni A, Albano M et al (2016) Cabozantinib in patients with advanced RET-rearranged non-small-cell lung cancer: an open-label, single-centre, phase 2, single-arm trial. Lancet Oncol 17(12):1653–1660

Einhorn LH (2008) First-line chemotherapy for non-small-cell lung cancer: is there a superior regimen based on histology? J Clin Oncol 26(21):3485–3486

Facchinetti F, Friboulet L (2019) Profile of entrectinib and its potential in the treatment of ROS1-positive NSCLC: evidence to date. Lung Cancer 10:87–94

Faller BA, Pandit TN (2011) Safety and efficacy of vinorelbine in the treatment of non-small cell lung cancer. Clin Med Insights Oncol 5:131–144

Fournel P, Robinet G, Thomas P, Souquet PJ, Léna H, Vergnenégre A et al (2005) Randomized phase III trial of sequential chemoradiotherapy compared with concurrent chemoradiotherapy in locally advanced non-small-cell lung cancer: Groupe Lyon-Saint-Etienne d'Oncologie Thoracique-Groupe Français de Pneumo-Cancérologie NPC 95-01 study. J Clin Oncol 23(25):5910–5917

Garrido-Castro AC, Felip E (2013) HER2 driven non-small cell lung cancer (NSCLC): potential therapeutic approaches. Transl Lung Cancer Res 2(2):122–127

Ghittoni R, Accardi R, Chiocca S, Tommasino M (2015) Role of human papillomaviruses in carcinogenesis. Ecancermedicalscience 9:1–9

Gil-Bazo I, Rolfo C (2016) AZD9291 in TKI EGFR resistance in non-small cell lung cancer and the new concept of phase I trials. Transl Lung Cancer Res 5:85–88

Goebel C, Louden CL, McKenna R, Onugha O, Wachtel A, Long T (2019) Diagnosis of non-small cell lung cancer for early stage asymptomatic patients. Cancer Genomics Proteomics 16(4):229–244

Goffin JR, Anderson IC, Supko JG, Eder JP Jr, Shapiro GI, Lynch TJ, Shipp M, Johnson BE, Skarin AT et al (2005) Phase I trial of the matrix metalloproteinase inhibitor marimastat combined with carboplatin and paclitaxel in patients with advanced non-small cell lung cancer. Clin Cancer Res 11(9):3417–3424

Goto Y, Nokihara H, Murakami H, Shimizu T, Seto T, Krivoshik AP, Keating AT, Uegaki K, Takeda K, Komatsu K, Morita S, Fukuoka M, Nakagawa K (2015) ASP8273, a mutant-selective irreversible EGFR inhibitor in patients (pts) with NSCLC harboring EGFR activating mutations: preliminary results of first-in-human phase I study in Japan. J Clin Oncol, 33(15_suppl):8014

Govindan R (2004) Cetuximab in advanced non-small cell lung cancer. Clin Cancer Res 10(12): 4241s–4244s

Greillier L, Tomasini P, Barlesi F (2015) Necitumumab for non-small cell lung cancer. Expert Opin Biol Ther 15(8):1231–1239

Gubens MA, Wakelee HA (2010) Docetaxel in the treatment of non-small cell lung carcinoma: an update and analysis. Lung Cancer Targets Ther 1:63–76

Hayashi H, Kurata T, Nakagawa K (2011) Gemcitabine: efficacy in the treatment of advanced stage nonsquamous non-small cell lung cancer. Clin Med Insights Oncol 5:177–184

Hida T, Velcheti V, Reckamp KL, Nokihara H, Sachdev P, Kubota T et al (2019) A phase 2 study of lenvatinib in patients with RET fusion-positive lung adenocarcinoma. Lung Cancer 138:124–130

Ho GF, Chai CS, Alip A, Wahid MIA, Abdullah MM, Foo YC, et al (2019) Real-world experience of first-line afatinib in patients with EGFR-mutant advanced NSCLC: A multicenter observational study. BMC Cancer [Internet]. [cited 2020 Jun 1];19(1):896. https://bmccancer.biomedcentral.com/articles/10.1186/s12885-019-6107-1

http://0-online.lexi.com.library.touro.edu/action/home

https://gilotrifhcp.com/sites/default/files/pdfs/PC-GF-0426-PROF_MVA_7_(Digital_Version)_091916_Digital_Proof.pdf

https://www.cancer.gov/about-cancer/treatment/types/targeted-therapies/targeted-therapies-fact-sheet

https://www.cancer.org/cancer/non-small-cell-lung-cancer/treating/surgery.html

https://www.epa.gov/sites/production/files/2016-02/documents/2012_a_citizens_guide_to_radon.pdf

https://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm279174.htm

Hughes B, Mileshkin L, Townley P, Gitlitz B, Eaton K, Mitchell P et al (2014) Pertuzumab and Erlotinib in patients with relapsed non-small cell lung cancer: a phase II study using 18F-Fluorodeoxyglucose positron emission tomography/computed tomography imaging. Oncologist 19(2):175–176

Jain RK, Duda DG, Willett CG, Sahani DV, Zhu AX, Loeffler JS et al (2011) Biomarkers of response and resistance to antiangiogenic therapy. Therapy 6(6):327–338

Janku F, Stewart DJ, Kurzrock R (2010) Targeted therapy in non-small-cell lung cancer—is it becoming a reality? Nat Rev Clin Oncol 7(7):401–414

Jia Y, Juarez J, Li J, Manuia M, Niederst MJ, Tompkins C et al (2016) EGF816 exerts anticancer effects in non-small cell lung cancer by irreversibly and selectively targeting primary and acquired activating mutations in the EGF receptor. Cancer Res 76(6):1591–1602

Johnston JB, Navaratnam S, Pitz MW, Maniate JM, Wiechec E, Baust H et al (2006) Targeting the EGFR pathway for cancer therapy. Curr Med Chem 13(29):3483–3492

Joshi A, Pande N, Noronha V, Patil V, Kumar R, Chougule A et al (2019) ROS1 mutation non-small cell lung cancer—access to optimal treatment and outcomes. Ecancermedicalscience 13:900

Julien S, Jacoulet P, Dubiez A, Westeel V, Depierre A (2000) Non-small cell lung cancer: a study of long-term survival after vinorelbine monotherapy. Oncologist 5(2):115–119

Kasibhatla S, Li J, Tompkins C, Vaillancourt M-T, Anderson J, Pferdekamper AMC, Li C, Long O, McNeill M, Epple R, Liao D, Murphy E, Bender S, Jia Y, Lelais G (2014) EGF816, a novel

covalent inhibitor of mutant-selective epidermal growth factor receptor, overcomes T790M-mediated resistance in NSCLC. Cancer Res 74(19 Suppl):1733
Kawamura T, Murakami H (2016) Dabrafenib in patients with BRAF-mutated non-small cell lung cancer. Transl Cancer Res 5(S2):S342–S344
Khunger A, Khunger M, Velcheti V (2018) Dabrafenib in combination with trametinib in the treatment of patients with BRAF V600-positive advanced or metastatic non-small cell lung cancer: clinical evidence and experience. Therap Adv Respir Dis 12:175346661876761
Kim ES (2016) Olmutinib: first global approval. Drugs 76(11):1153–1157
Konagai S, Sakagami H, Yamamoto H, Tanaka H, Matsuya T, Mimasu S, Tomimoto Y, Mori M, Koshio H, Hirano M, Kuromitsu S, Takeuchi M (2015) ASP8273 selectively inhibits mutant EGFR signal pathway and induces tumor shrinkage in EGFR mutated tumor models. Cancer Res 75(15_Suppl):2586
Korpanty G, Smyth E, Carney DN (2011) Update on anti-angiogenic therapy in non-small cell lung cancer: are we making progress? J Thorac Dis 3(1):19–29
Krewski D, Lubin JH, Zielinski JM, Alavanja M, Catalan VS, Field RW et al (2005) Residential radon and risk of lung cancer. Epidemiology 16(2):137–145
Kumar M, Ernani V, Owonikoko TK (2015) Biomarkers and targeted systemic therapies in advanced non-small cell lung cancer. Mol Aspects Med 45:55–66
Kuribayashi K, Tabata C (2016) Cutting-edge medical treatment for advanced non-small cell lung cancer. J Cancer Biol Res 2:1–9
Lara PN, Laptalo L, Longmate J, Lau DHM, Gandour-Edwards R, Gumerlock PH et al (2004) Trastuzumab plus docetaxel in HER2/neu-positive non-small-cell lung cancer: a California cancer consortium screening and phase II trial. Clin Lung Cancer 5(4):231–236
Lau SCM, Batra U, Mok TSK, Loong HH (2019) Dacomitinib in the management of advanced non-small-cell lung cancer. Drugs 79:823–831
Lavanya V, Adil M, Ahmed N, Rishi AK, Jamal S (2014) Small molecule inhibitors as emerging cancer therapeutics. Integr Cancer Sci Ther 1(3):39–46
Lee K-O et al (2014) Discovery of HM61713 as an orally available and mutant EGFR selective inhibitor. Cancer Res 74(19_Suppl):LB-100
Li S, Kussie P, Ferguson KM (2008) Structural basis for EGF receptor inhibition by the therapeutic antibody IMC-11F8. Structure 16(2):216–227
Lugowska I, Koseła-Paterczyk H, Kozak K, Rutkowski P (2015) Trametinib: a MEK inhibitor for management of metastatic melanoma. Onco Targets Ther 8:2251–2259
Ma C, Wei S, Song Y (2011) T790M and acquired resistance of EGFR TKI: a literature review of clinical reports. J Thorac Dis 3:10–18
Marimastat in treating patients with stage III non-small cell lung cancer—full text view—ClinicalTrials.gov [Internet]. [cited 2020 Jun 1]. https://clinicaltrials.gov/ct2/show/NCT00002911
Martin P, Stewart E, Pham NA, Mascaux C, Panchal D, Li M, et al (2016) Cetuximab inhibits T790M-mediated resistance to epidermal growth factor receptor tyrosine kinase inhibitor in a lung adenocarcinoma patient-derived xenograft mouse model. Clin Lung Cancer [Internet] 17(5):375–383.e2. https://doi.org/10.1016/j.cllc.2016.01.002
McCall NS, Lu B (2017) Brigatinib and the new generation of ALK-inhibitors for non-small cell lung cancer. Transl Cancer Res 6(S2):S380–S383
Merchant N, Nagaraju GP, Rajitha B, Lammata S (2009) Accepted manuscript
Merchant N, Nagaraju GP, Rajitha B, Lammata S, Jella KK, Buchwald ZS et al (2017) Matrix metalloproteinases: their functional role in lung cancer. Carcinogenesis 38(8):766–780
Mok T, Lee K, Tang M, Leung L (2014) Dacomitinib for the treatment of advanced or metastatic non-small-cell lung cancer. Future Oncol. 10(5):813–822
Molina JR, Yang P, Cassivi SD, Schild SE, Adjei AA (2008) Non-small cell lung cancer: epidemiology, risk factors, treatment, and survivorship. Mayo Clin Proc 83(5):584–594

Morgensztern D, Campo MJ, Dahlberg SE, Doebele RC, Garon E, Gerber DE, et al. (2015) Molecularly targeted therapies in non-small cell lung cancer annual update 2014. J Thorac Oncol 10:1–63 p

Neal JW, Gainor JF, Shaw AT (2015) Developing biomarker-specific end points in lung cancer clinical trials. Nat Rev Clin Oncol 12:135–146

Ogunleye F, Ibrahim M, Stender M, Kalemkerian G, Jaiyesimi I (2009) Epidermal growth factor receptor tyrosine kinase inhibitors in advanced non-small cell lung cancer. A paradigm shift in stage IV non-small cell lung cancer treatment Foluso. Expert Opin Investig Drugs 18(3): 293–301

osimertinib resistance.pdf

Pallis AG, Syrigos KN (2013) Targeting tumor neovasculature in non-small-cell lung cancer. Crit Rev Oncol Hematol 86(2):130–142

Pao W, Girard N (2011) New driver mutations in non-small-cell lung cancer. Lancet Oncol 12(2): 175–180

Pao W, Miller VA, Politi KA, Riely GJ, Somwar R, Zakowski MF et al (2005) Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. PLoS Med 2(3):e73

Park K, et al A global Phase II study of Olmutinib (HM61713) in patients with T790M-positive NSCLC after failure of first-line EGFR-TKI (412O). OncologyPRO [Internet]. [cited 2020 Jun 1]. https://oncologypro.esmo.org/meeting-resources/esmo-asia-2017-congress/A-global-Phase-II-study-of-Olmutinib-HM61713-in-patients-with-T790M-positive-NSCLC-after-failure-of-first-line-EGFR-TKI-412O

Peddi PF, Hurvitz SA (2013) Trastuzumab emtansine: the first targeted chemotherapy for treatment of breast cancer. Future Oncol 9(3):319–326

Peters S, Zimmermann S (2014) Targeted therapy in NSCLC driven by HER2 insertions. Transl Lung Cancer Res 3(2):84–88

Piccirillo MC, Daniele G, Di Maio M, Bryce J, De Feo G, Del Giudice A et al (2010) Vinorelbine for non-small cell lung cancer. Expert Opin Drug Saf 9:493–510

Pillai RN, Ramalingam SS (2014) Necitumumab: a new therapeutic option for squamous cell lung cancer? Transl Lung Cancer Res. 3(6):382–383

Piperdi M et al (2015) Targeting angiogenesis in squamous non-small cell lung cancer. Drugs 74(4): 403–413

Pirker R, Herth FJF, Kerr KM, Filipits M, Taron M, Gandara D et al (2010) Consensus for EGFR mutation testing in non-small cell lung cancer: results from a European workshop. J Thorac Oncol 5(10):1706–1713

Pirker R, Buder A, MF. (2017) Osimertinib in advanced EGFR T790M-positive non-small-cell lung cancer: the clinical impact of AURA3. Transl Cancer Res 6(2):S265–S269

Qiao X, Tullgren O, Lax I, Sirzén F, Lewensohn R (2003) The role of radiotherapy in treatment of stage I non-small cell lung cancer. Lung Cancer 41(1):1–11

Raben D, Bianco C, Damiano V, Bianco R, Melisi D, Mignogna C et al (2004) Antitumor activity of ZD6126, a novel vascular-targeting agent, is enhanced when combined with ZD1839, an epidermal growth factor receptor tyrosine kinase inhibitor, and potentiates the effects of radiation in a human non-small cell lung cancer xenograft. Mol Cancer Ther 3(8):977–983

Ramalingam S, Belani CP (2004) Paclitaxel for non-small cell lung cancer. Expert Opin Pharmacother 5(8):1771–1780

Rehman S, Dy GK. MET inhibition in non-small cell lung cancer. 2019;(March):100–11

Rhee MS, Wang Y, Nair MG, Galivan J (1993) Acquisition of resistance to antifolates caused by enhanced gamma-glutamyl hydrolase activity. Cancer Res [Internet]. [cited 2020 Jun 1];53 (10 Suppl):2227–30. http://www.ncbi.nlm.nih.gov/pubmed/7683570

Rolfo C, Caparica R (2016) B-RAF mutation in non-small cell lung cancer: the sleeping beauty is waking up. Transl Lung Cancer Res 5(4):367–369

Rosell R, Sa J, Lee JS, Brate AO, Gonza L, Alberola V et al (2019) Paclitaxel resistance in non-small-cell lung cancer associated with beta-tubulin gene mutations. J Clin Oncol 17(6): 1786–1793

Ross HJ, Blumenschein GR, Aisner J, Damjanov N, Dowlati A, Garst J et al (2010) Randomized phase II multicenter trial of two schedules of lapatinib as first- or second-line monotherapy in patients with advanced or metastatic non-small cell lung cancer. Clin Cancer Res 16(6): 1938–1949

Rothschild SI (2014) Ceritinib-a second-generation ALK inhibitor overcoming resistance in ALK-rearranged non-small cell lung cancer. Transl Lung Cancer Res 3(6):1–3

Rothschild SI (2015) Targeted therapies in non-small cell lung cancer—beyond EGFR and ALK. Cancers (Basel) 7(2):930–949

Ruiz-Ceja KA, Chirino YI (2017) Current FDA-approved treatments for non-small cell lung cancer and potential biomarkers for its detection. Biomed Pharmacother [Internet]. 90:24–37. https://doi.org/10.1016/j.biopha.2017.03.018

Sakagami H, Konagai S, Yamamoto H, Tanaka H, Matsuya T, Mori M, Koshio H, Yuri M, Masaaki Hirano SK (2014) ASP8273, a novel mutant-selective irreversible EGFR inhibitor, inhibits growth of non-small cell lung cancer (NSCLC) cells with EGFR activating and T790M resistance mutations. Cancer Res 74(19_Suppl):1728

Sánchez-Torres JM, Viteri S, Molina MA, Rosell R (2013) BRAF mutant non-small cell lung cancer and treatment with BRAF inhibitors. Transl Lung Cancer Res 2(3):244–250

Sandler A (2007) Bevacizumab in non-small cell lung cancer. Clin Cancer Res 13(15):4613–4617

Sandler A, Ettinger DS (1999) Gemcitabine: single-agent and combination therapy in non-small cell lung cancer. Oncologist 4:241–251

Sause WT (1999) The role of radiotherapy in non-small cell lung cancer. Chest 116(6 suppl):504S–508S

Scheffler M, Bos M, Gardizi M, König K, Michels S, Fassunke J et al (2015) *PIK3CA* mutations in non-small cell lung cancer (NSCLC): genetic heterogeneity, prognostic impact and incidence of prior malignancies. Oncotarget 6(2):1315

Schiller JH, Harrington D, Belani CP, Langer C, Sandler A, Krook J et al (2002) Comparison of four chemotherapy regimens for advanced non–small-cell lung cancer. N Engl J Med 346(2): 92–98

Sequist LV, Soria J-C, Goldman JW, Wakelee HA, Gadgeel SM, Varga A et al (2015) Rociletinib in *EGFR* -mutated non–small-cell lung cancer. N Engl J Med 372(18):1700–1709

Sgambato A, Casaluce F, Maione P, Rossi A, Ciardiello F, Gridelli C (2014) Cetuximab in advanced non-small cell lung cancer (NSCLC): the showdown? J Thorac Dis 6(6):578–580

Shaw AT, Kim D-W, Mehra R, Tan DSW, Felip E, Chow LQM et al (2014) Ceritinib in *ALK* -rearranged non–small-cell lung cancer. N Engl J Med 370(13):1189–1197

Shaw AT, Gandhi L, Gadgeel S, Riely GJ, Cetnar J, West H et al (2016a) Phase 2 prospective analysis of alectinib in ALK-positive, crizotinib-resistant non-small-cell lung cancer Alice. Lancet Oncol 17(2):234–242

Shaw AT, Friboulet L, Leshchiner I, Gainor JF, Bergqvist S, Brooun A et al (2016b) Resensitization to Crizotinib by the Lorlatinib *ALK* resistance mutation L1198F. N Engl J Med 374(1):54–61

Shen Q, Qu J, Chen Z, Zhou J (2021) Case report: Dacomitinib overcomes Osimertinib resistance in NSCLC patient harboring L718Q mutation: a case report. Front Oncol 11(December):1–4

Shien K, Toyooka S, Yamamoto H, Soh J, Jida M, Thu KL et al (2015) Acquired resistance to egfr inhibitors is associated with a manifestation of stem cell–like properties in cancer cells. Cancer Res 73(10):3051–3061

Simon GR, Somaiah N (2014) A tabulated summary of targeted and biologic therapies for non-small-cell lung cancer. Clin Lung Cancer 15(1):21–51

Socinski MA (1999) Single-agent paclitaxel in the treatment of advanced non-small cell lung cancer. Oncologist 4(5):408–416

Stinchcombe TE, Johnson GL (2014) MEK inhibition in non-small cell lung cancer. Lung Cancer [Internet] 86(2):121–125. https://doi.org/10.1016/j.lungcan.2014.09.005

Sullivan I, Planchard D (2016) ALK inhibitors in non-small cell lung cancer: the latest evidence and developments. Ther Adv Med Oncol 8(1):32–47

Tan FH, Putoczki TL, Stylli SS, Luwor RB (2019) Ponatinib: a novel multi-tyrosine kinase inhibitor against human malignancies. Onco Targets Ther [Internet] [cited 2020 Jun 2];12:635–45. https://www.dovepress.com/ponatinib-a-novel-multi-tyrosine-kinase-inhibitor-against-human-malign-peer-reviewed-article-OTT

Tanoue L (2008) Staging of non-small cell lung Cancer. Semin Respir Crit Care Med 29(3): 248–260

Thakur M, Wozniak A (2017) Spotlight on necitumumab in the treatment of non-small-cell lung carcinoma. Lung Cancer 8:13–19

Thatcher N, Hirsch FR, Luft AV, Szczesna A, Ciuleanu TE, Dediu M et al (2015) Necitumumab plus gemcitabine and cisplatin versus gemcitabine and cisplatin alone as first-line therapy in patients with stage IV squamous non-small-cell lung cancer (SQUIRE): an open-label, randomised, controlled phase 3 trial. Lancet Oncol 16(7):763–774

Toschi L, Finocchiaro G, Bartolini S, Gioia V, Cappuzzo F (2005) Role of gemcitabine in cancer therapy. Future Oncol 1(1):7–17

Tran PN, Klempner SJ (2016) Profile of rociletinib and its potential in the treatment of non-small-cell lung cancer. Lung Cancer 7:91–97

Tsao AS, Scagliotti GV, Bunn PA, Carbone DP, Warren GW, Bai C et al (2016) Scientific advances in lung cancer 2015. J Thorac Oncol 11(5):613–638

Vaishnavi A, Capelletti M, Le AT, Kako S, Butaney M, Ercan D et al (2013) Oncogenic and drug-sensitive NTRK1 rearrangements in lung cancer. Nat Med 19(11):1469–1472

Venorelbone resistance.pdf

Wakelee HA, Gettinger S, Engelman J, Jänne PA, West H, Subramaniam DS et al (2017) A phase Ib/II study of cabozantinib (XL184) with or without erlotinib in patients with non-small cell lung cancer. Cancer Chemother Pharmacol 79(5):923–932

Wang Q, Yang S, Wang K, Sun SY (2019) MET inhibitors for targeted therapy of EGFR TKI-resistant lung cancer. J Hematol Oncol 12(1):1–11

Wangari-Talbot J, Hopper-Borge E (2012) Drug resistance mechanisms in non-small cell lung carcinoma. J Can Res Updates 17(6):949–964

WHO (2018) Breast cancer. https://www.who.int/news-room/fact-sheets/detail/breast-cancer

Winer A, Adams S, Mignatti P (2018) Matrix metalloproteinase inhibitors in cancer therapy: turning past failures into future successes. Mol Cancer Ther 17(6):1147–1155

Wu YL, Zhou C, Liam CK, Wu G, Liu X, Zhong Z et al (2015) First-line erlotinib versus gemcitabine/cisplatin in patients with advanced EGFR mutation-positive non-small-cell lung cancer: analyses from the phase III, randomized, open-label, ENSURE study. Ann Oncol 26(9): 1883–1889

Wu J, Savooji J, Liu D (2016) Second- and third-generation ALK inhibitors for non-small cell lung cancer. J Hematol Oncol 9(1):1–7

www.NCCN.org/professionals/physician_gls/pdf/nscl.pdf

Yamamoto A, Yano S, Shiraga M, Ogawa H, Goto H, Miki T et al (2003) A third-generation matrix metalloproteinase (MMP) inhibitor (ONO-4817) combined with docetaxel suppresses progression of lung micrometastasis of mmp-expressing tumor cells in nude mice. Int J Cancer 103(6): 822–828

Yamasaki M, Murakami I, Nakano K, Doi M, Kitaguchi S, Kondo T et al (2017) Carboplatin plus weekly paclitaxel combined with bevacizumab as first-line treatment for non-small cell lung cancer. Anticancer Res 37(2):923–928

Yang JC, Reckamp K, Kim Y, Novello S, Smit E, Lee J et al (2017) P2.03-058 Tiger-3: a phase 3 randomized study of Rociletinib vs chemotherapy in EGFR-mutated non-small cell lung cancer (NSCLC). J Thorac Oncol 12:S2397

Yano S, Nishioka Y, Goto H, Sone S (2003) Molecular mechanisms of angiogenesis in non-small cell lung cancer, and therapeutics targeting related molecules. Cancer Sci 94(6):479–485

Yoh K, Seto T, Satouchi M, Nishio M, Yamamoto N, Murakami H et al (2017) Vandetanib in patients with previously treated RET-rearranged advanced non-small-cell lung cancer (LURET): an open-label, multicentre phase 2 trial. Lancet Respir Med 5(1):42–50

Yu HA, Oxnard GR, Spira AI, Horn L, Weiss J, Feng Y, West HJ, Giaccone G, Evans TL, Kelly RJ, Fleege T, Poondru S, Jie F, Aoyama K, Fole MA, Krivoshik AP (2015) Phase I dose escalation study of ASP8273, a mutant-selective irreversible EGFR inhibitor, in subjects with EGFR mutation positive NSCLC. J Clin Oncol 33(15_suppl):8083

Zappa C, Mousa SA (2016) Non-small cell lung cancer: current treatment and future advances. Transl Lung Cancer Res 5(3):288–300

Zeng L, Xiao L, Jiang W, Yang H, Hu D, Xia C, et al. (2020) Investigation of efficacy and acquired resistance for EGFR-TKI plus bevacizumab as first-line treatment in patients with EGFR sensitive mutant non-small cell lung cancer in a real world population. Lung Cancer [Internet]. 141(2019):82–8. https://doi.org/10.1016/j.lungcan.2020.01.009

Zukin M (2012) Epidermal growth factor receptor inhibitors in non-small cell lung cancer: current status and future perspectives. Rev Assoc Med Bras 58(2):263–268

Chapter 8
Drug Repurposing in Cancer

N. R. Shruthi, M. Samatha Jain, Harsha Ganesan, Antara Banerjee, Hong Zhang, Xiao-Feng Sun, and Surajit Pathak

Abstract The discovery of drug compounds has a long history in drug repurposing, notably by fortuitous findings. It has taken a new path in the creation of novel therapeutics based on existent or authorized drugs in recent years. Importantly, our knowledge of cancer biology and the related cancer hallmarks is growing. This, together with repurposing studies that use modern bioinformatics and comprehensive screening of the complete pharmacopeia, should lead to the discovery of novel medicines and targets. Furthermore, the usage of non-oncology pharmaceuticals, which make up most of our treatments, has the potential to speed up drug repurposing even further. We looked at both phenotypic-based and target-based methods of medication repurposing as well as described and assessed old non-oncology medications as prospective candidates for drug repurposing based on a broad knowledge of these principles and associated investigations of drug repurposing over the previous decade. Some of these medications successfully regulate at least one characteristic of cancer, whereas the others have a broad anticancer activity by regulating several targets through different signaling pathways, which is often brought on by various simultaneous signaling pathways.

N. R. Shruthi · M. Samatha Jain · H. Ganesan · A. Banerjee
Department of Medical Biotechnology, Faculty of Allied Health Sciences, Chettinad Academy of Research and Education (CARE), Chettinad Hospital and Research Institute (CHRI), Chennai, India

H. Zhang
Institute of Medical Sciences, School of Medicine, Örebro University, Örebro, Sweden

X.-F. Sun (✉)
Departments of Oncology and Biomedical and Clinical Sciences, Linköping University, Linköping, Sweden
e-mail: xiao-feng.sun@liu.se

S. Pathak (✉)
Department of Medical Biotechnology, Faculty of Allied Health Sciences, Chettinad Academy of Research and Education (CARE), Chettinad Hospital and Research Institute (CHRI), Chennai, India

Departments of Oncology and Biomedical and Clinical Sciences, Linköping University, Linköping, Sweden

R. C. Sobti et al. (eds.), *Drug Repurposing for Emerging Infectious Diseases and Cancer*, https://doi.org/10.1007/978-981-19-5399-6_8

Furthermore, the emergence of computerized databases of disease gene targets, functional readouts, and clinical data encompassing inter-individual genetic variants and toxicities has allowed an alternative “big data” approach to grow at an unheard-of rate during the past decade. Here, we review the sources that are now on hand and speculate on significant upside possibilities.

Keywords Drug repurposing · Colorectal cancer · Target-based · Signaling pathways · Bioinformatics

8.1 Introduction

Drug repurposing (DR) has captivated interest in research and the pharmaceutical industry due to its potential to discover novel applications for currently used medications and to develop new drugs, as well as its efficiency as a method of lessening the duration and financial savings compared to conventional de novo drug development methods. Various terms for drug repurposing include drug repositioning, drug reprofiling, drug redirecting, drug retasking, and therapeutic switching (Jarada et al. 2020). This entails identifying new therapeutic indications for existing prescription drugs or approved pro-drugs that have failed, been researched, are already marketed, or are FDA approved. It entails finding novel therapeutic applications for existing medications, including those that have been authorized, discontinued, abandoned, or are still in development (Badria 2020). Currently, the drug repositioning process has gained traction, with repurposed pharmaceuticals accounting for around one-third of new drug approvals, generating over 25% of the pharmaceutical industry’s yearly revenue (Cha et al. 2018). According to current estimates, the market for repurposed pharmaceuticals was worth $24.4 billion in 2015, with a forecast increase to $31.3 billion by 2020 (Badria 2020).

DR is based on two fundamental scientific foundations: (a) the finding, via the elucidation of the human genome, that some illnesses have biological targets that are sometimes shared and (b) the idea of pleiotropic drugs. Any structural alteration of the drug is thus excluded from the idea of drug repositioning. Instead, repositioning uses either the biological qualities for which the drug has previously been authorized perhaps in a new dosage, a new route of administration, or a modified formulation, or the drug’s side features that are responsible for its adverse effects in a new indication (Jourdan et al. 2020).

On-target and off-target DR are the two basic DR methods. A drug molecule’s established pharmacological action in on-target DR is applied to a novel therapeutic application. In this strategy, the biological target of the therapeutic molecule is the same, but the condition is different (Ferreira and Andricopulo 2016). The pharmacological mechanism in the off-target profile, on the other hand, is unknown (Badria 2020). The experiment-based method and the in silico-based approach are two different and complementary techniques for drug repositioning. The experiment-based technique is also known as activity-based repositioning, and it refers to the use

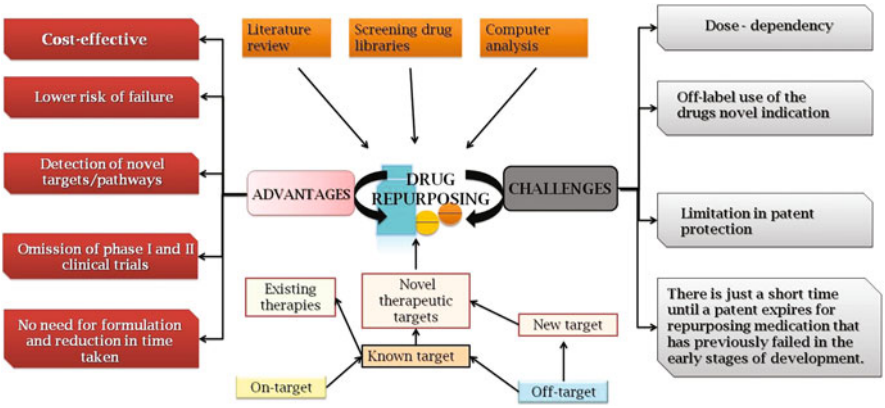


Fig. 8.1 A comprehensive overview of drug repurposing

of experimental assays to screen original drugs for new pharmacological indications. In silico repositioning, on the other hand, uses bioinformatics/cheminformatics and computational biology tools to perform virtual screenings of public databases of large drug/chemical libraries (Talevi 2018). In recent years, researchers and scientists have used a hybrid method to explore novel therapeutic indications for current drugs by combining in silico and experimental approaches. Preclinical biological experiments (in vivo and in vitro testing) and clinical studies are used to confirm the results of computer algorithms in the mixed approach (Badria 2020).

Due to the urgent need for innovative therapeutics, uncommon or terminal oncological symptoms provide less constraint on safety in the context of cancer. Furthermore, cancer is a multistage disease with treatment options available throughout the onset, fast heterogeneous growth, metastasis, and/or recurrence stages. These characteristics imply that cancer-focused drug repurposing might benefit both patients and pharmaceutical companies, with the sections that follow offer an overview of present prospects and possible hurdles in this field (Hernandez et al. 2017). This article gives a quick summary of repurposing, focusing on the most current scientific foundations of various repurposed drugs used in cancer therapy (Fig. 8.1).

8.1.1 Advantages

There are a variety of benefits to repositioning drugs. According to a recent analysis based on a survey of 30 pharmaceutical and biotechnology firms, the average cost of reintroducing a repurposed drug is $8.4 million, whereas the average cost of re-launching a new formulation of an existing drug in its original indication is $41.3 million. Because extensive information on their pharmacology, formulation,

possible toxicity, safety, and adverse drug response concerns is available, they have a greater success rate than the original medications, reducing attrition. As repurposing is based on prior research and development efforts, new drug candidates might be put into clinical trials swiftly, speeding up the FDA's review process and the approval of drugs (Agrawal 2015).

Clinical research and education programs offer in-depth knowledge of certain illness states, removing "activation barriers," and enabling projects to go fast into the preliminary stages. Clinical findings, on the other hand, can quickly lead to cellular and molecular pathway correlations and investigations. Drug repurposing initiatives can be quickly applied to diseases that lack viable treatments in this manner. Cell division, autophagy, apoptosis, and metabolism are examples of targets that can be manipulated therapeutically for a variety of clinically relevant purposes. Also, effective communication between fundamental scientists, physicians, and pharmaceutical scientists is required to gain a thorough understanding of pathway interdependencies and shunts, as well as the clinical repercussions of modulated treatment perturbations for such targets (Oprea et al. 2011).

In terms of chances of success, a repurposed drug has the benefit of being connected with a large body of information about human pharmacokinetics, bioavailability, and toxicity, which helps to reduce risk. Furthermore, the knowledge available about the repurposed drug is significantly more through and perhaps less risky than it was when the New Chemical Entity was first being developed (Pantziarka et al. 2015).

Finally, research on a repurposed drug directed at a new indication has the potential to inform the creation of novel New Chemical Entity, giving the notion of repurposing additional benefits (Islam et al. 2022).

8.1.2 Challenges

8.1.2.1 Dosing and Safety

Concerns about dose and delivery capacity frequently hamper the clinical utility of finding novel drug–target interactions. The new drugs are only licensed after a through examination reveals obvious therapeutic benefits within well-defined safety limitations (the potential to administer the medicine to specific targets in the illness foci). Dosing and delivery also consider safety concerns, as the targets must be exposed to the drugs (or its active metabolites) for a short period. Novel drug–target interactions are frequently reported in peer-reviewed or patent literature, especially for older drugs that haven't had their full effects defined before being approved. These data frequently reveal potency at the micromolar level. The burden of evidence and therapeutic relevance, on the other hand, is on the discovery teams, which must demonstrate that such effects may be observed in the clinic at dosages

within the allowed ranges. So far, it has been difficult to discover new drug–target interactions within the confines of the approved therapeutic window. Phase I clinical trials would have to be conducted by the discovery teams if the anticipated potency is outside range, thereby blending the lines between de novo and repurposed drug development (Oprea et al. 2011).

8.1.2.2 Data Availability

Even though the open-source concept is gaining traction in the drug research community (Talevi and Bellera 2020), public access to some types of useful data (e.g., clinical trials) is still restricted. Even if accessibility was not an issue, certain data (e.g., imaging data) are less conducive to data mining, integration, and modification, and are occasionally provided in an unstandardized format (Pushpakom et al. 2019). Integrating many types of data has proven to be computationally challenging, as it improves the analytical power (Ritchie et al. 2015).

Compound availability with generic active pharmaceutical components can sometimes be problematic, especially if the compound is no longer available on the international market. In such situations, finding a dependable source could be difficult.

8.1.2.3 Intellectual Property

Repositioners confront significant hurdles due to the lack of intellectual property protection. Some legal issues might make it more difficult to patent a new medical use and/or enforce patent rights, lowering the incentives for drug repurposing. Obtaining a patent for a second or subsequent medicinal use is hampered by certain national legislations (Talevi and Bellera 2020). This is since the drug in question has been patented as a new chemical entity, and future medicines comprising the same entity can only be provided by a novel application patent (Jourdan et al. 2020).

Many possible repurposing applications have already been documented in the specialized literature or are being used in clinical practice as off-label, unregistered applications (Pushpakom et al. 2019). Even though such applications have yet to be validated in controlled clinical studies, the knowledge is available in the public domain, affecting novelty and, as a result, patentability. One specific obstacle is a dearth of specialists in the legal aspects of drug repurposing, which is a relatively new topic for academics. Another limitation is the dissemination of novel drug-target-disease connections via PubChem or other internet databases, or by articles extending from peer-reviewed literature to blogs and other online publications. Such disclosures successfully thwart intellectual property protection efforts, leading to a reduction in the number of patent applications filed (Oprea et al. 2011).

8.2 Drug Repurposing in Cancer Therapy

In cancer therapy, repurposing existing drugs is a cost-effective and time-saving approach to drug discovery. The rapid identification of new therapeutic targets enabled by screening clinical drug collections will lead to the development of novel and more affordable treatment options.

8.2.1 Cytostatic Agents for Cancer Therapy

8.2.1.1 Aspirin

Aspirin an anti-inflammatory drug, commonly used to prevent and treat cardiovascular diseases, also contains other pharmacological properties including antiplatelet activity at low doses and anti-inflammatory action at high doses. Apart from these effects, the constant search to discover new mechanisms of action hasn't ceased (Thorat and Cuzick 2013). Kune et al. (1988) presented the first study relating aspirin use in colorectal cancer (CRC) reduction in 1988, finding a statistically significant reduction in cases taking aspirin-containing medicine in both men and women (Kune et al. 1988). The early reduction in cancer deaths in trials of daily aspirin versus control could be due to aspirin's ability to prevent distant metastasis. Similarly, the findings of Rothwell et al. (2012) suggest that aspirin could be beneficial in the treatment of certain cancers and provides proof of distant metastasis prevention (Rothwell et al. 2012). The use of aspirin on a regular and long-term basis has been linked to a significant reduction in the incidence of colorectal cancer, according to several studies. This is thought to be due to the inhibition of adenomas, which are the precursor lesions inhibited by aspirin in randomized trials (Tougeron et al. 2014).

8.2.1.2 Metformin

Metformin's origins date back to a plant extract whose primary ingredient was guanidine, which was used to treat diabetes symptoms throughout the Middle Ages. Metformin is an antihyperglycemic drug and insulin sensitizer that reduces fasting plasma insulin levels and improves insulin resistance. Its other benefits include reduction of cellular reactive oxygen species, down-regulation of proinflammatory cytokines, inhibiting of lipogenesis, and alleviating hyperlipidemia. Dysregulation of these processes is linked to an increased risk of cancer. Because of its low toxicity (the most common adverse event being lactic acidosis), metformin is becoming more popular as a cytostatic cancer treatment. Metformin's action could be explained by two mechanisms. It inhibits complex I of the mitochondrial respiratory chain, preventing oxidative phosphorylation resulting in an

increase in adenosine monophosphate (AMP) or the AMP/ATP ratio (Zhang et al. 2007; Pernicova and Korbonits 2014). Secondly, metformin inhibits ATP production by activating AMP-activated protein kinase (AMPK), providing a plausible and widely accepted model for gluconeogenic gene expression and glucose output suppression (Meng et al. 2017). Metformin, a commonly used antidiabetic medication, extends overall survival in diabetic CRC patients, though the effect on CRC-specific survival is still questionable (Tougeron et al. 2014).

8.2.1.3 Statins

Statins, or hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, are a commonly used medication for lowering serum total and low-density lipoprotein (LDL) cholesterol by inhibiting the conversion of HMG-CoA to mevalonic acid. Statins appear to have pleiotropic effects, including control of cell proliferation, death, and inflammation, in addition to their powerful pharmacologic suppression of cholesterol production. Statins can influence a wide range of disease processes, including cancer, via modulating these pathways. Statins appear to prevent CRC based on epidemiological research, but the molecular mechanism is yet to be understood. Statins have anabolic effects on bone by boosting the production of bone morphogenetic protein (BMP) 2 mRNA and protein by osteoblast-like cells by activating the BMP2 promoter. BMP expression is induced by statins in bone cells. (Mundy et al. 1999). The findings of Rutledge et al. (2019) implied that taking lipophilic statins has an anticarcinogenic impact and a lower risk of developing late-stage CRC, which could have implications for CRC prevention and management in the future (Rutledge et al. 2019).

8.3 Target Prediction in Cancer

The drug-centric approach allows for the formulation of innovative rational repositioning hypotheses based on the precise characterization of drug–target interactions. Due to their great efficiency and low costs, the development of numerous computer approaches has provided important strategies for the systematic prediction of possible drug–target interactions (DTIs) throughout the last decade. DTIs have become a significant aspect in drug discovery or repositioning, with the purpose of uncovering potential new treatments or novel targets for existing drugs. In contrast to expensive and delayed in vivo or biochemical experimental procedures for finding new DTIs, silico or computational approaches can quickly uncover promising DTI candidates for in vivo validation, decreasing the hassle and costs of drug development or repositioning (Luo et al. 2017). Target-based drug repurposing commences with high-throughput and/or high-content screening of drug compounds, following in silico screening of drug compounds through drug libraries, including ligand-based

screening or docking, based on proteins or biomarkers of interest (Yamanishi et al. 2010).

Traditional methodologies based on the three-dimensional (3D) structures of targets, such as molecular docking, have been widely used in DTI prediction. These approaches analyzed DTIs using scoring functions, which can offer quantitative docking scores that are related to binding affinities. Machine learning is a general method for DTI prediction that has been rapidly evolving in recent years. Deep learning approaches have lately been used in DTI prediction in addition to classic machine learning techniques, and molecular descriptors and protein sequence descriptors are used more often (Liu et al. 2010; Waszkowycz et al. 2011; Hwang et al. 2017).

8.3.1 Structure-Based Target Prediction

Since the inception of in silico molecular modeling, structure-based virtual screening technologies have aided the drug development process. Furthermore, numerous structure-based non-docking techniques have been widely used in drug repositioning to overcome docking's inefficiency and inaccuracy. Finding an appropriate drug target, identifying a prospective drug, and putting up experimental tests to test the efficacy of the proposed therapy is a time-consuming and expensive procedure in traditional drug development. Molecular docking, on the other hand, which mimics a drug's binding affinity in the 3D structure of a drug target, can be used as a higher resolution simulation method (Li and Jones 2012). The ligand-based approaches and molecular docking-based approaches are the two basic tactics used in traditional computational methods. As a result, in rational drug repositioning, computational tools for drug–target interaction prediction have gotten a lot of attention (Yan et al. 2019).

Below are the list of tools that can be used to predict new drug–target interactions based on structural data of protein targets with or without bound compounds for structure-based drug repositioning. This is not an extensive or complete list for docking, binding site prediction, pharmacophore-based screening, and interaction similarity.

8.3.1.1 Docking

AutoDock—The conformational space of the ligand is compared to a set of grids characterizing the target protein. It does not require the selection of atom kinds because the grids are calculated internally. It is possible to visualize the atomic affinity grids (Forli et al. 2016).

Karuppasamy et al. (2017) developed a multi-modal computational drug repositioning approach to identify anti-proliferative drugs. Flexible molecular docking was used with antiviral medications that met all of the thresholds with

native mutant beta-tubulin to analyze the free binding energy of the antiviral drug. The commonly preferred AutoDock was used to thoroughly evaluate ligand conformations and estimate the medicinal molecule's binding affinity (Karuppasamy et al. 2017).

FlexX—A virtual screening tool that explores the conformational space of ligands. In the study of Chittepu et al. (2019) protein–ligand interactions were predicted using FlexX molecular docking simulations to estimate possible draggability towards the dipeptidyl peptidase-4 mechanism of action, the FlexX algorithm was utilized to infer protein–ligand interaction residues and binding energy (Chittepu et al. 2019). In a study conducted by Lokhande et al. (2020) for a small-molecule checkmate to cancer therapy in which they came to a conclusion that according to FlexX's molecular docking experiment, the derivatives Qur8, Kem204, and Res183 of quercetin, resveratrol, and kaempferol, respectively, are more potent against PPAR-γ than other compounds (Lokhande et al. 2020).

8.3.1.2 Binding Site Prediction

Sitemap—SiteMap uses a novel search to identify the characteristics of binding sites and evaluates each one by calculating properties such as volume, size, amino acid exposure, contact, enclosure, hydrophilicity, hydrophobicity, and the acceptor/donor ratio (Halgren 2007). Recently, researchers have discovered a unique binding site for the E542K amino acid residue, which is extensively mutated in cancers of various sorts. This exploratory site is extended in two directions by SiteMap predicted binding sites, one of which includes the activator loop and the other another usually altered residue, E545K (Miller et al. 2017).

FindSite—Combinatorial regimens are required for the effective treatment of colorectal malignancies. A study conducted by Mitchel et al. (2021) used the computational structure-based drug discovery tool FINDSITE^{comb} to find a PDK1 inhibitor and conduct combinatorial trials with 5-FU for effective colorectal cancer treatment. Stearic acid was predicted to have few off-target effects, making it a promising colorectal cancer chemotherapeutic candidate (Mitchel et al. 2021).

8.3.1.3 Pharmacophore-Based Screening

LigandScout—Ligand topology interpretation and identification of integral amino acids as pharmacophore templates using explored and unexplored protein–ligand complexes (Wolber and Langer 2005). Patil et al. (2021) conducted a study on non-cancer to anticancer: potassium channel inhibitors based on the human ether-a-go-go gene as possible treatments where LigandScout 4.4 was used to investigate the binding poses of the ligands and the manner of interactions of the protein–ligand complex (Patil et al. 2021). To suggest substances that potentially have more effective fascin1 inhibitory characteristics than migrastatin, in silico screening was used. Migrastatin's core structure was subjected to pharmacophore modeling using

LigandScout in order to create a ligand-based pharmacophore model (Alburquerque-González et al. 2020).

8.3.1.4 Interaction Similarity

PLIP—PLIP generates a report profile that includes all discovered non-covalent interactions that define the ligand's binding affinity at the atomic level and are represented in 3D (Salentin et al. 2015). Through an in silico screening of all presently available structural data using PLIP, Salentin et al. (2017) discovered the FDA-approved malaria medicine amodiaquine as a possible repositioning candidate for cancer. The results of this study demonstrate the viability of PLIP interaction patterns as computational repositioning tools. They can be used to distinguish structurally unrelated candidates, such as FDA-approved medications with well-known safety and pharmacology profiles, by providing search query relevant data from a given drug and its target (Salentin et al. 2017).

SIFt—3D protein–ligand binding interactions are analyzed and represented. By translating 3D structural binding information onto a binary string, SIFt generates an interaction fingerprint (Deng et al. 2004). Given that BMP4 is a causative factor of CRC and the significance of protein-based modeling in elucidating rare disease-causing variations, Lubbe et al. (2011) used two in silico techniques, PolyPhen and SIFT, to estimate the putative influence of non-synonymous coding alterations in BMP4 on expressed protein function (Lubbe et al. 2011).

TIFP—TIFP allows you to compare interaction pattern resemblance to a ligand or binding site pairwise similarities. Protein–ligand coordinates are encoded into a fingerprint of 210 molecular interaction motifs (Desaphy et al. 2013). Any two protein–ligand complexes can be compared using the TIFP fingerprint to examine associations based on interaction pattern similarities and ligand or binding site pairwise similarities. Additionally, the researchers have combined the TIFP fingerprint with the recently released tools Ishape and Grim to align protein-ligand complexes in a biased manner toward interactions, post-process docking scores in accordance with recognized interaction patterns for a particular target, and conduct a virtual search for biometric scaffolds with comparable interaction patterns (Briels et al. 2020).

8.3.2 Cheminformatics-Based Target Prediction

Cheminformatics is a relatively new branch of information technology concerned with the gathering, storage, analysis, and manipulation of chemical data. Small molecule formulae, structures, properties, spectra, and activities are usually included in the chemical data of interest (biological or industrial). Unlike molecular docking approaches, the ligand-based cheminformatics approach does not require information on the target protein. Rather, features derived from a drug's chemical structure

graph, or any other appropriate description are linked to known target binding data (i.e., binding affinity) or phenotypic alterations (i.e., high-throughput cancer cell viability assays) (Issa et al. 2021). Using cheminformatics methodologies, medications with chemical structures like cancer drugs or genes connected with pharmaceuticals with similar chemical structures can be found.

KNIME-CDK includes functions for converting molecules to and from standard formats, as well as signature, fingerprint, and molecular property generation. It is built on the Chemistry Development Toolkit and makes use of the Chemical Markup Language to maintain consistency (Beisken et al. 2013). The Connectivity Map (CMap) project, as well as its expanded Library of Integrated Network-Based Cellular Signatures (LINCS), is widely regarded as a crucial concept in a number of well-known medication repurposing studies. The CMap's expanded effort results in large-scale gene expression profiles from human cancer cells that can be targeted by multiple treatment molecules in diverse conditions (Vidović et al. 2014; Subramanian et al. 2017).

Vásquez-Bochm et al. (2019) used a consensus gene profile that characterizes breast cancer stem cells to query CMap to repurpose medicines that target breast cancer stem cells. The structural similarity of the potential medications was investigated using the KNIME program in the CDK module, which generated fingerprints using circular and MACCS approaches and compared them using the Tanimoto coefficient (Vásquez-Bochm et al. 2019).

The Web Ontology Language (OWL), which is commonly used in bio-ontologies like Gene Ontology, is a sophisticated language for developing complicated domain ontologies (Tudose et al. 2013). Cheminformatics, a collection of computational technologies that may be utilized to tackle a variety of chemical challenges, can be used to find and assess new pharmacogenomics (PGx) connections.

8.4 Effect of DR in Different Pathways

For various cancer kinds, molecular pathways' operation and interaction are quite important. Previous research has shown that the dysregulation of numerous signaling pathways, such as Wnt, PI3K/Akt, AMPK, NF-B, and MAPK, is a major factor in the advancement of CRC, and other significant possible molecular processes and emphasize their impact on CRC are illustrated in Fig. 8.2. Furthermore, the way these channels interact is intricate and precise. Additionally, a growing body of evidence indicates that either cancer itself might cause genetic disturbance or epigenetic dysregulation, or that it can stimulate the formation of CRC. Recent advances in understanding these crucial potential molecular processes and highlighting how they relate to CRC will soon provide an appealing treatment approach for the disease.

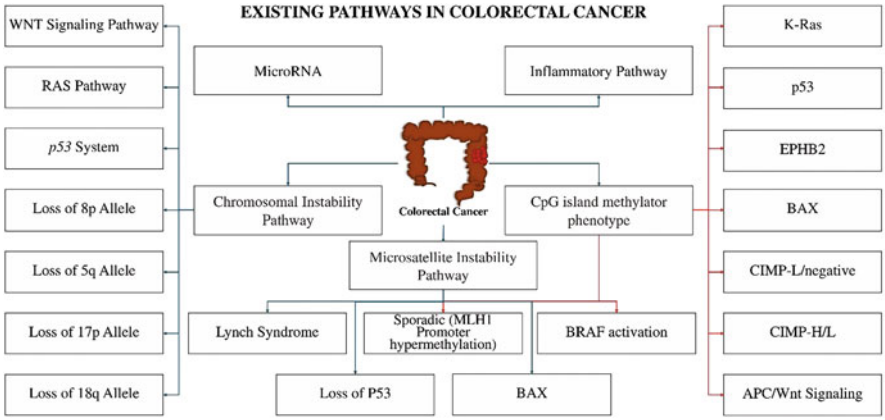


Fig. 8.2 Existing pathways in colorectal cancer: The three predominant pathways are chromosomal instability (alludes to an alarming pace of gains or losses of complete or significant sections of chromosomes that causes karyotypic diversity from cell to cell. It is seen in 65–70% of sporadic colorectal tumors), **CpG island methylator phenotype** (a subtype of colorectal tumors that develops via an epigenetic instability route and is distinguished by extensive hypermethylation of promoters CpG island sites, which results in the inactivation of numerous tumor suppressor genes or additional tumor-related genes)**,** and microsatellite instability pathway (molecular signature of the underactive mismatch repair (MMR) mechanism, which accounts for about 15% of colorectal malignancies)**,** while the two occasional pathways are microRNA and the inflammatory pathway

8.4.1 Wnt Pathway

The Wnt signaling pathway is important for cell growth and stem-cell differentiation. Multiple diseases have been linked to Wnt mutations, including breast and prostate cancer, glioma, and diabetes. Almost all CRCs begin with an activating mutation in the Wnt/β -catenin pathway (Miller et al. 1999). A Wnt protein often binds to the Frizzled family of receptors' domain. Through direct interactions, these transmembrane G-protein coupled receptors transmit a signal to the disheveled phosphoproteins in the cytoplasm. Wnt/β-catenin pathway reactivity is often induced by a mutation in the adenomatous polyposis coli (APC) gene. This causes a shortened APC protein that is incapable to complete its mechanism as a component of the β-catenin destruction complex, culminating in diminished β-catenin degradation and unusually high levels of cytoplasmic β-catenin. This stabilized β- catenin translocates to the nucleus and attaches to members of the T-cell factor (Tcf)/lymphoid enhancer factor transcription factor group, triggering transcription of Wnt targets gene expression such as Myc and cyclin D (Mann et al. 1999).

8.4.2 mTOR Signaling Pathway—The Role of AMPK Activation in Aspirin-Mediated mTOR Inhibition

Din et al. (2012) evaluated the impact of aspirin on adenosine monophosphate-activated protein kinase (AMPK) and the mechanistic target of rapamycin (mTOR) signaling in CRC cells. These findings, in addition to the siRNA results, proved that aspirin may suppress mTOR through both AMPK-dependent and independent pathways. The effects of aspirin on the mTORC1 target proteins and its substrate ribosomal protein were studied in three CRC cell lines: SW480, RKO, and HCT116. Phosphorylation of ACC provided a more accurate picture of AMPK enzyme activity. Increased AMPK phosphorylation was accompanied by enhanced ACC phosphorylation. Using quantitative kinase assays that mirrored the phosphorylation state of AMPK and ACC, Din et al. (2012) confirmed that aspirin enhances AMPK activity. This study's findings on AMPK and ACC phosphorylation, along with AMP kinase activity, demonstrate unequivocally that aspirin regulates AMPK in CRC cells (Din et al. 2012).

8.4.3 Inhibition of Ras/ERK and Ras/mTOR Pathways

The expression of Ras protein and the activity of its downstream effectors were investigated by Tsubaki et al. (2017) to determine the molecules implicated in statin-induced apoptosis. Statins reduced the levels of phosphorylated ERK and mTOR and repressed Ras membrane localization in HSC-3 and HEp-2 cells. Ras lowered the phosphorylation of mTOR and ERK1/2 and raised the expression of Bim because of suppressing geranylgeranyl pyrophosphate (GGPP) production. In addition, a combination of PD0325901 (a MEK inhibitor) and rapamycin (an mTOR inhibitor) was found to trigger cell death, inhibit ERK1/2 and mTOR, and increase Bim expression in cells (Tsubaki et al. 2017).

8.4.4 ERK/Akt Pathway

Statins trigger apoptotic cell death in various types of growing tumor cells in a cholesterol-independent way. Mevalonate is transformed to farnesyl pyrophosphate (FPP) or GGPP, which can be prenylated and attached to intracellular proteins, ensuring their relocalization in cell membranes (Wu et al. 2004). According to Qi et al. (2013) treatment with statin accelerated DNA fragmentation and the induction of proapoptotic proteins such as PARP, caspase 3, and Bax. N-acetylcysteine, an antioxidant, and metabolic precursors of the HMG-CoA reductase pathway, such as FPP, mevalonate, and GGPP, reduced statin-induced cytotoxicity, DNA fragmentation, as well as substantial changes in ERK, caspase-3, Akt, and p38 activation

(Qi et al. 2013). Statins inhibit GGPP production in the mevalonate pathway, then inhibit signal transduction in the Ras/ERK and Ras/Akt pathways (Tsubaki et al. 2016).

8.4.5 AMPK-NF-κB Signaling

The protein complex nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) works as a signal-induced transcription factor. Mutations in genes that encode NF-κB or govern NF-κB activity, such as the IκB genes, activate NF-κB in tumor cells. In recent decades, the NF-κB, a transcription factor involved in the regulation of immunological responses and inflammation, has been recognized as playing a key role in cancer formation and progression. Metformin treatment activates AMPK-NF-κB signaling in cancer cells, which participates in regulating M1 and M2 inducing cytokine expression by increasing AMPK and p65 phosphorylation (Li et al. 2017).

Kim et al. (2011) revealed metformin as an anticancer agent. In his findings, metformin decreased the activation of NF-κB and MDR1 promoter activity significantly and also reduced adriamycin-activated NF-κB and MDR1 promoter activity in a dose-dependent manner. Researchers investigated if metformin inhibits MDR1 and NF-κB via suppressing IκB- α phosphorylation, and it was found to suppress MDR1 protein and mRNA expression that was increased by TNF- α. TNF- α induced NF-κB activation which was reduced by metformin. Therefore, metformin decreased MDR1 by lowering NF-κB activation, according to these findings (Kim et al. 2011).

8.5 Large Data Analysis and Precise Personal Therapy

8.5.1 Genome-Wide Association Studies (GWAS)

The most reliable statistical tool for examining a person's genetic predisposition to multifaceted diseases is GWAS. A substantial amount of data analysis has shown that genetic targets frequently propose potent pharmacological targets, with many convincing instances coming from GWAS. By using a hypothesis-free methodology, GWAS enables simultaneously examining millions of single nucleotide polymorphisms (SNPs) throughout the genome, allowing for a comprehensive examination of the influence of specific genetic variants on a given characteristic. GWAS has been used extensively in studies on several forms of malignant tumors since its inception. With the use of GWAS, researchers have discovered numerous tumor-vulnerable locations and areas and have made significant progress (Plenge et al. 2013; Nelson et al. 2015).

According to GWAS, SMAD7, which is situated on chromosome 18, contains significant genetic variations that have now been linked to colorectal cancer. An SNP in SMAD7's intron 3 called rs4939827 and an adjacent intronic SNP called rs12953717 were both found to have a substantially considerable correlation with colorectal neoplasia. A strong relationship was also discovered by Broderick et al. (2007) for the intronic SNP rs4464148 (Broderick et al. 2007; Tenesa et al. 2008). In SLC22A3, Cui et al. (2011) discovered a unique susceptibility locus that raises the possibility of proximal colon cancer in an Asian population (Japanese and Korean). The new locus on 6q26-q27 was substantially related to distal colon cancer (rs7758229 in SLC22A3, $p = 7.9210–9$, OR of 1.28). The link involving CRC and SNPs on 8q24 (rs6983267 and rs7837328, $p = 1.51108$ and 7.44108, ORs of 1.18 and 1.17, respectively) was also observed. Additionally, synergistic impacts of three genetic factors (rs6983267, rs7758229, and rs4939827 in SMAD7) and one extrinsic component (alcohol use) seem to roughly double the risk of CRC were also revealed (Cui et al. 2011).

Tenesa et al. (2008) genotyped 555,510 SNPs in 1012 earlier-stage Scottish CRC patients and 1012 controls for genome-wide associate research to find loci linked to CRC susceptibility (phase 1). In phase 2, 2057 Scottish malignancies were genotyped and 2111 controls for the 15,008 top-ranked SNPs. A previously unreported link at rs3802842 on 11q23 that demonstrated population-specific risk differences was uncovered. Additionally, the validation and fine mapping of correlations were at 8q24 and 18q21 (rs7014346; OR = 1.19; $P = 8.6 \times 10^{-26}$, rs4939827; OR = 1.2; $P = 7.8 \times 10^{-28}$, respectively). In both rs4939827 (P 0.009) and rs3802842 (P 0.008), the risk of rectal cancer was higher than the risk of colon cancer (Tenesa et al. 2008). These findings expand overall knowledge of the extensive genetic variation that plays a role in the etiology of CRC.

8.5.2 Electronic Health Records (EHRs)

A growing body of research has shown how useful EHR data is for locating promising therapeutic drug side effects and drug–drug interactions. As a result of their constant and longitudinal monitoring of clinically important outcomes and pharmaceutical exposures, EHRs may be especially helpful when examining treatment effects. The "real-world" circumstances of patient medication use, and treatment trends are represented by EHR data, making it a valuable tool for identifying the clinical ramifications of drug–genome interactions (Shickel et al. 2017). According to a study conducted by Rodríguez-Miguel et al. (2019) low-dose aspirin may have an antiplatelet impact by inhibiting cyclooxygenase-1, which may help explain why it lowers the risk of CRC and measure how low-dose aspirin and clopidogrel affected a Mediterranean population's prevalence of CRC, where EHR was reviewed manually by 2 independent researchers blinded to drug prescriptions in a supplementary method (Rodríguez-Miguel et al. 2019). Using information from the Department of Veterans Affairs EHR, a case-control research was conducted on

US Veterans with prevalent diabetes who had colonoscopies between 1999 and 2014. In contrast to controls, cases were identified by the presence of CRC during colonoscopy. Metformin was linked to an 8% absolute decrease in CRC chances (Demb et al. 2019).

8.5.3 PheWAS

Precision medicine is now seen from a unique angle thanks to the unification of EHRs for diverse illness classes from diverse racial groups and genomic data. In light of this, the phenome-wide association study (PheWAS), which incorporates data from both GWAS and EHRs from sizable cohort studies, has surfaced as yet another cutting-edge paradigm. The PheWAS widened the scope of the genotype-phenotype association and allowed researchers to discover novel applications for previously used medications. Zhang et al. (2022) sought to ascertain regardless of whether such a risk of other traits is likewise influenced by a genetic predisposition to CRC. They analyzed 334,385 unrelated White British people from the UK Biobank cohort (excluding CRC patients) using the PheWAS and tree-structured phenotypic model. From CRC genome-wide correlation studies, a polygenic risk score was created as a gauge of CRC risk (Zhang et al. 2022). The study that used DrugBank and PheWAS to analyze potential medication repurposing candidates for treating both prevalent and unusual disorders serves as an example of it. In this, 52,966 drug-disease combinations were identified, with around one-third of these pairings being substantiated by current publications, clinical trials, and drug–disease relationships, while the rest matches may be candidates for forthcoming drug repurposing studies (Denny et al. 2013; Law et al. 2014).

8.6 Conclusion

Repurposing tactics on a functional, semi-mechanistic foundation, such as the one provided by pathway-based analysis, is an emerging and effective area of study and intervention. This is not surprising given that the main objective of pharmacological therapies is to modulate functional features and processes on both a physiological and functional level. As the importance of highly precise and personalized medicine grows, mechanism-based repurposing initiatives are anticipated to be broadened to identify new indications for individual patients. Also, pathway-based studies offer a good approximation of these functional processes, leading us to speculate that conclusions drawn from them may be more useful in developing successful anticancer therapies. The approaches designed for big data merge information from many perspectives, make it easier to screen drug candidates, and allow for the predicting of drug toxicity, greatly expediting the discovery of new drugs and enhancing their

efficacy. Precision medicine strives to develop prevention and treatment plans that take individual heterogeneity into consideration. Its reliance on big data will grow as personalized therapy gradually gains prominence. The generation of complicated datasets and substantially higher quantities for precision medicine is imminent.

Acknowledgments The authors are grateful to the Chettinad Academy of Research and Education (CARE), Chennai, India, and University of Linkoping, Sweden for providing the infrastructural support as well as carrying out the research work. Dr. Surajit Pathak acknowledges the Department of Science and Technology (DST) – Science and Engineering Research Board (SERB) to sanction the EMR Project (EMR/2017/001877).

Availability of Data NA.

Author Contribution Conceptualization XFS, SP, HG, AB (Writing, Review, and Editing)—XFS, SP, HG, AB, SR, SJ, HG. All authors revised and approved the content of the manuscript.

Funding Statement The authors are thankful to the Chettinad Academy of Research and Education and the University of Linkoping, Sweden for their support.

Conflict of Interest The authors declare no conflicts of interest.

References

Agrawal P (2015) Advantages and challenges in drug re-profiling. J Pharmacovigil 2:2–3

Alburquerque-González B, Bernabé-García M, Montoro-García S, Bernabé-García Á, Rodrigues PC, Ruiz Sanz J, López-Calderón FF, Luque I, Nicolas FJ, Cayuela ML, Salo T (2020) New role of the antidepressant imipramine as a Fascin1 inhibitor in colorectal cancer cells. Exp Mol Med 52:281–292

Badria FA (ed) (2020) Drug repurposing: hypothesis, molecular aspects and therapeutic applications. BoD–Books on Demand

Beisken S, Meinl T, Wiswedel B, de Figueiredo LF, Berthold M, Steinbeck C (2013) KNIME-CDK: workflow-driven cheminformatics. BMC Bioinform 14:1–4

Briels B, de Graaf C, Bender A (2020) Structural chemogenomics: profiling protein–ligand interactions in polypharmacological space. In: Structural biology in drug discovery: methods, techniques, and practices, pp 53–77

Broderick P, Carvajal-Carmona L, Pittman AM, Webb E, Howarth K, Rowan A, Lubbe S, Spain S, Sullivan K, Fielding S, Jaeger E (2007) A genome-wide association study shows that common alleles of SMAD7 influence colorectal cancer risk. Nat Genet 39:1315–1317

Cha Y, Erez T, Reynolds IJ, Kumar D, Ross J, Koytiger G, Kusko R, Zeskind B, Risso S, Kagan E, Papapetropoulos S (2018) Drug repurposing from the perspective of pharmaceutical companies. Br J Pharmacol 175:168–180

Chittepu VC, Kalhotra P, Osorio-Gallardo T, Gallardo-Velázquez T, Osorio-Revilla G (2019) Repurposing of FDA-approved NSAIDs for DPP-4 inhibition as an alternative for diabetes mellitus treatment: computational and in vitro study. Pharmaceutics 11:238

Cui R, Okada Y, Jang SG, Ku JL, Park JG, Kamatani Y, Hosono N, Tsunoda T, Kumar V, Tanikawa C, Kamatani N (2011) Common variant in 6q26-q27 is associated with distal colon cancer in an Asian population. Gut 60:799–805

Demb J, Yaseyyedi A, Liu L, Bustamante R, Earles A, Ghosh P, Gutkind JS, Gawron AJ, Kaltenbach TR, Martinez ME, Gupta S (2019) Metformin is associated with reduced odds for colorectal cancer among persons with diabetes. Clin Transl Gastroenterol 10:e00092

Deng Z, Chuaqui C, Singh J (2004) Structural interaction fingerprint (SIFt): a novel method for analyzing three-dimensional protein−ligand binding interactions. J Med Chem 47:337–344

Denny JC, Bastarache L, Ritchie MD, Carroll RJ, Zink R, Mosley JD, Field JR, Pulley JM, Ramirez AH, Bowton E, Basford MA (2013) Systematic comparison of phenome-wide association study of electronic medical record data and genome-wide association study data. Nat Biotechnol 31: 1102–1111

Desaphy J, Raimbaud E, Ducrot P, Rognan D (2013) Encoding protein–ligand interaction patterns in fingerprints and graphs. J Chem Inf Model 53:623–637

Din FV, Valanciute A, Houde VP, Zibrova D, Green KA, Sakamoto K, Alessi DR, Dunlop MG (2012) Aspirin inhibits mTOR signaling, activates AMP-activated protein kinase, and induces autophagy in colorectal cancer cells. Gastroenterology 142:1504–1515

Ferreira LG, Andricopulo AD (2016) Drug repositioning approaches to parasitic diseases: a medicinal chemistry perspective. Drug Discov 21:1699–1710

Forli S, Huey R, Pique ME, Sanner MF, Goodsell DS, Olson AJ (2016) Computational protein–ligand docking and virtual drug screening with the AutoDock suite. Nat Protoc 11:905–919

Halgren T (2007) New method for fast and accurate binding-site identification and analysis. Chem Biol Drug Des 69:146–148

Hernandez JJ, Pryszlak M, Smith L, Yanchus C, Kurji N, Shahani VM, Molinski SV (2017) Giving drugs a second chance: overcoming regulatory and financial hurdles in repurposing approved drugs as cancer therapeutics. Front Oncol 7:273

Hwang H, Dey F, Petrey D, Honig B (2017) Structure-based prediction of ligand–protein interactions on a genome-wide scale. Proc Natl Acad Sci U S A 114:13685–13690

Islam S, Wang S, Bowden N, Martin J, Head R (2022) Repurposing existing therapeutics, its importance in oncology drug development: kinases as a potential target. Br J Clin Pharmacol 88: 64–74

Issa NT, Stathias V, Schürer S, Dakshanamurthy S (2021) Machine and deep learning approaches for cancer drug repurposing. Semin Cancer Biol 68:132–142. Academic Press

Jarada TN, Rokne JG, Alhajj R (2020) A review of computational drug repositioning: strategies, approaches, opportunities, challenges, and directions. J Cheminform 12:1–23

Jourdan JP, Bureau R, Rochais C, Dallemagne P (2020) Drug repositioning: a brief overview. J Pharm Pharmacol 72:1145–1151

Karuppasamy R, Verma K, Sequeira VM, Basavanna LN, Veerappapillai S (2017) An integrative drug repurposing pipeline: switching viral drugs to breast cancer. J Cell Biochem 118:1412–1422

Kim HG, Hien TT, Han EH, Hwang YP, Choi JH, Kang KW, Kwon KI, Kim BH, Kim SK, Song GY, Jeong TC (2011) Metformin inhibits P-glycoprotein expression via the NF-κB pathway and CRE transcriptional activity through AMPK activation. Br J Pharmacol 162:1096–1108

Kune GA, Kune S, Watson LF (1988) Colorectal cancer risk, chronic illnesses, operations, and medications: case control results from the Melbourne colorectal cancer study. Cancer Res 48: 4399–4404

Law V, Knox C, Djoumbou Y, Jewison T, Guo AC, Liu Y, Maciejewski A, Arndt D, Wilson M, Neveu V, Tang A (2014) DrugBank 4.0: shedding new light on drug metabolism. Nucleic Acids Res 42:D1091–D1097

Li YY, Jones SJ (2012) Drug repositioning for personalized medicine. Genome Med 4:1–4

Li C, Zhao Z, Zhou J, Liu Y, Wang H, Zhao X (2017) Relationship between the TERT, TNIP1 and OBFC1 genetic polymorphisms and susceptibility to colorectal cancer in Chinese Han population. Oncotarget 8:56932

Liu X, Ouyang S, Yu B, Liu Y, Huang K, Gong J, Zheng S, Li Z, Li H, Jiang H (2010) PharmMapper server: a web server for potential drug target identification using pharmacophore mapping approach. Nucleic Acids Res 38:W609–W614

Lokhande KB, Ballav S, Thosar N, Swamy KV, Basu S (2020) Exploring conformational changes of PPAR-Ɣ complexed with novel kaempferol, quercetin, and resveratrol derivatives to understand binding mode assessment: a small-molecule checkmate to cancer therapy. J Mol Model 26:1–2

Lubbe SJ, Pittman AM, Matijssen C, Twiss P, Olver B, Lloyd A, Qureshi M, Brown N, Nye E, Stamp G, Blagg J (2011) Evaluation of germline BMP4 mutation as a cause of colorectal cancer. Hum Mutat 32:E1928–E1938

Luo Y, Zhao X, Zhou J, Yang J, Zhang Y, Kuang W, Peng J, Chen L, Zeng J (2017) A network integration approach for drug-target interaction prediction and computational drug repositioning from heterogeneous information. Nat Commun 8:573

Mann B, Gelos M, Siedow A, Hanski ML, Gratchev A, Ilyas M, Bodmer WF, Moyer MP, Riecken EO, Buhr HJ, Hanski C (1999) Target genes of β-catenin–T cell-factor/lymphoid-enhancer-factor signaling in human colorectal carcinomas. Proc Natl Acad Sci U S A 96:1603–1608

Meng F, Song L, Wang W (2017) Metformin improves overall survival of colorectal cancer patients with diabetes: a meta-analysis. J Diabetes Res 2017:5063239

Miller JR, Hocking AM, Brown JD, Moon RT (1999) Mechanism and function of signal transduction by the Wnt/β-catenin and Wnt/Ca2+ pathways. Oncogene 18:7860–7872

Miller MS, Maheshwari S, McRobb FM, Kinzler KW, Amzel LM, Vogelstein B, Gabelli SB (2017) Identification of allosteric binding sites for PI3Kα oncogenic mutant specific inhibitor design. Bioorg Med Chem 25:1481–1486

Mitchel J, Bajaj P, Patil K, Gunnarson A, Pourchet E, Kim YN, Skolnick J, Pai SB (2021) Computational identification of stearic acid as a potential PDK1 inhibitor and in vitro validation of stearic acid as colon cancer therapeutic in combination with 5-fluorouracil. Cancer Inform 20: 117693512110659

Mundy G, Garrett R, Harris S, Chan J, Chen D, Rossini G, Boyce B, Zhao M, Gutierrez G (1999) Stimulation of bone formation in vitro and in rodents by statins. Science 286:1946–1949

Nelson MR, Tipney H, Painter JL, Shen J, Nicoletti P, Shen Y, Floratos A, Sham PC, Li MJ, Wang J, Cardon LR (2015) The support of human genetic evidence for approved drug indications. Nat Genet 47:856–860

Oprea TI, Bauman JE, Bologa CG, Buranda T, Chigaev A, Edwards BS, Jarvik JW, Gresham HD, Haynes MK, Hjelle B, Hromas R (2011) Drug repurposing from an academic perspective. Drug Discov Today Ther Strateg 8:61–69

Pantziarka P, Bouche G, Meheus L, Sukhatme V, Sukhatme VP (2015) Repurposing drugs in your medicine cabinet: untapped opportunities for cancer therapy? Future Oncol 11:181–184

Patil VM, Gaurav A, Garg P, Masand N (2021) Non-cancer to anti-cancer: investigation of human ether-a-go-go-related gene potassium channel inhibitors as potential therapeutics. J Egypt Natl Canc Inst 33:1–6

Pernicova I, Korbonits M (2014) Metformin-mode of action and clinical implications for diabetes and cancer. Nat Rev Endocrinol 10:143–156

Plenge RM, Scolnick EM, Altshuler D (2013) Validating therapeutic targets through human genetics. Nat Rev Drug Discov 12:581–594

Pushpakom S, Iorio F, Eyers PA, Escott KJ, Hopper S, Wells A, Doig A, Guilliams T, Latimer J, McNamee C, Norris A (2019) Drug repurposing: progress, challenges, and recommendations. Nat Rev Drug Discov 18:41–58

Qi XF, Zheng L, Lee KJ, Kim DH, Kim CS, Cai DQ, Wu Z, Qin JW, Yu YH, Kim SK (2013) HMG-CoA reductase inhibitors induce apoptosis of lymphoma cells by promoting ROS generation and regulating Akt, Erk and p38 signals via suppression of mevalonate pathway. Cell Death Dis 4:e518

Ritchie MD, Holzinger ER, Li R, Pendergrass SA, Kim D (2015) Methods of integrating data to uncover genotype–phenotype interactions. Nat Rev Genet 16:85–97

Rodríguez-Miguel A, García-Rodríguez LA, Gil M, Montoya H, Rodríguez-Martín S, de Abajo FJ (2019) Clopidogrel and low-dose aspirin, alone or together, reduce risk of colorectal cancer. Clin Gastroenterol Hepatol 17:2024–2033

Rothwell PM, Price JF, Fowkes FG, Zanchetti A, Roncaglioni MC, Tognoni G, Lee R, Belch JF, Wilson M, Mehta Z, Meade TW (2012) Short-term effects of daily aspirin on cancer incidence, mortality, and non-vascular death: analysis of the time course of risks and benefits in 51 randomised controlled trials. Lancet 379:1602–1612

Rutledge BP, Desai P, Liu S, Luo J, Nassir R, Lihong Q, Arun M, Abdel-Rasoul M, Simon MS (2019) The association between statins and colorectal cancer stage in the Women's Health Initiative. Mol Clin Oncol 11:252–258

Salentin S, Schreiber S, Haupt VJ, Adasme MF, Schroeder M (2015) PLIP: fully automated protein–ligand interaction profiler. Nucleic Acids Res 43:W443–W447

Salentin S, Adasme MF, Heinrich JC, Haupt VJ, Daminelli S, Zhang Y, Schroeder M (2017) From malaria to cancer: computational drug repositioning of amodiaquine using PLIP interaction patterns. Sci Rep 7:1–3

Shickel B, Tighe PJ, Bihorac A, Rashidi P (2017) Deep EHR: a survey of recent advances in deep learning techniques for electronic health record (EHR) analysis. IEEE J Biomed Health Inform 22:1589–1604

Subramanian A, Narayan R, Corsello SM, Peck DD, Natoli TE, Lu X, Gould J, Davis JF, Tubelli AA, Asiedu JK, Lahr DL (2017) A next generation connectivity map: L1000 platform and the first 1,000,000 profiles. Cell 171:1437–1452

Talevi A (2018) Drug repositioning: current approaches and their implications in the precision medicine era. Expert Rev Precis Med Drug Dev 3:49–61

Talevi A, Bellera CL (2020) Challenges and opportunities with drug repurposing: finding strategies to find alternative uses of therapeutics. Expert Opin Drug Discov 15:397–401

Tenesa A, Farrington SM, Prendergast JG, Porteous ME, Walker M, Haq N, Barnetson RA, Theodoratou E, Cetnarskyj R, Cartwright N, Semple C (2008) Genome-wide association scan identifies a colorectal cancer susceptibility locus on 11q23 and replicates risk loci at 8q24 and 18q21. Nat Genet 40:631–637

Thorat MA, Cuzick J (2013) Role of aspirin in cancer prevention. Curr Oncol Rep 15:533–540

Tougeron D, Sha D, Manthravadi S, Sinicrope FA (2014) Aspirin and colorectal cancer: back to the future. Clin Cancer Res 20:1087–1094

Tsubaki M, Mashimo K, Takeda T, Kino T, Fujita A, Itoh T, Imano M, Sakaguchi K, Satou T, Nishida S (2016) Statins inhibited the MIP-1α expression via inhibition of Ras/ERK and Ras/Akt pathways in myeloma cells. Biomed Pharmacother 78:23–29

Tsubaki M, Fujiwara D, Takeda T, Kino T, Tomonari Y, Itoh T, Imano M, Satou T, Sakaguchi K, Nishida S (2017) The sensitivity of head and neck carcinoma cells to statins is related to the expression of their Ras expression status, and statin-induced apoptosis is mediated via suppression of the Ras/ERK and Ras/mTOR pathways. Clin Exp Pharmacol Physiol 44:222–234

Tudose I, Hastings J, Muthukrishnan V, Owen G, Turner S, Dekker A, Kale N, Ennis M, Steinbeck C (2013) Ontoquery: easy-to-use web-based owl querying. Bioinformatics 29:2955–2957

Vásquez-Bochm LX, Velázquez-Paniagua M, Castro-Vázquez SS, Guerrero-Rodríguez SL, Mondragon-Peralta A, La Fuente-Granada D, Pérez-Tapia SM, González-Arenas A, Velasco-Velázquez MA (2019) Transcriptome-based identification of lovastatin as a breast cancer stem cell-targeting drug. Pharmacol Rep 71:535–544

Vidović D, Koleti A, Schürer SC (2014) Large-scale integration of small molecule-induced genome-wide transcriptional responses, Kinome-wide binding affinities and cell-growth inhibition profiles reveal global trends characterizing systems-level drug action. Front Genet 30:5–342

Waszkowycz B, Clark DE, Gancia E (2011) Outstanding challenges in protein–ligand docking and structure-based virtual screening. Wiley Interdiscip Rev Comput Mol Sci 1:229–259

Wolber G, Langer T (2005) LigandScout: 3-D pharmacophores derived from protein-bound ligands and their use as virtual screening filters. J Chem Inf Model 45:160–169

Wu J, Wong WW, Khosravi F, Minden MD, Penn LZ (2004) Blocking the Raf/MEK/ERK pathway sensitizes acute myelogenous leukemia cells to lovastatin-induced apoptosis. Cancer Res 64: 6461–6468

Yamanishi Y, Kotera M, Kanehisa M, Goto S (2010) Drug-target interaction prediction from chemical, genomic and pharmacological data in an integrated framework. Bioinformatics 26: i246–i254

Yan XY, Zhang SW, He CR (2019) Prediction of drug-target interaction by integrating diverse heterogeneous information source with multiple kernel learning and clustering methods. Comput Biol Chem 78:460–467

Zhang L, He H, Balschi JA (2007) Metformin and phenformin activate AMP-activated protein kinase in the heart by increasing cytosolic AMP concentration. Am J Physiol Heart Circ Physiol 293:H457–H466

Zhang X, Li X, He Y, Law PJ, Farrington SM, Campbell H, Tomlinson IP, Houlston RS, Dunlop MG, Timofeeva M, Theodoratou E (2022) Phenome-wide association study (PheWAS) of colorectal cancer risk SNP effects on health outcomes in UK biobank. Br J Cancer 126:822–830

Chapter 9
Targeting the Ubiquitin Machinery for Cancer Therapeutics

Janvi Patel and Ekta Tripathi

Abstract The ubiquitin–proteasome system (UPS) is an important player in various biological processes maintaining cellular homeostasis. The regulation of all essential components of UPS (Ubiquitinating enzymes-E1, E2, E3, deubiquitinases, and proteasome machinery) is complex and multifaceted, and their dysregulation has been implicated in several diseases including cancer. Increasing number of studies has established UPS as a cancer therapeutic target. In this chapter, we discuss the role of each component of the ubiquitin pathway in maintaining physiological function and their deregulation in cancer. In addition, we also discuss the enzymes that could serve as promising drug targets for anticancer therapy.

Keywords Ubiquitination · Deubiquitination · Proteasome · Cancer therapy

9.1 Introduction

Protein metabolism, including protein synthesis and degradation, is crucial for the maintenance of cellular homeostasis and viability. In eukaryotic cells, proteins are degraded through three different mechanisms: lysosomes degrade endocytosed proteins, mitochondrial proteases degrade majority of mitochondrial proteins, and ubiquitin–proteasome system (UPS) degrades short-lived intracellular proteins in an ATP-dependent manner (Mitch and Goldberg 1996). It was first discovered from biochemical studies that ubiquitin modification provides proteolytic signals for the degradation of misfolded proteins (Etlinger and Goldberg 1977). The UPS system is a major pathway for maintaining protein homeostasis thereby regulating several biological functions (Pickart 2004). Dysregulation of UPS has been observed in

J. Patel
Centre for Infectious Disease Research, Department of Microbiology and Cell Biology, Indian Institute of Science, Bengaluru, India

E. Tripathi (✉)
Department of Biotechnology, Faculty of Life and Allied Health Sciences, M.S. Ramaiah University of Applied Sciences, Bengaluru, India
e-mail: ektatripathi.bt.ls@msruas.ac.in

R. C. Sobti et al. (eds.), *Drug Repurposing for Emerging Infectious Diseases and Cancer*, https://doi.org/10.1007/978-981-19-5399-6_9

many diseases, including cancer, making it a promising target for anticancer therapy. The discovery of ubiquitin-mediated protein degradation fetched scientists Aaron Ciechanover, Avram Hershko, and Irwin Rose, the Nobel Prize in Chemistry in 2004.

9.1.1 Ubiquitin–Proteasome System

The ubiquitin–proteasome system consists of 2 components—an array of enzymes that tag the protein to be destructed with a small molecule ubiquitin (Ub) and a giant cytosolic protease: Proteasome that degrades these Ub-tagged proteins/substrates. Ubiquitin-mediated degradation of protein occurs in two consecutive steps: (1) covalent addition of multiple ubiquitin molecules to the cellular proteins and (2) recognition and degradation of conjugated proteins by the 26S proteasome complex (Ciechanover et al. 2000; Coux et al. 1996). The ubiquitination of protein begins with the ordered action of three enzymes (Fig. 9.1). First, the ubiquitin is activated by the E1-activating enzyme in an ATP-dependent manner. The activated ubiquitin is then transferred from E1 to the E2-conjugating enzyme, forming an intermediate complex E2-ubiquitin. Finally, the specific E3 ligase binds to the E2–Ub complex and allows the transfer of ubiquitin onto the targeted protein (Nalepa et al. 2006). UPS is involved in the regulation of various cellular processes such as DNA damage repair, cell cycle, apoptosis, modulation of the inflammatory response, transcription, development, and differentiation, and therefore defects in this pathway result in a number of diseases, including development of different types of tumors (Hoeller et al. 2006).

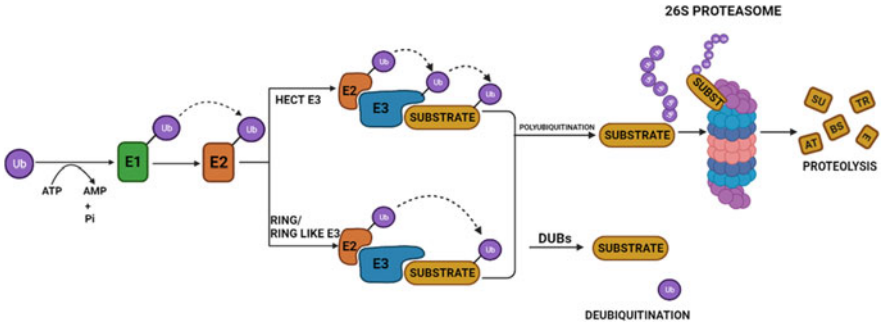


Fig. 9.1 The ubiquitin proteasome system: In the UPS system, first the ubiquitin is activated by E1 and then transferred to E2 which is then conjugated to the substrate with specific E3 ligase. Further polyubiquitination of the substrate leads to proteasomal degradation of the substrate via 26S proteasome and this activity is reversed in the presence of DUBs

9.1.1.1 Ubiquitin

Ubiquitin, a highly conserved polypeptide consisting of 76 amino acid residues, was discovered in 1975. In the human genome, there are four genes that code for ubiquitin—UBB, UBC, UBA52, and RPS27A. Each ubiquitin molecule has seven lysine residues (K6, K11, K27, K29, K33, K48, and K63), all of which can be ubiquitinated to form ubiquitin chains (Fig. 9.2). The C-terminus of one ubiquitin can be covalently linked to second ubiquitin via ɛamino-terminal of lysyl residues forming ubiquitin chains. Several studies have observed that K-11 and K-48 polyubiquitin linkages are the markers for protein degradation in a proteasome-dependent manner, while K-63 is a nonproteolytic modification that regulates signal transduction and other functions (Akutsu et al. 2016). K27 linkages are shown to be important for modulating the immune response. Hence, different polyubiquitination modifications decide the distinct fates of the substrate proteins.

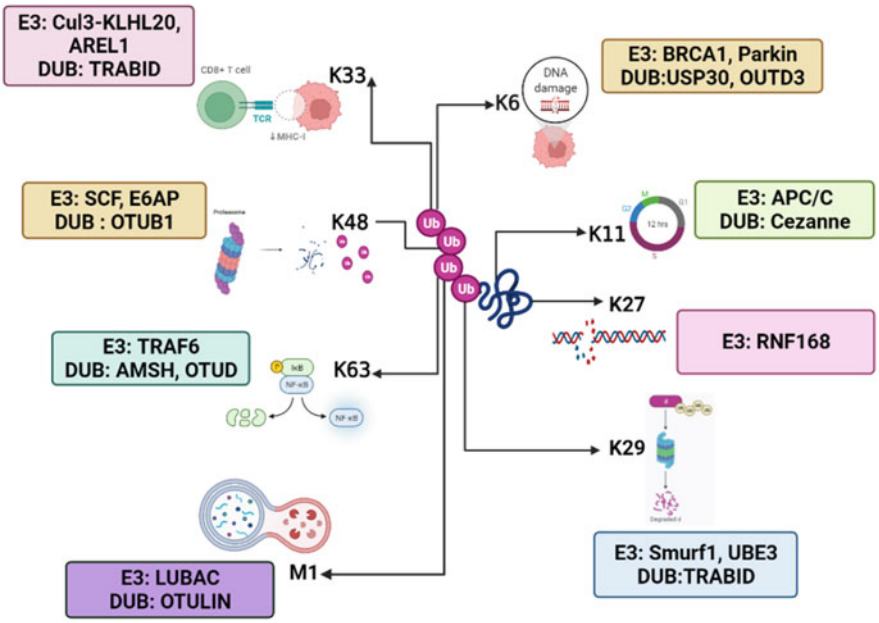


Fig. 9.2 Ubiquitin linkages and their functions: This figure shows various polyubiquitination modifications via different E3 ligase enzymes. Polyubiquitination of K6 is involved in DNA damage repair, K11 is involved in cell cycle regulation, K27 plays important role in DNA damage response. K29 is associated with Ub fusion degradation. K33 is involved with TCR signaling. K48 gives a canonical signal for proteasomal degradation. K63 is involved in NF-κB signaling and M1 plays a key role in angiogenesis and selective autophagy

9.1.1.2 Proteasome Machinery

The 26S proteasome is a giant (2.5 MDa) complex that catalyzes the degradation of polyubiquitinated proteins into small peptides. It consists of two subcomplexes: a cylindrical proteolytic core complex (20S proteasome) and one or two regulatory complexes (19S complex). The 19S RP binds to the 20S proteasome to form an active enzyme complex with the sedimentation coefficient of 26S, hence referred to as the 26S proteasome (Groll et al. 2000). The proteasome possesses three types of catalytic activities: caspase-like, trypsin-like, and chymotrypsin-like, which allow them to cleave peptide bonds at the C-terminus of acidic, basic, and hydrophobic amino-acid residues, respectively. 19S RP recognizes the ubiquitinated protein, and after binding, there is a change in conformation of 19S that allows the protein to enter the 20S chamber where the ubiquitin chain is cleaved. After the removal of the ubiquitin chain, ATPase subunits of 19S enable the substrate to enter the proteolytic chamber, where the polypeptide is further degraded into short peptides in an ATP-dependent manner (Kleiger and Mayor 2014).

9.1.2 Classification of Ubiquitination and Deubiquitination Enzymes

The ubiquitin proteasome system is highly dynamic and consists of both ubiquitination and deubiquitination machinery to favor controlled regulation of cellular homeostasis. The protein substrate can be modified by (1) Monoubiquitination: a protein attached with a single ubiquitin molecule, (2) multiubiquitination: a protein attached with multiple ubiquitins, (3) polyubiquitination: a protein is attached with a polyubiquitin chain. Polyubiquitination that occurs on the amino-terminal of the lysine residue emerged as a novel mode of modification (Ciechanover and Ben-Saadon 2004). Formation of K48-linked polyubiquitin chain is so far the best-studied ubiquitination which tags the target protein for 26S proteasome degradation (Rahighi and Dikic 2012). In recent studies, K-6 linkages have been identified in ubiquitinating mitochondrial outer membrane proteins upon depolarization of the organelle (Ordureau et al. 2014). It has also been observed that RNF168 promotes K-27-linked polyubiquitylation of Histone 2A upon DNA damage (Gatti et al. 2015). Also, K-33 linkages have been described so far in the endocytic and secretory pathways (Yuan et al. 2014). Furthermore, these ubiquitination modifications are reversible and can be removed by deubiquitinating enzymes (DUBs) (Fig. 9.1) (Finley 2009). Hence, the fine balance between protein ubiquitination and deubiquitination is tightly controlled to regulate protein turnover and function.

9.1.2.1 E1-Activating Enzyme

Ubiquitination is a multistep enzymatic cascade in which ubiquitin is covalently bound to target proteins. In the first step, ubiquitin-activating enzyme UBA1 (E1) uses ATP and binds to an ubiquitin molecule. In the second step, the activated ubiquitin is transferred to the active site of the E2 enzyme and finally, the E3 ubiquitin ligase cause ubiquitination of the target proteins. These polyubiquitinated proteins are then recognized and degraded by the 26S proteasome complex depending on the Ub linkages (Hershko 2005). Previous studies suggest that the E1 enzyme plays a vital role in yeast as a deletion in the E1 enzyme was found to be lethal (McGrath et al. 1991). Moreover, a hypomorphic allele of E1 was identified, which caused impairment of ubiquitin conjugation to the substrate proteins (Swanson and Hochstrasser 2000). In-vivo studies on protein ubiquitination inhibition demonstrated that malignant cell lines increased the activity of the ubiquitination pathway. Blocking this pathway with chemical inhibitors of the E1 enzyme delayed tumor growth in a mouse model of leukemia and induced ER stress-causing cell death and unfolded protein response (Xu et al. 2010).

9.1.2.2 E2-Conjugating Enzyme

The E2 ubiquitin-conjugating enzymes (UBC), also called ubiquitin carrier enzymes, are characterized by the presence of a highly conserved ubiquitin-conjugating domain (UCD), which provides a binding platform for E1s, E3s, and activated Ub/UBL (ubiquitin-like) protein. E2 enzymes play main role in the selection of the lysine to construct ubiquitin chains and thus control the cellular fate of the substrate. In humans, 40 active E2 enzymes have been identified so far, out of which some possess N and C-terminal extensions mediating E2-specific processes (Wijk and Timmers 2010). Several studies suggest that mutation or alterations in E2 are associated with several disease conditions, including cancer and immunological disorders.

9.1.2.3 E3 Ubiquitin Ligase

E3 ubiquitin ligase plays an essential role in substrate recognition and specificity in ubiquitin conjugation (Reinstein and Ciechanover 2006). E3 enzymes are divided into four classes based on their structural features: HECT (homologous to E6-AP carboxy terminus), RING (really interesting new gene) fingers, U-box type, and RBR (RING between RING) (Glickman and Ciechanover 2002). HECT E3 forms a thioester intermediate with ubiquitin, which is conjugated to the substrate directly from the Ub-charged E3 (Kee and Huibregtse 2007), while the RING-finger E3 allows the transfer of ubiquitin to the target substrate directly from the Ub-charged E2. RING E3 ligases can be of two types: monomeric RING fingers such as Mdm2,

COP1, and TRAF6 and multisubunit E3s such as APC/C and CRLs (cullin-RING ligases). The U-box E3 ligases contain a conserved U box domain of about 70 amino acid residues at C terminus that are necessary for enzymatic activity. RBR come under a unique family of RING-HECT hybrid E3 ligases, which consists of a conserved catalytic region, including a RING1, a central in between-RINGs (IBR) and a RING2 domain.

9.1.2.4 Deubiquitination Enzymes (DUBs)

Ubiquitination of proteins is a highly dynamic and reversible process that can be reversed by a class of isopeptidases called deubiquitinating enzymes (DUBs). They catalyze the cleavage of the isopeptide bond between the C-terminal glycine of Ub and the amino group of lysine of the target protein. To date, 100 DUBs have been identified, and based on their structures and sequence, they are categorized into seven families: USPs (ubiquitin-specific proteases), OTUs (ovarian tumor proteases), UCHs (ubiquitin C-terminal hydrolases), MJDs (Machado-Josephin domain proteases), JAMMs (JAB1/MPN/MOV34 metalloproteases), MINDYs (motif interacting with ubiquitin (MIU)-containing novel DUB family), and ZUFSP family. All DUBs are cysteine proteases except JAMMs, which are zinc metalloproteases. DUBS have three distinct mechanisms of action: (1) Generation/release of free ubiquitin (de novo ubiquitin synthesis) (Meray and Lansbury 2007). (2) Cleavage of polyubiquitinated chains. Ubiquitin chains of different lengths and patterns can modify the localization of the degradation of the ubiquitinated protein. Therefore, specific cleavage of ubiquitin chains can have differential effects on the fate of the substrate protein (Millard and Wood 2006). (3) Complete removal of ubiquitin from ubiquitinated proteins stabilizes the protein and prevents it from UPS degradation (Komander et al. 2009; Bonifacino and Weissman 1998). The free ubiquitin is then recycled to maintain the ubiquitin homeostasis. DUBs regulate apoptosis, cell cycle, gene expression, DNA repair, and cytokine signaling.

9.1.3 Role of Ubiquitination in Tumorigenesis

Apart from phosphorylation, the second most common post-translational modification is ubiquitination. Thus, disruptions in ubiquitination are shown to be associated with disease development and progression, especially cancer (Morrow et al. 2015). One fundamental characteristic of cancer is the deregulation of the cell cycle checkpoints, which is highly regulated by the constant proteolysis of cyclins and CDKs that are executed by the UPS. Various other biological processes that are altered in tumorigenesis, including tumor metabolism, tumor microenvironment (TME), cancer stem cell (CSC) stemness, etc. are also regulated by the ubiquitination system (Fig. 9.3).

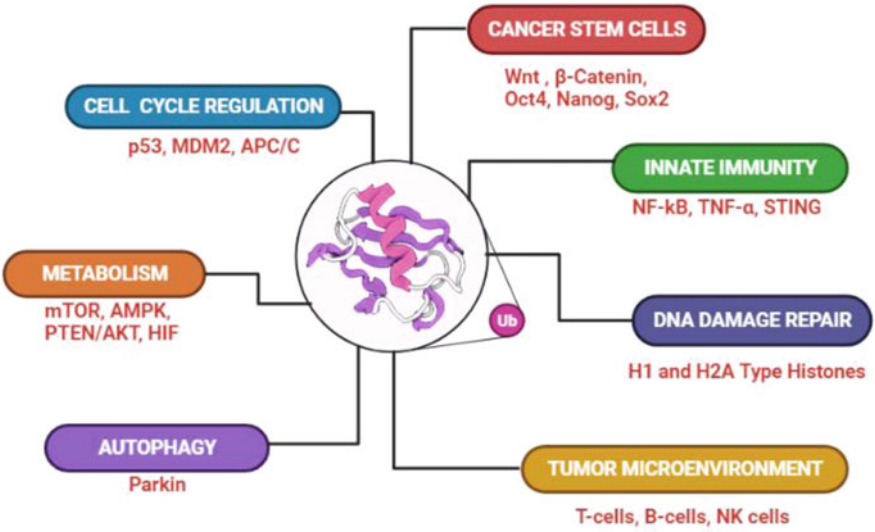


Fig. 9.3 Roles of UPS in cancer: Ubiquitination is the post-translational modification that regulates key proteins of many cellular processes such as innate immunity, cell cycle regulation, DNA-damage repair, tumor metabolism, tumor microenvironment, cancer stem cells' stemness

9.1.3.1 Tumor Metabolism

Metabolic reprogramming is an intricately regulated process that occurs in cancer cells to satisfy the nutrient and energy requirements for cell growth and survival. Recent studies show that ubiquitination is involved in the regulation of mTOR-signaling pathway (Deng et al. 2015), AMPK pathway (Han et al. 2018), PTEN-AKT-signaling pathway (Wang et al. 2007), and it also regulates key transcriptional regulators of cell metabolism (Denko 2008). E3 ligase TRAF6 is upregulated in cancer cells and mediates K-63-linked polyubiquitination of mTOR by interacting with p62, promoting its activation, and subsequently exerting an oncogenic effect in cancer (Linares et al. 2013). Hence, an mTOR inhibitor like everolimus is used in breast cancer patients (Mimoto et al. 2017). During mitochondrial stress, E3 ligase PARKIN targets mTOR for ubiquitination, thereby enhancing cell survival (Park et al. 2014). DUB USP9X negatively modulates the mTOR function (Agrawal et al. 2014). Adenylate-activated protein kinase (AMPK), which is the core regulator of intracellular metabolic homeostasis, is also shown to be associated with tumorigenesis (Steinberg and Carling 2019). When ATP levels in the cells decrease, AMP levels increase which leads to activation of AMPK. AMPK enhances glucose uptake and utilization by regulating essential proteins required in fatty acid-oxidation to produce more energy (Green et al. 2014). Activation of AMPK is regulated by K-48- and K-63-linked polyubiquitination via E3 ligase MAGE-A3/6-TRIM28 (Pineda et al. 2015). Ubiquitination also plays an important role in the regulation of the calcium/Calmodulin-dependent protein kinase 2 (CaMKK2)-AMPK-signaling

pathway. E3 ligase Fbxl12 targets CaMKK2 for degradation by ubiquitination (Mallampalli et al. 2013). PTEN, a tumor suppressor, inhibits the PI3K-signaling pathway and is associated with tumorigenesis. PTEN is polyubiquitinated by Nedd1–4 E3 ligase at K13 and K289, leading to the degradation of PTEN (Wang et al. 2007). While this polyubiquitin chain is removed by USP13 and OTUD3 that inhibits degradation of PTEN, leading to inhibition of the activity of AKT-signaling pathway and tumor growth (Yuan et al. 2015). The transcription factors such as hypoxia-inducible factor-1α (HIF-1α), Myc, and p53 are also regulated by ubiquitination and deubiquitination.

9.1.3.2 Tumor Microenvironment Modulation

The tumor microenvironment (TME) constitutes both cellular components such as various stromal cells and noncellular components, including extracellular matrix and secreted factors. The stromal cells, including cancer-associated fibroblasts (CAFs) and infiltrating immune cells, provide a permissive environment for tumor progression. CAFs have been reported to be involved in cancer progression by secreting pro-inflammatory factors like TGF-β, HGF, and CXCL12 (Kalluri 2016). The UPS regulates the activity of these immune cells such as tumor-associated macrophages, myeloid suppressive cells, T cells, B cells, Dendritic cells, and natural killer cells (NK) cells and contributes to providing suppressive or permissive environments for tumor growth.

9.1.3.3 Cancer Stem Cells (CSCs) Stemness Maintenance

Stemness is the key ability of stem cells for self-renewal and differentiation into the germline. Cancer stem cells play a crucial role in tumor recurrence, resistance, and progression. The stemness-specific genes including Oct4, Sox2, and Nanog and stemness maintenance pathways, including the Hippo and Wnt-signaling pathway, are regulated by ubiquitination. The Cullin3-dependent E3 ligase, SPOP, cause Nanog degradation (Jeong et al. 2006), whereas USP21 maintains the self-renewal ability of embryonic stem cells by stabilizing Nanog (Liu et al. 2016). Two different E3 ligase WWP2 and ITCH mediates Oct4 degradation by polyubiquitination (Liao et al. 2013). Moreover, the Wnt-signaling pathway is critical in regulating CSCs (Clevers 2006). In the absence of Wnt, β-catenin is recognized by the destructive complex and promotes its ubiquitination and degradation. In contrast, the presence of Wnt inhibits phosphorylation and ubiquitination of β-catenin and hence maintains CSCs stemness (Ling et al. 2009).

9.1.4 *Deregulation of the Ubiquitin System and Cancer*

The UPS is an essential player in maintaining protein homeostasis; thereby its deregulation affects various cellular processes that are linked to multiple diseases, including cancer. Indeed, all the necessary components of UPS, including proteasome, E3 ligases, and DUBs or their targets, are found to be expressed abnormally or mutated in different cancers (Fig. 9.4).

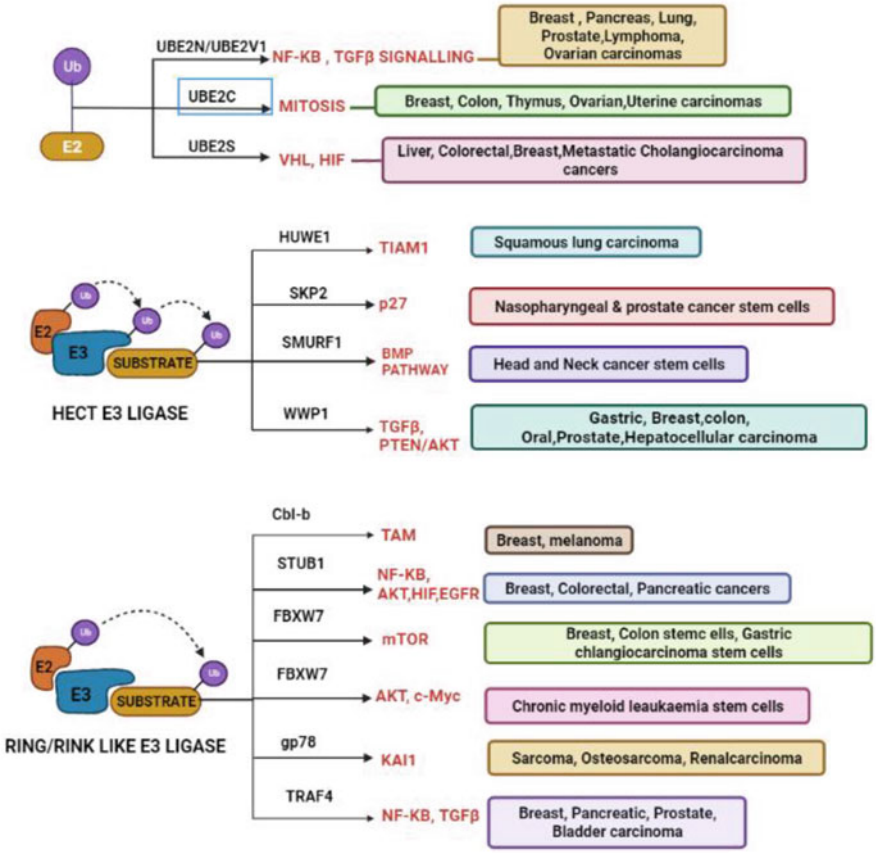


Fig. 9.4 Regulation of various pathways by ubiquitinating enzymes in cancer: Ub–E2 complex ubiquitinates proteins necessary for NF-κB, TGF-β signaling, and Mitosis. Ubiquitination by the HECT E3 enzyme regulates the BMP pathway, PTEN/AKT pathway. RING E3 enzyme ubiquitinates key proteins involved in mTOR signaling, AKT, NF-κB, and TGF signaling

9.1.4.1 Dysregulation of E3 Ligases

Several studies have reported overexpression or mutation of E3 ligases in various cancers (Table 9.1). The E3 ligase MDM2 ubiquitinates and degrades p53, a major tumor suppressor gene regulating diverse biological processes, including cell cycle, DNA damage repair, and apoptosis. It has been shown that 10% of tumors have overexpression of MDM2, consistent with the fact that inhibition of p53 is linked to developing tumors (Toledo and Wahl 2006).

The multisubunit E3 ligase SCF (SKP-cullin-F box protein) are essential players in multiple cellular processes. SCF^{Skp2} mediates the proteasomal degradation of p27,

Table 9.1 Aberrations of the E3 ubiquitin ligases in human cancers

E3 ligase	Type	Substrates	Cancer association	Gene expression
Mdm2/ MdmX	RING	DHFR, FOXO4,P73, IGF1R, pRB, p53	Glioblastoma, colorectal cancer, breast cancer	Overexpression
FBXW7	RING	c-Myc, c-Jun, cyclin E, mTOR, Notch-1, Mcl-1	Colorectal adenocarcinoma, melanoma, T-cell acute lymphoblastic leukemia, non–small-cell lung cancer, gastric cancer	Downregulation
SKP2	RING	P27, P21, P57, cyclin A, cyclin E, cyclin D1	Hepatocellular carcinoma, lung cancer, oral squamous cell carcinoma	Overexpression
βTrCPs	RING	Mcl-1, BimEL, PDCD4, pro-caspase-3, Snail, STAT1	Breast and prostate cancer, colorectal cancer, pancreatic cancer, melanoma	Tissue dependent
APC/C-Cdc20	RING	NEK2A, cyclin A, securin, cyclin B1	Advanced non–small-cell lung cancer patients, colorectal cancer, skin cancer	Overexpression
APC/C-Cdh1	RING	Cdc20, Aurora A and B, Tpx2, PLK1, JNK, HEC1, Cdc25A, Skp2	Colorectal and breast cancer, mammary gland adenocarcinomas and fibroadenomas	Downregulation
NEDD4	HECT	HER3, PTEN, Ras, Grb10, βTrCP, IGF-R, EGFR, TRAF3, Mdm2, p63, Notch	Breast cancer, prostate cancer, colorectal cancer	Tissue dependent
HERC2	HECT	XPA, BRCA1, USP20, RNF8, NEURL4, USP33, FBXL5, NCOA4	Non–small-cell lung cancer, breast cancer	Overexpression
E6AP	HECT	BLK, MCM7, MECP2, p53	Cervical cancer, non–small-cell lung cancer, prostate cancer	Tissue dependent
Parkin	RBR	Miro1, Mfn1, Mfn2, Tomm20, cyclin D, cyclin E	Glioma, colorectal cancer, ovarian cancer, breast cancer, lung cancer, neuroblastoma	Downregulation

Adapted from Wang et al. (2017)

which is an inhibitor of cell cycle regulatory protein CDK (cyclin-dependent kinases). In various cancers, overexpression of Skp2 is correlated with reduced p27 expression and tumor formation (Gstaiger et al. 2001). Interestingly, another E3 ligase SCF^{Fbw7} ubiquitinates and degrades many oncoproteins like cyclin E, c-Myc, Notch, and c-Jun. In various human cancers including breast, colorectal, and gastric cancers, downregulation or mutations of Fbw7 and its target genes that abolish their binding are observed (Minella et al. 2005). Furthermore, its deficiency is correlated with increased metastasis and poor prognosis and survival. Anaphase-promoting complex (APC), another E3 ligase required for the cell cycle, works by forming two complexes, APC^{Cdc20} and APC^{Cdh1}. Studies have shown that Cdc20 acts as an oncoprotein by targeting multiple substrates, including Cyclin B1, Cyclin A, securing, p21, and Mcl-1. In support of this, Cdc20 overexpression has been reported in various human cancers (Wang et al. 2013).

The neuronally expressed developmentally downregulated 4 (NEDD4), a HECT-type E3 ligase, is upregulated in a variety of cancers where it plays an oncogenic role (Ye 2012). One of the subunits of the hypoxia-inducible factor (NEDD4) family, HIF-α, is regulated by ubiquitination-mediated degradation by cullin E3 ligase VHL (von Hippel–Lindau protein). HIF regulates the expression of various genes in response to low oxygen levels and is also required for cancer progression. Deficiency or mutations in VHL enzyme increases HIF-α levels by preventing its ubiquitination and degradation contributing to pathological changes (Kapitsinou and Haase 2008).

The breast cancer susceptibility gene BRCA1 is an E3 ligase for the ubiquitination of multiple varied substrates and also for its own ubiquitination. The tumor suppressor function of BRCA1 requires the E3 activity, and mutations that alter the ligase activity are found in cancer cells (Ruffner et al. 2001).

9.1.4.2 Dysregulation of Deubiquitinating Enzymes

The DUBs perform numerous roles in DNA damage repair, cell cycle, protein stabilization, localization, and cell signaling. DUBs play a dual role in cancer by either acting as tumor suppressors or oncogenes (Table 9.2).

USP2a has been shown to have oncogenic properties by promoting cell proliferation by deubiquitinating and stabilizing multiple targets like MDM2, fatty acid synthase (FASN), and cyclin D1 (Graner et al. 2004; Wang et al. 2014). Overexpression of USP2a can induce migration, invasion, and resistance to chemotherapeutic drugs and is also correlated with poor prognosis. Similarly, USP7 is associated with tumor progression by targeting MDM2, PTEN, and DNMT1, and its overexpression is directly correlated with the development of multiple cancers (Tavana and Gu 2017; Bhattacharya et al. 2018).

On the contrary, CYLD acts as a tumor suppressor in several cancers such as melanoma, colon cancer, HCC, and breast cancer. Downregulation of CYLD promotes the accumulation of β-catenin and proto-oncogene Bcl-3, which is associated with cell proliferation. In addition, CYLD removes K63-polyubiquitin chains from

Table 9.2 Deregulation of deubiquitinating enzymes in human cancers

DUBs	Pathway involved	Cancer type
USP5	Wnt/b-catenin	Promotes colorectal cancer
USP11, USP21	ERK/MAPK	Promotes colorectal cancer
Ubiquitin carboxyl-terminal hydrolase 37	TGF-b signaling	Promotes esophageal cancer
USP1	Stabilize inhibitors of DNA binding	Promotes osteosarcoma
USP39	p21	Inhibits osteosarcoma
USP22	PI3K/AKT	Promotes osteosarcoma
USP37	c-Myc	Promotes lung cancer
USP22	ERK/AKT	Promotes lung cancer
CYLD	NF-κB	Inhibits liver cancer
UCHL1	Apoptotic resistance	Promotes liver cancer
USP22	Stabilize BMI1	Promotes leukemia

Adapted from Sharma et al. (2021)

the intermediate proteins of NF-κB cascade like TRAF-2, TRAF-6, and NEMO, thereby negatively regulating NF-κB signaling. Downregulation of CYLD is associated with increased cell survival and tumor progression by sustained NF-κB signaling (Tauriello et al. 2010). Similarly, A20 negatively regulates the NF-κB pathway and inhibits cell growth, and promotes apoptosis. Mutations or deletions in A20 are associated with cancer progression in lymphomas. Interestingly, A20 is also overexpressed in some tumors, where it promotes resistance to chemotherapy and radiation. Strikingly, along with DUB activity, A20 can also display E3 ligase activity, making it unique. Lee et al. reported that A20 could ubiquitinate and stabilize Snail1, which promotes metastasis in breast cancers (Lee et al. 2017). The expression level of A20 inversely correlates with metastasis-free patient survival.

The 3 DUBs: USP14, UCH37 (or UCHL5), and RPN11 (or POH1) associated with the 19S RP of the proteasome have a key role in maintaining ubiquitin homeostasis. USP14 and UCH37 are associated with various cancers by regulating various target proteins, such as the androgen receptor, vimentin, Dishevelled, cyclin D1, c-Myc, TGF-β receptors, etc (Zhu et al. 2017; Jung et al. 2013; Liu et al. 2020). In addition, overexpression of UCH37 is associated with a low survival rate and high recurrence in cancer patients. Similarly, RPN11 overexpression promotes cancer progression by E2F transcription factor stabilization, whereas its downregulation induces apoptosis in cancer cells (Liu et al. 2020).

9.1.5 *Targeting the Ubiquitin–Proteasome System*

The delicate balance between tumor suppressor proteins and oncoproteins is disrupted in cancer cells partly due to deregulated UPS leading to either degradation of tumor suppressors or increased stabilization of oncoproteins contributing to tumorigenesis. Therefore, all the essential components of the ubiquitin pathway, including E1, E2, E3 ligases, DUBs, and proteasome, could serve as promising drug targets for therapeutic strategies against cancer (Fig. 9.5).

9.1.5.1 Targeting the E1/E2 Enzyme

E1 enzyme activates ubiquitin molecules required for proteasome-mediated degradation, which makes it an effective target. There are various compounds reported to inhibit the E1 enzyme. The first compound discovered was Panepophenanthrin which inhibited the binding of E1 to ubiquitin; however, this molecule did not show inhibition of cell growth (Sekizawa et al. 2002). Later, a cell-permeable compound, PYR-41, was discovered, which prevented the degradation of p53 and effectively restricted cancer cell growth. Thereafter, several additional E1 inhibitors were reported that blocked the E1–E2 association. However, further studies are required to check their effectiveness in inhibiting cancer cell proliferation (Chen et al. 2011a). The compound CC0651, an inhibitor of the E2 enzyme Cdc34, suppressed cell growth in various cancer cell lines. The additional inhibitors, Leucettamol A and Manadosterols A/B, inhibit the interaction between the heterodimeric UBC13–UEV1A complex (Tsukamoto et al. 2008).

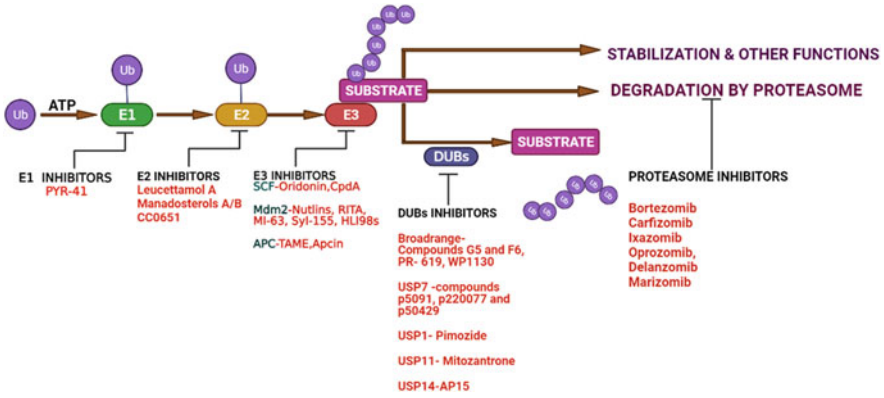


Fig. 9.5 Targeting ubiquitin proteasome system: The different components of UPS-E1/E2/E3, DUBs, and proteasome are altered in various cancers and are potential targets for anticancer therapy. Inhibitors of each component (marked in red) have been identified and are being tested

9.1.5.2 Targeting the E3 Enzyme

Since E3 ligases can function either as oncoproteins (Skp2 and MDM2) or tumor suppressors (Fbw7), their inhibition or activation can stabilize or degrade specific tumor suppressors and oncogenes, respectively. Henceforth, these E3 ligases are attractive targets for anticancer therapies as they act more specifically without altering the function of other proteins.

Fbw7 degrades multiple important oncoproteins, and its loss or mutation is observed in various cancers. Therefore, restoring the function of Fbw7 could be used to treat cancers. The natural compound Oridonin is shown to upregulate Fbw7, which degrades c-Myc, cyclin E, and mTOR, and subsequently blocks the cell cycle and induces apoptosis in cancer cells (Huang et al. 2012). On the contrary, most of the targets of Skp2 are tumor suppressors such as E-cadherin, p21, p27, p57, and FOXO1. The compound CpdA prevents p27 degradation by the Skp2 complex. Additional Skp2 inhibitors, including natural compounds such as curcumin, quercetin, lycopene, and Vitamin D3 or those discovered through a high-throughput screening like SMIP0004 and Compound 25 (SZL-P1–41), have been shown to suppress tumor growth (Chen et al. 2008; Rico-Bautista et al. 2010). However, further studies are required to validate their antitumor effects.

Interestingly, β-TRCP has both oncogenic and antitumor activity depending on the cell type or context-dependent manner. In support of this, both deficiency and overexpression of β-TRCP have been associated with cancers. Erioflorin, a small molecule, inhibits the β-TRCP and PDCD4 (a tumor suppressor) interaction repressing NF-κB activity (Schmid et al. 2008). The Mdm2-mediated p53 ubiquitination can be inhibited either by blocking interaction or inhibiting the ubiquitin ligase activity. A variety of small molecules and peptides, such as MI-63, Nutlins, RITA, and SyI-155, that block p53–Mdm2 binding, have been established. In addition, a family of small molecules, HLI98s, inhibits Hdm2's E3 ligase activity (Yang et al. 2005). TAME (tosyl-L-arginine methyl ester), a small molecule inhibitor of APC, blocks its activation by Cdc20 or Cdh1. Apcin (APC inhibitor) is a small molecule Cdc20 inhibitor that prevents ubiquitination of cyclin B in an APC-dependent manner (Sackton et al. 2014).

The proteolysis-targeting chimeras (PROTACs) are better as compared to small molecule inhibitors. PROTACs consist of two ligands connected by a linker, one binding to the protein of interest and the other to the specific E3 ligase. This allows them to bring the ligase enzyme close to the target protein and target it for degradation by UPS. The first PROTAC was designed to target the protein methionine aminopeptidase-2 by recruiting the β-TRCP. Using PROTAC, more than 50 proteins have been targeted so far, some of which are in clinical trials for cancer therapy (Yang et al. 2021).

9.1.5.3 Targeting DUBs

Like E3 ligases, DUBs are substrate-specific, which makes them an excellent therapeutic target for cancers. A range of DUB inhibitors from broad-spectrum to specific ones has been developed and considered prospective anticancer agents. Compounds G5 and F6, PR-619, WP1130, and NSC632839 are broad-spectrum inhibitors that induce apoptosis in various cancer cell lines. Pimozide and Mitozantrone, a specific inhibitor of USP1 and USP11, have been shown to inhibit the survival of glioblastoma stem-like cells and pancreatic cancer cells, respectively (Chen et al. 2011b; Burkhart et al. 2013).

Many small-molecule inhibitors of USP7 like p5091, p220077, p50429, FT671, XL188, and FT827 have been developed, which stabilize or increase the expression of p53 and subsequently inhibit tumor growth (Chauhan et al. 2012; Turnbull et al. 2017). The inhibitor IU1 was identified by chemical library screening to bind to proteasome-associated DUB USP14 and abolish its enzymatic activity (Wang et al. 2018). b-AP15 selectively inhibits the deubiquitinating activity of USP14 and UCHL5 associated with 19S RP of proteasome without altering the proteasome activity. This molecule can induce apoptosis in many solid tumors and multiple myeloma cells (Tian et al. 2014).

CSCs are a subset of cancer cells responsible for tumor formation, heterogeneity, metastasis, drug resistance, and tumor relapse. Accumulating evidence has reported the role of DUBs in maintaining CSC activity and stemness by regulating several signaling pathways leading to the survival of CSCs. In cancer treatment, targeting CSCs directly is considered a better approach than treating the tumor as a whole. Therefore, DUBs are more promising as they target multiple steps in cancer progression (Lei et al. 2017).

9.1.5.4 Targeting Proteasome Activity

The first compounds shown to inhibit proteasome activity were peptidyl aldehydes. Thereafter, several compounds have been identified that inhibit the proteasome activity, most of which bind to the 19S RP of the proteasome. Bortezomib (BTZ), a proteasome inhibitor, was the first drug approved by Food and Drug Administration (FDA) in 2003 to treat multiple myeloma (MM). However, it was found to be toxic to normal cells (Kubiczkova et al. 2014). Carfilzomib, another proteasome inhibitor, is more selective and stable, resulting in fewer side effects. Both bortezomib and carfilzomib inhibit the chymotrypsin-like activity of the 20S proteasome. However, CFZ cannot be administered orally. Other inhibitors such as ixazomib, delanzomib, oprozomib, and marizomib developed subsequently and are suitable for oral administration and have been tested for clinical trials (Ruschak et al. 2011). In the field of clinical therapeutics, multidrug resistance remains one of the major obstacles in cancer treatment. Numerous studies demonstrated that

inhibition of proteasome potentiates the efficiency of chemotherapeutic drugs by inducing apoptosis and inhibiting drug efflux transporters (Narayanan et al. 2020).

9.2 Conclusions and Future Perspectives

The research on UPS has been going on for around 50 years, since its first discovery in 1975. The ubiquitination–deubiquitination is an essential post-translational modification that regulates the "quality" and "quantity" of various proteins to maintain cellular homeostasis. The regulation of UPS components, including ubiquitinating enzymes E1/E2/E3, DUBs, and proteasome, is highly complex and multifaceted, and therefore, any dysfunction of these components can lead to various diseases, cancer being one of them. As the UPS activity is shown to be altered in a wide range of human cancers, this system has become apparent for molecular targeting in advancing anticancer therapeutics (Table 9.3). However, only a few UPS inhibitors are in clinical practice, and many challenges, including side effects, limited efficacy, and resistance, exist. Some of the FDA-approved proteasome inhibitors, such as bortezomib, carfilzomib, oprozomib, and ixazomib are in the clinic; however, their

Table 9.3 Selected compounds targeting the UPS components in clinical trials

Inhibitors	Target	Biological function
MLN4924	E1 enzyme	Blocks the neddylation of all CRLs, leading to apoptosis in cancer cells
NSC697923	Ubc13-Uev1A (E2)	Blocks the formation of the E2-Ub thioester conjugate and inhibits NF-κB signaling
DS-3032	E3 ligase Mdm2	MDM2-p53 Interaction. Leads to p53 activation
Compound #25	E3 ligase SKP2	Suppresses SKP2 ligase activity
AT-406	XIAP, cIAP1/2	An antagonist of IAPs, Activates apoptosis
Bortezomib	Proteasome	Inhibits the chymotrypsin-like activity of the proteasome and leads to the accumulation of polyubiquitinated proteins
Ixazomib	Proteasome	Binds to catalytic subunits and inhibits proteasome activity
Pimozide	DUB-USP1	Reversible, noncompetitive inhibition of USP1
Mitoxantrone	DUB-USP11	Not clear
b-AP15	DUB-USP14, UCHL5	Inhibition of 19S RP deubiquitinating activity
Orthophenanthroline (OPA)	DUB-RPN11	Inhibits RPN11 activity but does not affect proteasome activity

Adapted from Park et al. (2020)

use is limited because of side-effects. Similarly, E2- and E3-specific inhibitors have been identified that work well in vitro but not in animal models and clinical trials.

One reason could be the deregulation of other cell-signaling pathways during tumorigenesis, making targeted therapies inefficient. Thus, multitarget combination treatment can be considered as a future direction. Further, an in-depth understanding of UPS functions is required to elucidate their roles in cancer initiation and progression and to develop novel drugs for the treatment and prevention of human cancers.

References

Agrawal P, Chen Y-T, Schilling B, Gibson BW, Hughes RE (2014) Ubiquitin-specific peptidase 9, X-linked (USP9X) modulates activity of mammalian target of rapamycin (mTOR). J Biol Chem 289:14965. https://doi.org/10.1074/jbc.a111.328021

Akutsu M, Dikic I, Bremm A (2016) Ubiquitin chain diversity at a glance. J Cell Sci 129:875–880. https://doi.org/10.1242/jcs.183954

Bhattacharya S, Chakraborty D, Basu M, Ghosh MK (2018) Emerging insights into HAUSP (USP7) in physiology, cancer and other diseases. Signal Transduct Target Ther 3:17. https://doi.org/10.1038/s41392-018-0012-y

Bonifacino JS, Weissman AM (1998) Ubiquitin and the control of protein fate in the secretory and endocytic pathway. Annu Rev Cell Dev Biol 14:19–57. https://doi.org/10.1146/annurev.cellbio.14.1.19

Burkhart RA et al (2013) Mitoxantrone targets human ubiquitin-specific peptidase 11 (USP11) and is a potent inhibitor of pancreatic cancer cell survival. Mol Cancer Res 11:901–911. https://doi.org/10.1158/1541-7786.MCR-12-0699

Chauhan D et al (2012) A small molecule inhibitor of ubiquitin-specific Protease-7 induces apoptosis in multiple myeloma cells and overcomes Bortezomib resistance. Cancer Cell 22:345–358. https://doi.org/10.1016/j.ccr.2012.08.007

Chen Q et al (2008) Targeting the p27 E3 ligase SCFSkp2 results in p27and Skp2-mediated cell-cycle arrest and activation of autophagy. Blood 111:4690–4699. https://doi.org/10.1182/blood-2007-09-112904

Chen JJ et al (2011a) Mechanistic studies of substrate-assisted inhibition of ubiquitin-activating enzyme by adenosine sulfamate analogues. J Biol Chem 286:40867. https://doi.org/10.1074/jbc.M111.279984

Chen J et al (2011b) Selective and cell-active inhibitors of the USP1/ UAF1 deubiquitinase complex reverse cisplatin resistance in non-small cell lung cancer cells. Chem Biol 18:1390–1400. https://doi.org/10.1016/j.chembiol.2011.08.014

Ciechanover A, Ben-Saadon R (2004) N-terminal ubiquitination: more protein substrates join in. Trends Cell Biol 14:103–106. https://doi.org/10.1016/j.tcb.2004.01.004

Ciechanover A, Orian A, Schwartz AL (2000) The ubiquitin-mediated proteolytic pathway: mode of action and clinical implications. J Cell Biochem 77:40–51. https://doi.org/10.1002/(SICI)1097-4644(2000)77:34+<40::AID-JCB9>3.0.CO;2-6

Clevers H (2006) Wnt/beta-catenin signaling in development and disease. Cell 127:469–480

Coux O, Tanaka K, Goldberg AL (1996) Structure and functions of the 20S and 26S proteasomes. Annu Rev Biochem 65:801–847. https://doi.org/10.1146/annurev.bi.65.070196.004101

Deng L et al (2015) The ubiquitination of RagA GTPase by RNF152 negatively regulates mTORC1 activation. Mol Cell 58:804–818. https://doi.org/10.1016/j.molcel.2015.03.033

Denko NC (2008) Hypoxia, HIF1 and glucose metabolism in the solid tumour. Nat Rev Cancer 8:705–713. https://doi.org/10.1038/nrc2468

Etlinger JD, Goldberg AL (1977) A soluble ATP dependent proteolytic system responsible for the degradation of abnormal proteins in reticulocytes. Proc Natl Acad Sci U S A 74:54–58. https://doi.org/10.1073/pnas.74.1.54

Finley D (2009) Recognition and processing of ubiquitin-protein conjugates by the proteasome. Annu Rev Biochem 78:477–513. https://doi.org/10.1146/annurev.biochem.78.081507.101607

Gatti M et al (2015) RNF168 promotes noncanonical K27 ubiquitination to signal DNA damage. Cell Rep 10:226–238. https://doi.org/10.1016/j.celrep.2014.12.021

Glickman MH, Ciechanover A (2002) The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. Physiol Rev 82:373–428. https://doi.org/10.1152/physrev.00027.2001

Graner E et al (2004) The isopeptidase USP2a regulates the stability of fatty acid synthase in prostate cancer. Cancer Cell 5:253–261. https://doi.org/10.1016/S1535-6108(04)00055-8

Green DR, Galluzzi L, Kroemer G (2014) Metabolic control of cell death. Science 345:1250256. https://doi.org/10.1126/science.1250256

Groll M et al (2000) A gated channel into the proteasome core particle. Nat Struct Biol 7:1062–1067. https://doi.org/10.1038/80992

Gstaiger M et al (2001) Skp2 is oncogenic and overexpressed in human cancers. Proc Natl Acad Sci U S A 98:5043–5048. https://doi.org/10.1073/pnas.081474898

Han F et al (2018) The critical role of AMPK in driving Akt activation under stress, tumorigenesis and drug resistance. Nat Commun 9:4728. https://doi.org/10.1038/s41467-018-07188-9

Hershko A (2005) The ubiquitin system for protein degradation and some of its roles in the control of the cell division cycle. Cell Death Differ 12:1191–1197. https://doi.org/10.1038/sj.cdd.4401702

Hoeller D, Hecker CM, Dikic I (2006) Ubiquitin and ubiquitin-like proteins in cancer pathogenesis. Nat Rev Cancer 6:776–788. https://doi.org/10.1038/nrc1994

Huang HL et al (2012) Triggering Fbw7-mediated proteasomal degradation of c-Myc by oridonin induces cell growth inhibition and apoptosis. Mol Cancer Ther 11:1155–1165. https://doi.org/10.1158/1535-7163.MCT-12-0066

Jeong EK et al (2006) BTB domain-containing speckle-type POZ protein (SPOP) serves as an adaptor of Daxx for ubiquitination by Cul3-based ubiquitin ligase. J Biol Chem 281(18):12664–12672. https://doi.org/10.1074/jbc.M600204200

Jung H et al (2013) Deubiquitination of dishevelled by usp14 is required for wnt signaling. Oncogenesis 2:e64. https://doi.org/10.1038/oncsis.2013.28

Kalluri R (2016) The biology and function of fibroblasts in cancer. Nat Rev Cancer 16:582–598. https://doi.org/10.1038/nrc.2016.73

Kapitsinou PP, Haase VH (2008) The VHL tumor suppressor and HIF: insights from genetic studies in mice. Cell Death Differ 15:650–659. https://doi.org/10.1038/sj.cdd.4402313

Kee Y, Huibregtse JM (2007) Regulation of catalytic activities of HECT ubiquitin ligases. Biochem Biophys Res Commun 354:329–333. https://doi.org/10.1016/j.bbrc.2007.01.025

Kleiger G, Mayor T (2014) Perilous journey: a tour of the ubiquitin-proteasome system. Trends Cell Biol 24:352–359. https://doi.org/10.1016/j.tcb.2013.12.003

Komander D, Clague MJ, Urbé S (2009) Breaking the chains: structure and function of the deubiquitinases. Nat Rev Mol Cell Biol 10:550–563. https://doi.org/10.1038/nrm2731

Kubiczkova L, Pour L, Sedlarikova L, Hajek R, Sevcikova S (2014) Proteasome inhibitors—molecular basis and current perspectives in multiple myeloma. J Cell Mol Med 18:947–961. https://doi.org/10.1111/jcmm.12279

Lee JH et al (2017) A20 promotes metastasis of aggressive basal-like breast cancers through multi-monoubiquitylation of Snail1. Nat Cell Biol 19:1260–1273. https://doi.org/10.1038/ncb3609

Lei H, Shan H, Wu Y (2017) Targeting deubiquitinating enzymes in cancer stem cells. Cancer Cell Int 17:101. https://doi.org/10.1186/s12935-017-0472-0

Liao B et al (2013) Itch, an E3 ligase of Oct4, is required for embryonic stem cell self-renewal and pluripotency induction. J Cell Physiol 228:1443–1451. https://doi.org/10.1002/jcp.24297

Linares JF et al (2013) K63 polyubiquitination and activation of mTOR by the p62-TRAF6 complex in nutrient-activated cells. Mol Cell 51:283–296. https://doi.org/10.1016/j.molcel.2013.06.020

Ling L, Nurcombe V, Cool SM (2009) Wnt signaling controls the fate of mesenchymal stem cells. Gene 433:1–7. https://doi.org/10.1016/j.gene.2008.12.008

Liu X et al (2016) USP21 deubiquitylates nanog to regulate protein stability and stem cell pluripotency. Signal Transduct Target Ther 1:16024. https://doi.org/10.1038/sigtrans.2016.24

Liu D, Song Z, Wang X, Ouyang L (2020) Ubiquitin C-terminal hydrolase L5 (UCHL5) accelerates the growth of endometrial cancer via activating the Wnt/β-catenin signaling pathway. Front Oncol 10:865. https://doi.org/10.3389/fonc.2020.00865

Mallampalli RK et al (2013) Fbxl12 triggers G1 arrest by mediating degradation of calmodulin kinase I. Cell Signal 25:2047–2059. https://doi.org/10.1016/j.cellsig.2013.05.012

McGrath JP, Jentsch S, Varshavsky A (1991) UBA1: an essential yeast gene encoding ubiquitin-activating enzyme. EMBO J 10:227–236. https://doi.org/10.1002/j.1460-2075.1991.tb07940.x

Meray RK, Lansbury PT (2007) Reversible monoubiquitination regulates the Parkinson disease-associated ubiquitin hydrolase UCH-L1. J Biol Chem 282:10567–10575. https://doi.org/10.1074/jbc.M611153200

Millard SM, Wood SA (2006) Riding the DUBway: regulation of protein trafficking by deubiquitylating enzymes. J Cell Biol 173:463–468. https://doi.org/10.1083/jcb.200602082

Mimoto R, Nihira NT, Hirooka S, Takeyama H, Yoshida K (2017) Diminished DYRK2 sensitizes hormone receptor-positive breast cancer to everolimus by the escape from degrading mTOR. Cancer Lett 384:27–38. https://doi.org/10.1016/j.canlet.2016.10.015

Minella AC, Welcker M, Clurman BE (2005) Ras activity regulates cyclin e degradation by the Fbw7 pathway. Proc Natl Acad Sci U S A 102:9649–9654. https://doi.org/10.1073/pnas.0503677102

Mitch WE, Goldberg AL (1996) Mechanisms of muscle wasting—the role of the ubiquitin–proteasome pathway. N Engl J Med 335(25):1897–1905. https://doi.org/10.1056/nejm199612193352507

Morrow JK, Lin HK, Sun SC, Zhang S (2015) Targeting ubiquitination for cancer therapies. Future Med Chem 7:2333–2350. https://doi.org/10.4155/fmc.15.148

Nalepa G, Rolfe M, Harper JW (2006) Drug discovery in the ubiquitin—proteasome system. Nat Rev Drug Discov 5:596–613. https://doi.org/10.1038/nrd2056

Narayanan S et al (2020) Targeting the ubiquitin-proteasome pathway to overcome anticancer drug resistance. Drug Resist Updat 48:100663. https://doi.org/10.1016/j.drup.2019.100663

Ordureau A et al (2014) Quantitative proteomics reveal a feedforward mechanism for mitochondrial PARKIN translocation and ubiquitin chain synthesis. Mol Cell 56:360–375. https://doi.org/10.1016/j.molcel.2014.09.007

Park D et al (2014) Parkin ubiquitinates mTOR to regulate mTORC1 activity under mitochondrial stress. Cell Signal 26:2122–2130. https://doi.org/10.1016/j.cellsig.2014.06.010

Pickart CM (2004) Back to the future with ubiquitin. Cell 116:181. https://doi.org/10.1016/S0092-8674(03)01074-2

Pineda CT et al (2015) Degradation of AMPK by a cancer-specific ubiquitin ligase. Cell 160:715–728. https://doi.org/10.1016/j.cell.2015.01.034

Rahighi S, Dikic I (2012) Selectivity of the ubiquitin-binding modules. FEBS Lett 586:2705–2710. https://doi.org/10.1016/j.febslet.2012.04.053

Reinstein E, Ciechanover A (2006) Narrative review: protein degradation and human diseases: the ubiquitin connection. Ann Intern Med 145:676–684. https://doi.org/10.7326/0003-4819-145-9-200611070-00010

Rico-Bautista E, Yang CC, Lu L, Roth GP, Wolf DA (2010) Chemical genetics approach to restoring p27Kip1 reveals novel compounds with antiproliferative activity in prostate cancer cells. BMC Biol 8:153. https://doi.org/10.1186/1741-7007-8-153

Ruffner H, Joazeiro CAP, Hemmati D, Hunter T, Verma IM (2001) Cancer-predisposing mutations within the RING domain of BRCA1: loss of ubiquitin protein ligase activity and protection from

radiation hypersensitivity. Proc Natl Acad Sci U S A 98:5134–5139. https://doi.org/10.1073/pnas.081068398
Ruschak AM, Slassi M, Kay LE, Schimmer AD (2011) Novel proteasome inhibitors to overcome bortezomib resistance. J Natl Cancer Inst 103:1007–1017. https://doi.org/10.1093/jnci/djr160
Sackton KL et al (2014) Synergistic blockade of mitotic exit by two chemical inhibitors of the APC/C. Nature 514:646–649. https://doi.org/10.1038/nature13660
Schmid T et al (2008) Translation inhibitor Pdcd4 is targeted for degradation during tumor promotion. Cancer Res 68:1254–1260. https://doi.org/10.1158/0008-5472.CAN-07-1719
Sekizawa R et al (2002) Panepophenanthrin, from a mushroom strain, a novel inhibitor of the ubiquitin-activating enzyme. J Nat Prod 65:1491–1493. https://doi.org/10.1021/np020098q
Steinberg GR, Carling D (2019) AMP-activated protein kinase: the current landscape for drug development. Nat Rev Drug Discov 18:527–551. https://doi.org/10.1038/s41573-019-0019-2
Swanson R, Hochstrasser M (2000) A viable ubiquitin-activating enzyme mutant for evaluating ubiquitin system function in Saccharomyces cerevisiae. FEBS Lett 477:193–198. https://doi.org/10.1016/S0014-5793(00)01802-0
Tauriello DVF et al (2010) Loss of the tumor suppressor CYLD enhances Wnt/β-catenin signaling through K63-linked ubiquitination of Dvl. Mol Cell 37:607–619. https://doi.org/10.1016/j.molcel.2010.01.035
Tavana O, Gu W (2017) Modulation of the p53/MDM2 interplay by HAUSP inhibitors. J Mol Cell Biol 9:45–52. https://doi.org/10.1093/jmcb/mjw049
Tian Z et al (2014) A novel small molecule inhibitor of deubiquitylating enzyme USP14 and UCHL5 induces apoptosis in multiple myeloma and overcomes bortezomib resistance. Blood 123:706–716. https://doi.org/10.1182/blood-2013-05-500033
Toledo F, Wahl GM (2006) Regulating the p53 pathway: in vitro hypotheses, in vivo veritas. Nat Rev Cancer 6:909–923. https://doi.org/10.1038/nrc2012
Tsukamoto S et al (2008) Leucettamol A: a new inhibitor of Ubc13-Uev1A interaction isolated from a marine sponge, Leucetta aff. microrhaphis. Bioorganic Med Chem Lett 18:6319–6320. https://doi.org/10.1016/j.bmcl.2008.10.110
Turnbull AP et al (2017) Molecular basis of USP7 inhibition by selective small-molecule inhibitors. Nature 550:481–486. https://doi.org/10.1038/nature24451
Wang X et al (2007) NEDD4-1 is a proto-oncogenic ubiquitin ligase for PTEN. Cell 128:129–139. https://doi.org/10.1016/j.cell.2006.11.039
Wang Z et al (2013) Cdc20: a potential novel therapeutic target for cancer treatment. Curr Pharm Des 19:3210–3214. https://doi.org/10.2174/1381612811319180005
Wang CL et al (2014) Ubiquitin-specific protease 2a stabilizes MDM4 and facilitates the p53-mediated intrinsic apoptotic pathway in glioblastoma. Carcinogenesis 35:1500–1509. https://doi.org/10.1093/carcin/bgu015
Wang Y et al (2018) Small molecule inhibitors reveal allosteric regulation of USP14 via steric blockade. Cell Res 28:1186–1194. https://doi.org/10.1038/s41422-018-0091-x
Wijk SJL, Timmers HTM (2010) The family of ubiquitin-conjugating enzymes (E2s): deciding between life and death of proteins. FASEB J 24:981–993. https://doi.org/10.1096/fj.09-136259
Xu GW et al (2010) The ubiquitin-activating enzyme E1 as a therapeutic target for the treatment of leukemia and multiple myeloma. Blood 115:2251–2259. https://doi.org/10.1182/blood-2009-07-231191
Yang Y et al (2005) Small molecule inhibitors of HDM2 ubiquitin ligase activity stabilize and activate p53 in cells. Cancer Cell 7:547–559. https://doi.org/10.1016/j.ccr.2005.04.029
Yang Q, Zhao J, Chen D, Wang Y (2021) E3 ubiquitin ligases: styles, structures and functions. Mol Biomed 2:23. https://doi.org/10.1186/s43556-021-00043-2

Ye X (2012) NEDD4: a promising target for cancer therapy Xiantao. Changes

Yuan WC et al (2014) K33-linked Polyubiquitination of Coronin 7 by Cul3-KLHL20 ubiquitin E3 ligase regulates protein trafficking. Mol Cell 54:586–600. https://doi.org/10.1016/j.molcel.2014.03.035

Yuan L et al (2015) Deubiquitylase OTUD3 regulates PTEN stability and suppresses tumorigenesis. Nat Cell Biol 17:1169–1181. https://doi.org/10.1038/ncb3218

Zhu Y et al (2017) USP14 de-ubiquitinates vimentin and miR-320a modulates USP14 and vimentin to contribute to malignancy in gastric cancer cells. Oncotarget 8:48725–48736. https://doi.org/10.18632/oncotarget.10706

Chapter 10
Repurposing of Serotonin Pathway Influencing Drugs for Potential Cancer Therapy and Antimicrobial Functions

Madhurya Ray and Vipin Kumar

Abstract The conventional methods for the discovery and development of new drugs are a time-consuming and complex process that expands over several stages and decades. The repurposing of existing drugs for newer roles offers time and cost-saving benefits. Serotonin receptors are the means by which serotonin performs a variety of functions in the human body and several pharmacological classes of drugs, such as antidepressants, sedatives, antipsychotics, and migraine medications, can target the serotonin system. Many of these psychotropic drugs show antimicrobial functions. Studies have connected serotonin to the proliferation of cancer cells, and the serotonin pathway has been recognized as a potential target for cancer treatment. This chapter provides an overview of the literature on the relationship between serotonin (5-HT) and the immune system, and, sheds light on the anticancer and antimicrobial properties of some serotonin-pathway influencing drugs which can add to the understanding of the repurposing of these classes of drugs.

Keywords Serotonin pathway · SSRIs · Drug repurposing · Serotonin receptors · Anticancer · Antimicrobial

10.1 Introduction

Alternative applications of approved drugs could result in breakthrough treatment of a number of diseases and disorders. Drug repurposing is a useful approach toward finding suitable drugs for rare diseases and diseases with limited treatment options (Roessler et al. 2021). Successful examples of repurposed drugs include the use of erythromycin for gastric disorders and aspirin for coronary artery disease (Martinez 2022). Knowledge about repurposing/repositioning of drugs can be garnered by biological, chemical, and computational methods followed by clinical trials. The high cost of several drugs used for cancer also limits the options for treatment of

M. Ray · V. Kumar (✉)
Laboratory of Applied Microbiology, Indian Institute of Technology (Indian School of Mines), Dhanbad, Jharkhand, India
e-mail: vipinmicro1@iitism.ac.in

R. C. Sobti et al. (eds.), *Drug Repurposing for Emerging Infectious Diseases and Cancer*, https://doi.org/10.1007/978-981-19-5399-6_10

many patients. Preclinical and clinical studies take long durations of time before they are accepted by regulatory authorities. It is estimated that the development and synthesis of a new drug for therapeutic purposes can take up to 18 years, while the assignment of repurposed drugs can significantly reduce this length of time (Malik et al. 2022). The development of new drug also comes with the risk of failure in clinical trials, low tolerability, and extreme side effects. Thus, the repurposing of drugs provides the advantages of cost and time-saving.

Diseases can impact the body system at different points and via different pathways. When specific chemicals or pathways are inhibited, alternative networks may be activated. These chemical agents or pathways could be treated as biomarkers (Stenvang et al. 2013). Alternative medical uses of existing drugs can be found by different approaches which could be broadly classified as drug-focused, disease-focused, and treatment-focused.

The use of bioinformatics is a very efficient and commonly used method for target-based drug repurposing. Molecular docking is an important step in the process of drug discovery against targets. Potential drugs which can act as antagonist or agonist are identified based on their structure. High throughput virtual screening is a method based on the use of chemical databases for identifying chemical molecules against biological targets (Ramaswamy et al. 2021). The structure of the ligand or target along with information about the bioactivity of the drug is required for pharmacophore modeling. Other factors which help in predicting the ideal drugs include Food and Drug Administration (FDA) approval labels, clinical trial information, information about drug toxicity, and knowledge about side effects and pathways (Anwar et al. 2021). A study by Zhou et al. 2020 investigated drug target networks and discovered 16 repurposable drugs against human corona virus.

Serotonin is well known as a mood-stabilizer. It acts as a neurotransmitter in the central nervous system (CNS). Additionally, serotonin synthesis has also been observed in intestinal enterochromaffin cells and, platelets. Serotonin plays diverse roles in the human body which are mediated through serotonin receptors. While serotonin is relevant in psychological disorders, recent investigations have linked this neurotransmitter with much broader functional roles toward several diseases and disorders. Serotonin has been linked to cancer cell proliferation in several studies and the serotonin pathway has been identified as a potential therapeutic target for cancer therapy (Balakrishna et al. 2021). Serotonin is influenced by a broad range of psychotrophic drugs, some of which have been found to display antimicrobial properties as well (Munoz-Bellido et al. 2000).

Cancer is a complex disease and requires intensive research on its pathophysiology and treatment. It is characterized by uncontrolled cell growth in the body which can impact several tissues (Huang et al. 2018). Different kinds of cancers are widely observed on a global scale and are noted for their high mortality rates. The most common types of cancers include breast cancer, skin cancer, lung cancer, prostate cancer, colorectal cancer, and bladder cancer. Along with the development and discovery of new drug moieties, the repurposing of approved drugs can also target the disease. Apart from cancer, drug repurposing of the same agent can also be used for treatment of other diseases, such as microbial infections. The antimalarial drug

chloroquine has been shown to cause cytotoxicity against MCF-7 cell line with gold-conjugated chloroquine nanoparticles, while the drug is also known to have antiviral properties (Joshi et al. 2012; Shaik et al. 2022).

Antibiotic resistance has been a widely occurring situation in the twenty-first century. Several pathogenic strains of microorganisms have grown resistant to current antibiotics, while the development of newer antibiotics has been sluggish. Particularly, the development of drugs against Gram-negative bacteria is of concern. Antibiotic resistance could also have devastating financial impacts. Reports by the World Bank suggest that antibiotic resistance could impact up to 3.8% points of the world GDP by 2050 (The World Bank 2017). A particularly concerning case against antibiotic resistance is that of *Mycobacterium tuberculosis* which causes the disease tuberculosis. This causative agent has acquired resistance against several of the leading first and second line of drugs. Tuberculosis has now been categorized as multidrug-resistant, extremely drug-resistant, and totally drug-resistant leading to concerns about the future treatment of this disease (Sahu et al. 2019). Biofilm production by some microbial species limits the efficacy of several drugs against them. For example, the Mu50 biofilms of *Staphylococcus aureus* acts as a barrier for many antibiotics (Cui et al. 2006). An antipsychotic thioridazine was identified as a potent drug against *S. aureus* Mu50. Changes to the synthetic pathways of dopamine and serotonin have shown links to viral infections like COVID-19 (Pashaei 2021). The angiotensin converting enzyme II or ACE-2 receptor is the target for the S protein of the virus via which it can penetrate into human systems (Chung et al. 2020). The present chapter focuses on repurposing of drugs influencing serotonergic receptors and the serotonergic pathway for treatment of cancer and microbial diseases.

10.2 Role of Serotonin and Serotonin Receptors in the Immunomodulation

Serotonin or 5-hydroxytryptamine (5-HT) is a monoamine neurotransmitter produced by the tryptophan (TRP) metabolism. Serotonin also plays a prime role in the human immune system. Serotonin pathway is involved with the synthesis and transport of 5-HT. Tryptophan (trp) is the precursor of serotonin. In the serotonergic pathway, it is hydroxylated to 5-hydroxytryptophan followed by decarboxylation to 5-HT (Fig. 10.1). Serotonin activities influence several bodily functions like memory, emotions, sleep, and appetite. Thus, serotonin system can be targeted by many different classes of drugs including antidepressants, anxiolytics, antipsychotics, and antimigraine drugs (Golden et al. 2021). The stimulation of nerve impulses results in the release of the 5-HT neurotransmitter into the synapse where it binds reversibly to the postsynaptic receptor. Serotonin can also bind to the presynaptic neuron autoreceptors to control its production and release.

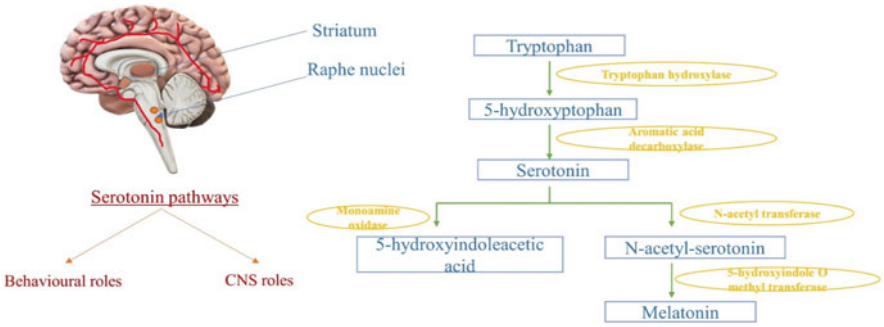


Fig. 10.1 Biosynthetic pathway of serotonin (5-HT) and its functional roles

Various drugs used primarily for the treatment of psychiatric disorders have been found to influence the neurotransmitter directly or indirectly. These drugs, used for treating psychiatric disorders like major depressive disorder, anxiety, and schizophrenia, operate on serotonin receptors, either directly or indirectly. The effects of drugs on mechanisms of the body depend on gene–drug interactions. The 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, 5-HT_{1B}, 5-HT_4, 5-HT_6, and 5-HT_7 are some of the important receptors playing a key role in the serotonin pathway (McGowan and Reynolds 2020). The 5-HT_{1A} receptors are presynaptic autoreceptors located in the raphe nuclei. They have shown moderate efficacy for antidepressants. It is the site of action for several anxiolytic drugs and antipsychotic drugs (Díaz-Mataix et al. 2005). These drugs inhibit the reuptake of serotonin and increase extraneuronal 5-HT. The 5-HT_{2A} receptors stimulate the phospholipase-c and are primarily postsynaptic. Some antipsychotic drugs function by inhibiting the 5-HT_{2A} receptors (Sharp and Barnes 2020). The 5-HT_{2C} receptor is also primarily postsynaptic excitatory and regulates several aspects of the serotonin. They are found in the choroid plexus, basal ganglia, and limbic regions of the brain from where they can interact with antipsychotics and antidepressants. They also have a role in hypothalamus and can regulate endocrine activities like food intake. The 5-HT_{1B} receptors are found in the brain of human beings and are expressed in the striatum, cortex, lateral geniculate nucleus, and raphe nuclei (Yevtushenko and Reynolds 2010). They can be found in the terminal axons of serotonergic receptors. The 5-HT_4 are found in high densities in the basal ganglia, the hippocampal formation, and regions of neocortex. They are involved in activities like learning and memory. The 5-HT_6 are found in striatum, cortex, nucleus accumbens, and cerebellum. The 5-HT_7 receptors are expressed in high densities in layers of the cortex and some subcortical structures, and they is commonly associated with mood regulation (Dayer 2014). The 5-HT_7 receptor is of special importance in neuro-oncology studies because of its expression in glioma cells (malignant).

Based on their structural and functional properties, antidepressants can be classified as selective serotonin reuptake inhibitors (SSRIs), tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), serotonin antagonist and reuptake

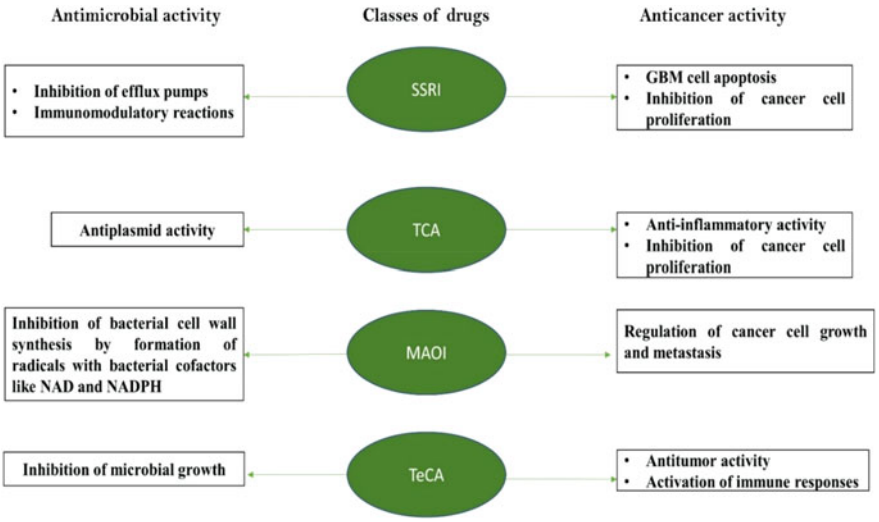


Fig. 10.2 Possible mechanisms for anticancer and antimicrobial activity

inhibitors (SARIs), tetracyclic antidepressants (TeCA), norepinephrine and dopamine inhibitors (NDRIs), and some miscellaneous classes of drugs (Song et al. 2022). Several studies have identified anticancer properties of antidepressants. Anticancer activities of these drugs are implemented by mechanisms like apoptosis, metastasis, regulation of cell proliferation, and oxidative stress (Fig. 10.2).

An adverse side effect of drugs which interfere with synaptic serotonin is the serotonin syndrome. Serotonin toxicity might also be caused by drugs which do not have direct serotonergic activity. Serotonin syndrome is characterized by the presence of high levels of 5-HT in the synaptic cleft (Kruijt et al. 2020). The overactivation of serotonin receptors can be potentially fatal. Such high concentrations of serotonin are often seen as an effect of two or more serotonergic agents acting together at the same time. Serotonin syndrome can be caused by MAOIs, SSRIs, and SNRIs. It could also be a result of conjoint administration of any of the mentioned category of drugs with medicines which inhibit cytochromes P450.

10.3 Classes of Drugs Involved with the Serotonin Pathway

10.3.1 Antidepressants

Selective serotonin reuptake inhibitors are a class of drugs which are used for the treatment of several psychological conditions including anxiety and depression. SSRIs are widely prescribed as antidepressants and antianxiety medicines due to their tolerability. Fluoxetine, citalopram, fluvoxamine, sertraline, escitalopram, and

paroxetine are the FDA-approved SSRIs. These drugs are highly tolerable even above their daily prescribed limits. All the SSRIs have similar tolerability but differ in their pharmacokinetic properties**.** SSRIs show a high binding affinity toward 5-HT transporters and also inhibit 5-HT reuptake. They can be differentiated on the basis of their interaction with adrenergic, muscarinic, cholinergic, and serotonergic receptors. The volume of distribution for SSRIs can reach up to 45 L/kg and thus, drugs of this class can be highly distributed in body tissues (Pashaei 2021). A study conducted on citalopram showed the generation of immunomodulatory reactions in HIV seropositive patients (Benton et al. 2010). The effects included increased NK cell innate immunity and reduction in HIV replication in macrophage cell lines. The study by Greeson et al. 2016 showed that citalopram downregulates CD4 expression and CCR5 expression, thus decreasing inflammation. Similar observations were also made for sertraline which reduced the replication of HIV in cerebrospinal fluid. Chronic inflammation is commonly observed in cancer patients; hence, drugs with anti-inflammatory properties can play possibly positive roles in the prevention and treatment of cancer.

Antidepressants target the monoaminergic neurotransmission pathway, i.e., serotonergic, dopaminergic, and noradrenergic. Some SSRIs have shown antifungal properties against pathogens like *Candida* sp. and *Aspergillus* sp. Their antifungal/antimicrobial potency can directly be linked to their lipophilicity, implying that the most vigorous antifungal agent will also be the most lipophilic (Young et al. 2003). Systematic drug delivery is often limited by insufficient supply of drug molecules to the target sites, impeding the treatment of the infected sites. The desirable condition for treatment includes high concentrations of drug at the targeted locations while also keeping the concentrations lower than the toxicity level. One approach toward meeting this objective is the use of medicine which allows sustained release of the drug at targeted sites. The use of nanocarriers ensures that high solubilization capacity is retained after drug administration (Patra et al. 2018). Unlike formulation-based solubilization, nanocarriers do not require the use of cosolvents when it comes to lipophilic compounds.

10.3.2 Antiemetics

Nausea and vomiting are generally linked to underlying causes. When a direct correlation with the cause is not found, the symptoms can be treated directly. Therapeutic modalities target the pathways of the neurotransmitters-serotonin, dopamine, histamine, and acetylcholine. Serotonin antagonists are often very effective in treating nausea. Some antiemetics can be used to boost innate and adaptive immunity against viral infections. Functional and structural links could be found between dopamine and serotonin systems. Serotonin receptors in brain play a role in the modulation of dopaminergic pathways. In certain studies, dopamine production has been linked to lower levels of serotonin. Dopamine agonist drugs like domperidone are commonly used antiemetics. These drugs can be used to control the blood

prolactin levels and boost innate and adaptive immunity. Dopamine antagonists are used to increase blood prolactin levels, which favor increased CD4 cell count against HIV (Sen 2020). Another antiemetic drug metoclopramide (MCP) has been successfully used against viral infections. MCP was found to inhibit dengue virus infection by targeting the dopamine 2 receptor (Shen et al. 2021). The antiemetic drug Rolapitant was investigated for repurposing in a study by Kabil et al. (2022), which suggested Rolapitant loaded nanovesicles as a possible treatment modality for lung cancer.

10.3.3 Antipsychotics

Several of the commonly used psychiatric medicines have been explored for their role in processes like chemotherapy. Studies have reported a link between the use of antipsychotic drugs for the treatment of schizophrenia and the lower occurrence rates of cancer in the drug-administered population. Primary observations suggest that psychiatric medicines could have an effect on cancerous cells (Elmaci and Altinoz 2018). Driscoll et al. (1993) performed an in vivo screening of several compounds which act on the CNS and found that psychotropic molecules could possess up to 18 times more antiproliferative potential then other agents. The administration of schizophrenia-related drugs like SSRIs, tricyclic antidepressants, and phenothiazines which induce glioblastoma (GBM) cell apoptosis and prevent GBM cell proliferation resulted in lower rates of colon, rectum, and prostate cancer (Jandaghi et al. 2016). These drugs have also been found to have a role in the upregulation of genes involved in lipid homeostasis. The upregulation of lipogenic genes is caused by the disruption of intracellular trafficking and results in the treatment as well as generation of side effects in psychiatric disorders. Antipsychotic drugs have been screened for their anticancer activity in various studies. One study explored the potential anticancer effects of the drugs chlorpromazine, olanzapine, risperidone, haloperidol, reserpine, and pimozide on tumor cell lines from neuroblastoma, lymphoblastoma, and breast adenocarcinoma (Wiklund et al. 2010). While all the drugs displayed anticancer activity by inhibiting cancer cells, pimozide was found to be highly effective. Pimozide acts as an antagonist of 5-HT_7 receptor and has the highest affinity toward the receptor when compared to other drugs. The drug inhibited cancer cell growth and proliferation and suppressed colony formation and STAT3 activity in the case of prostate cancer (Zhou et al. 2016). It also induced apoptosis in MCF-7 line of breast cancer (Strobl et al. 1990).

SSRIs modify the behavior of 5-HT in the synaptic space. Antidepressants and antipsychotic drugs target metabolisms related to monoamines. They regulate the amount and direction of serotonin in the synaptic spaces. By inhibiting the movement of serotonin toward the presynaptic neurons, SSRIs increase 5-HT concentrations in synapse space. These drugs can also be utilized for their antimicrobial properties, primarily against Gram-positive bacteria with the exception of *Pseudomonas* sp. and *Citribacter* sp. Antimicrobial activity was also shown against

H. Influenzae, *C. Jejuni*, and *Acinetobacter* sp. (Munoz-Bellido et al. 2000). Their antimicrobial functions can be limited by the maximum serum level attainable. However, they can be used in combination with other antibiotics to increase efficiency. The maximum serum levels were found to be around 1 mg/L, which is sufficient to inhibit bacterial metabolism although the serum levels mostly remain below the required concentration for inhibiting microbial growth.

10.4 Probable Repurposing/Repositioning of Antidepressants and Antipsychotics for Cancer Therapy and Antimicrobial Treatment

Fluoxetine: Fluoxetine was found to be helpful in treatment of human infections and can be used as an antiviral drug. Infections of bronchial epithelial cell line, observed in SARS CoV-2 were also reduced by the drug. Fluoxetine, prescribed for major depressive disorder (sold as Prozac, Rapiflux, Sarafem, and Selfemra), has also been shown to generate antitumor effects on certain types of cancers such as colon cancer, gastric cancer, lung cancer, and ovarian cancer. It suppresses tumor growth and has the ability to stimulate apoptosis by altering mitochondrial membrane permeability (Kannen et al. 2015).

Sertraline: The antidepressant sertraline has shown antitumor activities against colon cancer, breast cancer, and oral cancer. Sertraline can induce apoptosis toward certain types of cancer by regulating Ca^{+} concentrations (Chien et al. 2011). Anticancer activity has also been linked to changed serotonin levels induced by the drug.

Tranylcypromine: Tranylcypromine (TCP) was approved as an antidepressant by the FDA in 1961. It is a MAOI, which inhibits catabolism of serotonin and thus can alter concentrations of serotonin in the brain. TCP inhibits prostacyclin synthase CYP8A1 which results in anti-inflammatory activity and regulation of cardiovascular hemostasis (Ulrich et al. 2017). Thakare et al. (2020) highlighted that MAOIs like TCP can display antimicrobial activity by inhibiting efflux pumps.

Amitriptyline: Another of the tricyclic antidepressants is Amitriptyline. This drug has been in use since the 1950s and its mechanism of action includes inhibition of uptake of serotonin in the CNS. Amitriptyline displayed antimicrobial activity against bacterial (Gram-positive and Gram-negative) and fungal strains. Inhibitory effects were observed against *Bacillus*, *Staphylococcus*, *Salmonella*, *Shigella,* and *E. coli* with an MIC range of 25–100 μg/mL (Mandal et al. 2010). Antiplasmid activity can be shown by TCAs (Molnár 1988). Microbial plasmids are the primary reason behind antibiotic resistance as they carry the favorable genes. In addition to plasmid DNA, TCAs target the DNA gyrase enzyme. Thus, TCAs might be crucial for evading antibiotic resistance of bacteria. TCAs were also found to be effective against *Leishmania* sp. (Zilberstein and Dwyer 1984).

Olanzapine: Olanzapine, used in the treatment of psychotic disorders like depression and schizophrenia, is another serotonin receptor antagonist. It has high affinity for the 5-HT_{2A} receptor. This drug reduced the expression of survivin in cancer cell lines of pancreas and lungs, making them more responsive to chemotherapeutic drugs like 5-fluorouracil, cisplatin, and gemcitabine (Sanomachi et al. 2017). Survivin is a protein which is a part of inhibitor of apoptosis (IAP) family. It regulates cell proliferation and the cell cycle (Table 10.1). Reduction in survivin expression was also observed in noncancer stem cells of ovarian cancer.

Mirtazapine: The drug Mirtazapine, which plays a role in recovering serotonin levels and is used for treating clinical depression, displays antitumor responses against some types of cancer. Mirtazapine is a drug which falls in the class of TeCAs. Their mechanism of action involves increasing levels of serotonin and norepinephrine. Mirtazapine exhibits antitumor activities against gastric cancer, colon cancer, and human squamous cell carcinoma cells. Along with recovery of serotonin levels, it also activates immune responses. Mirtazapine also displayed antimicrobial properties, as it inhibited the growth of microorganisms *B. bifidum* 791, *L. rhamnosus* ATCC 53103, and *E. coli* ATCC 25922 at the concentration of 1000 ug/mL (Ivashchenko et al. 2021).

Duloxetine: Duloxetine, an SNRI drug, was patented in 1990. They inhibit the reuptake of norepinephrine and serotonin and hence are known as dual-acting antidepressants. The drug was found to have anticancer effects against lung and gastric cancer. It has comparatively lower toxicity levels (Hassani et al. 2019).

Vortioxetine: The antidepressant vortioxetine is a serotonin transporter antagonist. It plays a role in regulation of apoptosis and acts against gastric cancer by activating the phosphoinositide 3-kinase/ATK pathway (Taciak et al. 2018). It has a low IC_{50} value.

10.5 Conclusion

Drug repurposing is an important approach toward finding effective alternative uses of existing drugs against critical diseases. Serotonin, a neurotransmitter, plays an important role in maintaining cognitive and behavioral functions of the body. Many antidepressants and antipsychotics target the 5-HT receptors for treatment of mental disorders. Over the years, several studies have found that psychotropic drugs which interact with serotonin and serotonin receptors could be effective against several diseases like different types of cancers, infections, Alzheimer's, Parkinson's disease, diabetes, etc. Serotonin pathway influencing drugs were particularly found to be useful against different kinds of cancer including breast cancer, oral cancer, colon cancer, and gastric cancer. They were also found to display antimicrobial activity against several strains of bacteria, virus, and fungi. This implies that these drugs can used for probable repurposing on a much broader spectrum in the future.

Table 10.1 Drugs targeting the serotonergic pathway and their probable repurposable roles in the treatment of cancer and microbial infections

Drug	Brand name (s)	Class	Primary use	Anticancer activity	Antimicrobial activity
Citalopram	C Pram S, Celexa, Citrol	SSRI	Antidepressant	Downregulation of CD4 expression reducing inflammation	Immunomodulatory reactions in HIV seropositive patients
Fluoxetine	Prozac, Sarafem	SSRI	Antidepressant	Suppresses tumor growth, stimulates apoptosis	Anti-viral effect against SARS CoV -2
Sertraline	Zoloft	SSRI	Antidepressant	Suppresses tumor growth	Reduced the replication of HIV in cerebrospinal fluid
Amitriptyline	Elavil	TCA	Antidepressant	Inhibition of cellular proliferation, causes cell apoptosis	Inhibitory effects against some microorganisms
Tranylcypromine	Parnate	MAOI	Antidepressant and anxiolytic	Inhibits prostacyclin synthase CYP8A1 which results in anti-inflammatory activity	Acts as efflux pump inhibitors
Olanzapine	Zyprexa, Aedon	Phenothiazines	Antipsychotic	Reduces the expression of survivin in cancer cell lines	–
Mirtazapine	Remeron	TeCA	Antidepressant	Exhibits antitumor activities against gastric cancer, colon cancer and human squamous cell carcinoma cells	Inhibited the growth of certain microorganisms
Duloxetine	Cymbalta	SNRI	Antidepressant	Suppresses cell growth of MKN45 cells	–
Vortioxetine	Trintellix, Brintellix	Miscellaneous antidepressants	Antidepressant	Acts against gastric cancer by activating the phosphoinositide 3-kinase/ATK pathway, induces apoptosis	–

References

Anwar T, Kumar P, Khan AU (2021) Modern tools and techniques in computer-aided drug design. In: Molecular docking for computer-aided drug design. Academic, pp 1–30

Balakrishna P, George S, Hatoum H, Mukherjee S (2021) Serotonin pathway in cancer. Int J Mol Sci 22(3):1268

Benton T, Lynch K, Dubé B, Gettes DR, Tustin NB, Lai JP et al (2010) Selective serotonin reuptake inhibitor suppression of HIV infectivity and replication. Psychosom Med 72(9):925

Chien JM, Chou CT, Pan CC, Kuo CC, Tsai JY, Liao WC et al (2011) The mechanism of sertraline-induced [Ca2+] i rise in human OC2 oral cancer cells. Hum Exp Toxicol 30(10):1635–1643

Chung MK, Karnik S, Saef J, Bergmann C, Barnard J, Lederman MM et al (2020) SARS-CoV-2 and ACE2: the biology and clinical data settling the ARB and ACEI controversy. EBioMedicine 58:102907

Cui L, Iwamoto A, Lian JQ, Neoh HM, Maruyama T, Horikawa Y, Hiramatsu K (2006) Novel mechanism of antibiotic resistance originating in vancomycin-intermediate Staphylococcus aureus. Antimicrob Agents Chemother 50(2):428–438

Dayer A (2014) Serotonin-related pathways and developmental plasticity: relevance for psychiatric disorders. Dialogues Clin Neurosci 16(1):29

Díaz-Mataix L, Scorza MC, Bortolozzi A, Toth M, Celada P, Artigas F (2005) Involvement of 5-HT1A receptors in prefrontal cortex in the modulation of dopaminergic activity: role in atypical antipsychotic action. J Neurosci 25(47):10831–10843

Driscoll MS, Rothe MJ, Grant-Kels JM, Hale MS (1993) Delusional parasitosis: a dermatologic, psychiatric, and pharmacologic approach. J Am Acad Dermatol 29(6):1023–1033

Elmaci I, Altinoz MA (2018) Targeting the cellular schizophrenia. Likely employment of the antipsychotic agent pimozide in treatment of refractory cancers and glioblastoma. Crit Rev Oncol Hematol 128:96–109

Golden SR, Rosenstein DL, Belhorn T, Blatt J (2021) Repurposing psychotropic agents for viral disorders: beyond COVID. Assay Drug Dev Technol 19(6):373–385

Greeson JM, Gettes DR, Spitsin S, Dubé B, Benton TD, Lynch KG et al (2016) The selective serotonin reuptake inhibitor citalopram decreases human immunodeficiency virus receptor and coreceptor expression in immune cells. Biol Psychiatry 80(1):33–39

Hassani M, Ghassemi-Barghi N, Modanloo M, Mohammadpour A, Shokrzadeh M (2019) Cytotoxic effects of duloxetine on MKN45 and NIH3T3 cell lines and genotoxic effects on human peripheral blood lymphocytes. Arq Gastroenterol 56:372–376

Huang J, Zhao D, Liu Z, Liu F (2018) Repurposing psychiatric drugs as anti-cancer agents. Cancer Lett 419:257–265

Ivashchenko D, Buromskaya N, Kachanova A, Akmalova K, Grishina E, Shevchenko Y, Sychev D (2021) P. 0602 pharmacokinetic genetic factors are associated with antipsychotic's safety in adolescents experiencing acute psychotic episodes. Eur Neuropsychopharmacol 53:S441–S442

Jandaghi P, Najafabadi HS, Bauer AS, Papadakis AI, Fassan M, Hall A et al (2016) Expression of DRD2 is increased in human pancreatic ductal adenocarcinoma and inhibitors slow tumor growth in mice. Gastroenterology 151(6):1218–1231

Joshi P, Chakraborti S, Ramirez-Vick JE, Ansari ZA, Shanker V, Chakrabarti P, Singh SP (2012) The anticancer activity of chloroquine-gold nanoparticles against MCF-7 breast cancer cells. Colloids Surf B: Biointerfaces 95:195–200

Kabil MF, Nasr M, Ibrahim IT, Hassan YA, El-Sherbiny IM (2022) New repurposed rolapitant in nanovesicular systems for lung cancer treatment: development, in-vitro assessment and in-vivo biodistribution study. Eur J Pharm Sci 171:106119

Kannen V, Garcia SB, Silva WA Jr, Gasser M, Moench R, Alho EJL et al (2015) Oncostatic effects of fluoxetine in experimental colon cancer models. Cell Signal 27(9):1781–1788

Kruijt N, van den Bersselaar LR, Wijma J, Verbeeck W, Coenen MJH, Neville J et al (2020) HyperCKemia and rhabdomyolysis in the neuroleptic malignant and serotonin syndromes: a literature review. Neuromuscul Disord 30(12):949–958

Malik JA, Ahmed S, Jan B, Bender O, Al Hagbani T, Alqarni A, Anwar S (2022) Drugs repurposed: an advanced step towards the treatment of breast cancer and associated challenges. Biomed Pharmacother 145:112375

Mandal A, Sinha C, Jena AK, Ghosh S, Samanta A (2010) An investigation on in vitro and in vivo antimicrobial properties of the antidepressant: amitriptyline hydrochloride. Braz J Microbiol 41(3):635–642

Martinez MA (2022) Efficacy of repurposed antiviral drugs: lessons from COVID-19. Drug Discov Today 27:1954

McGowan OO, Reynolds GP (2020) Functional pharmacogenetics of serotonin receptors in psychiatric drug action. In: Handbook of behavioral neuroscience, vol. 31. Elsevier, pp 941–957

Molnár J (1988) Antiplasmid activity of tricyclic compounds. Methods Find Exp Clin Pharmacol 10(7):467–474

Munoz-Bellido JL, Munoz-Criado S, Garcıa-Rodrıguez JA (2000) Antimicrobial activity of psychotropic drugs: selective serotonin reuptake inhibitors. Int J Antimicrob Agents 14(3):177–180

Pashaei Y (2021) Drug repurposing of selective serotonin reuptake inhibitors: could these drugs help fight COVID-19 and save lives? J Clin Neurosci 88:163–172

Patra JK, Das G, Fraceto LF, Campos EVR, Rodriguez-Torres MDP, Acosta-Torres LS et al (2018) Nano based drug delivery systems: recent developments and future prospects. J Nanobiotechnol 16(1):1–33

Ramaswamy A, Balasubramanian S, Rajagopalan M (2021) Biomolecular talks—part 2: applications and challenges of molecular docking approaches. In: Molecular docking for computer-aided drug design. Academic, pp 245–269

Roessler HI, Knoers NV, van Haelst MM, van Haaften G (2021) Drug repurposing for rare diseases. Trends Pharmacol Sci 42(4):255–267

Sahu NU, Shah CP, Machhar JS, Kharkar PS (2019) Drug repurposing in search of anti-Infectives: need of the hour in the multidrug resistance era! In: In silico drug design. Academic, pp 399–426

Sanomachi T, Suzuki S, Kuramoto K, Takeda H, Sakaki H, Togashi K, Seino S, Yoshioka T, Okada M, Kitanaka C (2017) Olanzapine, an atypical antipsychotic, inhibits Survivin expression and sensitizes cancer cells to chemotherapeutic agents. Anticancer Res 37(11):6177–6188

Sen A (2020) Repurposing prolactin as a promising immunomodulator for the treatment of COVID-19: are common antiemetics the wonder drug to fight coronavirus? Med Hypotheses 144: 110208

Shaik FB, Swarnalatha K, Mohan MC, Thomas A, Chikati R, Sandeep G, Maddu N (2022) Novel antiviral effects of chloroquine, hydroxychloroquine, and green tea catechins against SARS-CoV-2 main protease and 3C-like protease for COVID-19 treatment. Clin Nutr Open Sci 42:62–72

Sharp T, Barnes NM (2020) Central 5-HT receptors and their function; present and future. Neuropharmacology 177:108155

Shen TJ, Hanh VT, Nguyen TQ, Jhan MK, Ho MR, Lin CF (2021) Repurposing the antiemetic metoclopramide as an antiviral against dengue virus infection in neuronal cells. Front Cell Infect Microbiol 10:606743

Song Y, Yang X, Yu B (2022) Repurposing antidepressants for anticancer drug discovery. Drug Discov Today 27(7):1924–1935

Stenvang J, Kümler I, Nygård SB, Smith DH, Nielsen D, Brünner N, Moreira JMA (2013) Biomarker-guided repurposing of chemotherapeutic drugs for cancer therapy: a novel strategy in drug development. Front Oncol 3:313

Strobl JS, Kirkwood KL, Lantz TK, Lewine MA, Peterson VA, Worley JF (1990) Inhibition of human breast cancer cell proliferation in tissue culture by the neuroleptic agents pimozide and thioridazine. Cancer Res 50(17):5399–5405

Taciak PP, Lysenko N, Mazurek AP (2018) Drugs which influence serotonin transporter and serotonergic receptors: pharmacological and clinical properties in the treatment of depression. Pharmacol Rep 70(1):37–46

Thakare R, Kaul G, Shukla M, Kesharwani P, Srinivas N, Dasgupta A, Chopra S (2020) Repurposing nonantibiotic drugs as antibacterials. In: Drug discovery targeting drug-resistant bacteria. Academic, pp 105–138

The World Bank (2017) Current health expenditure (% of GDP). https://data.worldbank.org/indicator/SH.XPD.CHEX.GD.ZS

Ulrich S, Ricken R, Adli M (2017) Tranylcypromine in mind (part I): review of pharmacology. Eur Neuropsychopharmacol 27(8):697–713

Wiklund ED, Catts VS, Catts SV, Ng TF, Whitaker NJ, Brown AJ, Lutze-Mann LH (2010) Cytotoxic effects of antipsychotic drugs implicate cholesterol homeostasis as a novel chemotherapeutic target. Int J Cancer 126(1):28–40

Yevtushenko OO, Reynolds GP (2010) Functional pharmacogenetics of serotonin receptors in psychiatric drug action. In: Handbook of behavioral neuroscience, vol. 21. Elsevier, pp 791–806

Young TJ, Oliver GP, Pryde D, Perros M, Parkinson T (2003) Antifungal activity of selective serotonin reuptake inhibitors attributed to non-specific cytotoxicity. J Antimicrob Chemother 51(4):1045–1047

Zhou W, Chen MK, Yu HT, Zhong ZH, Cai N, Chen GZ et al (2016) The antipsychotic drug pimozide inhibits cell growth in prostate cancer through suppression of STAT3 activation. Int J Oncol 48(1):322–328

Zhou Y, Hou Y, Shen J, Huang Y, Martin W, Cheng F (2020) Network-based drug repurposing for novel coronavirus 2019-nCoV/SARS-CoV-2. Cell Discov 6(1):1–18

Zilberstein D, Dwyer DM (1984) Antidepressants cause lethal disruption of membrane function in the human protozoan parasite Leishmania. Science 226(4677):977–979

Chapter 11
Drug Repurposing for Hematological Malignancies

Antony Anista Michael, Pavithra Balakrishnan, and Thirunavukkarasu Velusamy

Abstract Hematological malignancies are cancer that affects blood, bone marrow, and lymph nodes. They are classified as leukemia, lymphoma, and myeloma based on the type of cells it is affected. Despite numerous clinical drugs available, achieving complete remission for hematological malignancies still remains challenging, and therefore novel potential therapies are demanded. Drug repurposing opens up new horizons in the discovery of novel drugs by utilizing the existing medicines with an innovative approach. It facilitates the exploration of multitarget actions of a single drug and its involvement in other important biological pathways. Drug repurposing has helped to overcome failures in drug discovery process and with greater therapeutic breakthroughs.

Keywords Cancer · Hematological malignancies · Clinical trials · Drug discovery · Drug repurposing

11.1 Introduction

Cancer is characterized by the abnormal development of cells with uncontrolled division and has the ability to infiltrate the body and destroy normal body tissue (National Institutes of Health (US) 2007). Cancer has been perceived as a major public health concern worldwide for decades and its impact is increasing significantly day by day (World Health Organization (WHO) 2021). Moreover, it is found to be a pivotal cause of morbidity and mortality from a global perspective, regardless of the socio-economic level of human development. Globally, cancer has ranked second in the cause of mortality next to cardiovascular diseases. The WHO and World Cancer Report in 2018 have estimated that the cancer burden in India is about 1.8 million new cancer cases and 784,800 cancer deaths annually. It is noted that

A. A. Michael · P. Balakrishnan · T. Velusamy (✉)
Department of Biotechnology, School of Biotechnology and Genetic Engineering, Bharathiar University, Coimbatore, India
e-mail: thirunavukkarasu@buc.edu.in

R. C. Sobti et al. (eds.), *Drug Repurposing for Emerging Infectious Diseases and Cancer*, https://doi.org/10.1007/978-981-19-5399-6_11

there has been a growing trend in the incidences of cancer deaths and the global burden of cancer is anticipated to rise in the coming years.

According to Global Cancer Statistics 2020, around ten million cancer deaths occurred globally from the estimated 19.3 million incidences. With 2.3 million new cases annually, female breast cancer (11.7%) surpasses lung cancer (11.4%) to become the most commonly diagnosed cancer. Lung, colorectal (10%), prostate (7.3%), and stomach (5.6%) cancers are the next most frequently diagnosed cancers. However, lung cancer, which accounts for 18% of all deaths, is the leading cause of death, followed by colorectal (9.4%), liver (8.3%), stomach (7.7%), and female breast (6.9%) cancers (Sung et al. 2021). In 2025, the cancer prevalence shoots up to more than 20 million people as per the statistical predictions. Remarkably, malignant neoplasms such as liver, colorectal, prostate, and breast cancer will significantly contribute to cancer incidences and deaths due to difficulty in curing at terminal stages with contemporary treatments (Sleire et al. 2017). Even though a great volume of treatment paradigms is indeed existing, there are a multitude of challenges narrowing down the treatment efficacy in clinical oncology which includes physiological variation in tumors, their varied response to therapy, and multidrug resistance (MDR) (Hernández-Lemus and Martínez-García 2021). Thus, confronting these present and future limitations necessitates more effective alternatives and translational novel therapeutics.

Hematological malignancies are collectively defined as malignant neoplasms that affect the blood, bone marrow, and lymph nodes, with clinical presentation as leukemia, lymphoma, or myeloma (Jurlander 2011). Leukemia is the proliferation of immature, abnormal leucocytes in the bone marrow which limits the growth of normal blood cells. Based on the disease progression, it may be acute or chronic leukemia, and based on the type of cell involved, it is termed myeloid and lymphoid leukemia (Lyengar and Shimanovsky 2021). It includes major types such as acute lymphoid leukemia (ALL), chronic lymphoid leukemia (CLL), acute myeloid leukemia (AML), and chronic myeloid leukemia (CML). Other rare forms of leukemia are hairy cell leukemia (HCL), prolymphocytic leukemia (PLL), myelodysplastic syndrome (MDS), and large granular lymphocytic (LGL) leukemia. Lymphoma refers to the clonal proliferation of lymphocytes (B-cells and T-cells) in the lymph system and comprises about 5% of malignancies (Jamil and Mukkamalla 2021). It is broadly classified as Hodgkin's lymphoma (HL) and non-Hodgkin's lymphoma (NHL). In HL, there is a distinct pathognomonic feature of pleomorphic, abnormal, bi- or multinucleated Reed Sternberg cells found (Shanbhag and Ambinder 2018) and represent about 10% of all types of lymphoma. NHL accounted for the remaining 90% of lymphoma cases and it includes indolent follicular lymphoma, aggressive diffuse large B-cell, and Burkitt's lymphomas (Shankland et al. 2012). Multiple myeloma is a malignancy that occurs in the subset of B-lymphocytes known as plasma cells (Bird and Boyd 2019). The GLOBOCON in 2020 reports that new cases of hematological malignancies are constituted by 5,44,352 of non-Hodgkin's lymphoma, 4,74,519 of leukemia, 1,76,404 of multiple myeloma, and 83,087 of Hodgkin lymphoma, and their respective deaths are estimated as 2,59,793 and 3,11,594 and 1,17,077, and 23,376, respectively (Sung et al. 2021).

Despite several clinical drugs and therapies available, discovering a complete cure for hematological malignancies poses a greater challenge and hence new novel therapeutic options are direly needed. There are many hurdles for clinical drugs to becoming available in the market, most importantly: Intricate drug developmental process, high expenditure, adverse effects, and impotence of novel drug compounds. The estimated cost for developing a novel drug from bench to bedside is around $1.8–2.6 billion; and it takes 10–15 years till the new drug reaches the market (Paul et al. 2010; DiMasi et al. 2016). Therefore, an emerging interest aroused among clinicians and researchers in the recent decades to find additional potential for existing drugs and repurpose them as a cure for emerging as well as chronic diseases. The evident success of a few noncancer drugs in repurposing for cancer has enlightened clinical research, and such repurposing will be favorable in reducing considerable timeline and expense inevitable to develop new anticancer drugs (Kale et al. 2021).

11.2 How Drug Repurposing Can Help in Oncotherapeutics?

"The most fruitful basis for the discovery of a new drug is to start with an old drug" famously quoted by Sir James Black, the Nobel Prize Laureate in Physiology and Medicine, 1998.

The opportunity for drug repurposing comes from the route of *de nova* drug discovery and development (Patel 2020). Drug discovery and development is one of the intricate and arduous ventures in clinical applications. Apart from the complications behind therapeutic drug products, there are also myriad of issues associated with scientific, regulatory, legal, and marketable settings (Hernández-Lemus and Martínez-García 2021). Therefore, such an arduous environment has made conventional drug development to be an undeniably tedious and decelerated process.

Expenditure in research and development has increased twice in the OECD countries (Organization for Economic Co-operation and Development) and the number of drug compounds in development has increased 62% ever since the human genome was sequenced (Hay et al. 2014). Despite these endeavors, success in developing potential anticancer drug has been a quite difficult. The average duration taken for a drug to be publically accessible from the identification of a drug candidate to get cleared legal regulations vary between 10 and 15 years. Overall, the estimated cost for developing a new drug compound from the scratch to market authorization is $1.8–2.6 billion (Kale et al. 2021). Due to the limited efficacy of current cancer therapies, huge investment has been invested in alternative drug developmental approaches. Currently, there have been more than 10,000 clinical trials being under process on investigating drug interventions in neoplastic diseases. Despite that, the number of drugs achieving the final phase is extremely limited (Sleire et al. 2017). As per statistics, 1 in 10,000 compounds synthesized will

be ultimately approved by the Food and Drug Administration (FDA) (Salazar and Gormley 2017). Notably, Center for Drug Evaluation and Research (CDER), division of FDA, approved 59 drugs in 2018, 48 in 2019, 53 in 2020, 50 in 2021, and 16 in 2022 (till June) (FDA 2021). Specifically, oncology is a challenging pathological condition and there are several bottle necks in progressing the drugs to phase 3 clinical trials. The FDA demands overall patient survival rate as the primary objective in crucial oncology studies (Hay et al. 2014). Recently, drugs for rare, orphan indications and cancer are being emphasized in development. In 2017, FDA approved 18 drugs for orphan indications (39% of the total approved drugs), 12 anticancer drugs (26% of the total) in which 8 drugs are designated for orphan cancer implications (Mullard 2018). Generally, FDA-approved clinical drugs are highly priced to cover the expenditure invested for both failed and successful drug candidates (Sleire et al. 2017). Hence, the cost of cancer drugs is expensive which significantly increases the worldwide economic burden. Overall, these issues have driven for new approaches to identify optimistic candidates for cancer drug development, while lowering costs and speeding up the process.

11.2.1 De Novo Drug Synthesis

The de novo approach is broadly presented in five stages:

- **Drug discovery**
- **Preclinical studies/development**
- **Clinical studies/development**
- **FDA approval**
- **FDA postmarket surveillance**

Conventionally, novel anticancer drug development is a multifaceted process which begins with screening and generation of lead compounds, succeeded by extensive testing and characterization of their pharmacologic effects, antineoplastic activities, and adverse events in preclinical and clinical studies (Sleire et al. 2017). The overall process involves establishing a promising drug candidate for primary indications and conducting various experiments to retrieve detailed information on pharmacokinetics (ADME characteristics, its therapeutic effects, mode of action, optimal dosage, best route of administration, side effects, its interaction with other drugs, its comparative efficiency with similar design of drugs, and how it reacts to different populations of people).

11.2.1.1 Drug Discovery

Drug discovery aims at exploring a therapeutic compound which helps in curing and treating disease. This process involves target identification, target characterization, lead screening, and lead generation (Deore et al. 2019). When a lead molecule has

displayed satisfactory significance in these evaluations, the next phases of drug development will be initiated. The developmental and validation process of drug discovery has not changed over the past century. However, some of the stages of drug development have evolved in various ways as a result of revolutionary advances in biotechnology and informatics. For instance, rather than pharmacokinetics (ADME) and toxicological profiling, the process of lead optimization is now carried out using high-throughput screening (HTS) experiments (Salazar and Gormley 2017).

11.2.1.1.1 Target Identification/Discovery

It is the foremost process in the drug discovery. The significance of this phase is to perform strategies in identifying targets involved in specific clinical implications. A drug target is a key molecule, generally a protein or nucleic acid sequence which is intrinsically involved in gene regulation or signaling pathway specific to a disease or pathological indication (Rao and Srinivas 2011). A better and obvious comprehension of the molecular mechanism behind a disease and the specific role a drug target implicated in that disease should be considered in designing a "good" drug. A good target needs to be druggable and have universal advantageous properties such as possessing clinical niche to drive the disease. Drugs are designed in such a way to act either as inhibitors or activators of integral target molecules in a pathological indication. Researchers all over the world are working in collaboration to find therapeutic drug candidates that targets for a specific medical complication and improvise them to elicit desirable effect on a specific clinical target involved in a disease/condition, which can be evaluated through in vitro and in vivo functional experiments (Hughes et al. 2011). The techniques used for target identification is interdisciplinary in nature. In the typical drug discovery process, primary step is to decide which strategy and approach of drug discovery to be followed. With this, test assays such as enzyme assay, reporter assay, cellular assay, etc., are then developed to evaluate compounds (Kubota et al. 2019).

There are various approaches that led to the discovery of potential targets. They are,

- Bioinformatics—data mining tools for exploring therapeutic druggable targets
- Genetic association studies—to identify genetic variations associated with the disease such as single nucleotide polymorphisms (Deore et al. 2019)
- Genome-wide association studies
- mRNA and protein expression profiling—gene expression and their correlation with disease progression or exacerbation (Hughes et al. 2011)
- Phenotypic analysis/screening—developing cell-based assays to identify disease relevant targets
- Functional screening—Using knockdown, knockout studies

In advanced drug discovery process, two distinguishable strategies that can be commonly categorized as mechanism-first/molecular approach and compound-first/

empirical approach are termed respectively as target-based drug discovery (TBDD) and phenotype-based drug discovery (PBDD). The strategy to be employed should be considered prior based on the characteristics of drug compound for successful target identification.

Target-Centric Drug Discovery

Target-based drug discovery starts with the identification of a drug target molecule and subsequent validation by development of specified experimental assays (Kubota et al. 2019). After target identification, researchers can utilize various technologies like crystallography, computational modeling, genetics, bioinformatics, pharmacokinetics, biochemistry, and mutational analysis. This allows for understanding drug–target interaction, structure–activity relationship for effective drug design, biomarker development, and directing to the findings of next-generation drugs. The advantage of this strategy is that they are generally simpler, quicker, and affordable to establish and validate. Some of the successful drugs discovered based on the concept of target-based discovery are tyrosine kinase inhibitors for cancer including gefitinib (targets EGFR), imatinib (targets BCR-ABL fusion gene), sorafenib (targets Raf), and sunitinib (targets VEGFR/PDGFR), and antiviral drugs including raltegravir (HIV integrase inhibitor), and zanamivir (neuraminidase inhibitor which targets influenza) (Swinney 2013). The drawback of this method is that the assays performed are simplified and may not always reflect the complexities of clinical phenotype which could substantially reduce the likelihood of drug development success (Swinney and Anthony 2011).

Phenotype-Centric Drug Discovery

It is an empirical approach which relies on phenotypic measures of response. Drugs can be serendipitously identified and without having any prior knowledge or bias toward a specific biological target in the case of phenotypic screening. Moreover, pharmacological activities of drugs are initially determined in cells, tissues, or animals (Croston 2017). It starts with the establishment of standard cellular assays, which helps to monitor various characteristics such as cellular proliferation, differentiation, protein phosphorylation, receptor translocation, expression of genes, and proteins (Kubota et al. 2019). In the recent decade, phenotypic drug discovery (PDD) was reintroduced as a key strategy to provide successful first-in-class medicines (Moffat et al. 2017). The success of PDD is based on its unbiased characteristic identification of the mechanism of action involved in a disease (Swinney 2013). The process of identification of drug targets from phenotypic response is termed as target deconvolution. Yet, they also have issues in hit validation and target deconvolution. It is usually a time-consuming and expensive process with considerable significance in elucidating the molecular mechanism of action (MMOA) of drug candidates. Thus, it aids in designing structure-based drug for optimization. This generally cut the losses contributed by later-stage attrition efficiently (Terstappen et al. 2007). Indirect and direct methods for target deconvolution are

available. The indirect technique involves comparing the impact of test and known compounds on gene expression profiles. They can provide better pathway-level information. The direct techniques seek out proteins that bind to the test compound directly (Kubota et al. 2019).

Direct Approach of Target Deconvolution

- Chemical proteomics—Affinity chromatography and activity-based protein profiling (ABPP) technique
- Expression cloning techniques—Phage display, ribosomal display, mRNA display, and three-hybrid systems
- "Reverse transfected" cell microarray
- Biochemical suppression
- Protein microarray
- Chemoproteomics method—Cellular thermal shift assay (CETSA)

Indirect Approach of Target Deconvolution

- In silico approach for target screening such as utilizing public databases, Similarity ensemble approach, connectivity map, etc.,
- RNAi/CRISPR screening

Some of the successful drugs discovered based on phenotypic drug discovery concept are ezetimibe (Zetia) to reduce blood cholesterol levels using animal models and the first histone deacetylase inhibitor like vorinostat (Zolinza) which was discovered through cellular assays (Swinney 2013).

11.2.1.1.2 Target Validation

Following the selection of a prospective target, a thorough validation process should be followed to show that it is a factor in the pathogenesis and that its activity can be modulated. For the success of drug development in the subsequent phases, extensive and accurate target validation trials are required. Target is also ensured for its appropriateness to develop a pharmaceutical product prior to screening "hits." It entails evaluating the structural–activity relationship (SAR) of small drug analogs, creating drug-resistant mutants, knocking down or overexpressing the defined target, and also monitoring its downstream signaling in the pathways (Deore et al. 2019).

11.2.1.1.3 Lead Identification

Subsequently, "hits" or compounds that can react with the target to elicit desirable therapeutic effects are discovered through screening. For hit identification and the subsequent hit-to-lead selection process, it is critical to frame biologically and

pharmaceutically relevant screening assays. The hit-to-lead process aims to boost a compound's efficiency, specificity, and physicochemical properties (such as compound's stability and solubility) to proceed for in vitro and in vivo studies, as well as lead optimization process. There are various approaches employed to screen for hit molecules such as high-throughput screening assays (HTS), selective compound library screen, phenotypic screen, fragment-based screen, virtual screen, structural-based drug design, physiological screen, and NMR screen (Hughes et al. 2011). Once hit molecules have been found, analogs can be evaluated to assess the compound's structure–activity relationships (SAR). SAR data could be utilized for designing potential analogs. From the collection of "hits," lead compounds are prioritized by honing the screening assays to scrutinize the most promising drug compounds for further optimization. At this phase, the secondary assays for determining off-target effects, thermodynamic, physical, chemical, and pharmacokinetic (ADME—absorption, distribution, metabolism, and excretion) characteristics should be employed for lead selection. Potential toxicity of lead compounds must be assessed by passing through early-phase safety studies such as cytotoxicity and genotoxicity screening (Deore et al. 2019). Once the favorable lead compound is selected as drug candidate, it undergoes optimization process.

11.2.1.1.4 Lead Optimization

Optimization causes precise chemical modifications in the structure of desired lead molecules for improving further therapeutic effects by altering their target specificity, selectivity, drug metabolism, and biosafety profiles while retaining their favorable characteristics. Sufficient high-quality data on these aspects are required which can be retrieved through combined in silico and experimental approaches. Importantly, accurate prediction of target biomolecule-ligand binding affinities is essential for lead optimization using computational tools (Jorgensen 2009). Among many tools, mass spectrometry and fragment-based screening of nuclear magnetic resonance (NMR) are preferred to study protein–ligand interactions and thus aid in structure-based drug discovery (Deore et al. 2019).

11.2.1.2 Preclinical Development

Preclinical procedure entails assessing a drug's potency and biological safety before administering it in humans through a series of experiments. Typically, this involves in vitro, in vivo, and recently, also in silico experiments for concluding potential human outcome. In vitro studies are relatively rapid, simple, and affordable way of preclinical testing. They are based on utilizing cells, tissues, scaffolds, organ cultures, or biological components under confined experimental environments. In vitro-based studies can generate mechanistic data for the investigational compound's mechanism of action. On the other hand, in vivo studies utilize complete organism. Generally, it includes the use of rodent model (like rat, mouse, guinea pig, and

hamster), nonrodent model and primates (like monkeys, apes, etc.) which should comply with FDA requirements. Recently, noninvasive methods such as imaging technologies, microsampling, and telemetric monitoring are developed to use in animal model studies in drug development. In-vivo testing of animals is highly governed in most of the countries and requires ethical clearance from local ethical review committees by ensuring that no unnecessary harm is done to the experimental subjects (Honek 2017).

A typical preclinical development involves (Steinmetz and Spack 2009):

- Synthesis of active pharmaceutical ingredients (API)
- Pharmaceutical drug preformulation and formulation
- Pharmacokinetics/ADME studies
- Toxicology and safety studies

Preclinical trials must also be approved by the appropriate regulatory bodies. Regulatory agencies make sure that the studies are undertaken in a sound and ethical manner, and only those drugs that have been confirmed to be safe and effective will be approved (Deore et al. 2019). Up to this stage, an approximate cost of $500 million is invested in research and development sector for a single drug. Since the expenditure of rest of the phases in drug development will be doubled, it is mandatory that preclinical testing can be accurate to the maximum in establishing the therapeutic success of the drug and minimize the loss.

11.2.1.2.1 Investigational New Drug Application (IND) Filing

IND filling is the initial submission of obtaining permit to a clinical development. The transition from preclinical studies to clinical trial depends on the filing of an Investigational New Drug Application.

Drug developers must state the following details in the IND application (Deore et al. 2019):

- Data on preclinical studies
- Drug formulation information
- Setting up of clinical trial protocols
- Available data from any previous findings
- Investigator information

Later, the FDA surveys a comprehensive examination of the IND and issues one of the below-mentioned three action letters (FDA 2018):

- Approval letter—approval of the IND application and green signal for initiating clinical trials
- Approvable letter—the drug will be approved eventually after some essential rectifications and incorporation of labeling changes
- Not approvable letter—stated with a list of supporting reasons for disapproval

11.2.1.3 Clinical Trials/Development

Clinical trials adhere to a predetermined study protocol devised by the researcher, investigator, or manufacturer. They use current treatments to address basic concerns about the biosafety and potency of drugs, vaccines, and other mode of therapies in human subjects (volunteer). They follow systematic studies as phases. According to FDA, each stage of a clinical trial in investigating a drug or biologics is designated as phase. It is mainly based on the objective of study, participants involved, and other parameters. Totally, there are five defined clinical phases classified by FDA. Besides, there are trials that do not come under FDA-defined phases called as "not applicable" such as trials of devices or behavioral interventions. Their primary objective is to examine the therapeutic effect of the approved drug intervention in the general population and to generate data on side effects associated with widespread use over longer duration. Generally, if the drug has proven its efficacy in phase III trials, it receives FDA approval. However, 1 out of every 5000–10,000 potential anticancer drug compounds get authorization and only 5% of cancer drugs reaching phase I clinical trials are ultimately approved (Zhang et al. 2020).

11.2.1.3.1 Phase 0

Phase 0 trials are exploratory and first-in-human (FIH) trials to perform in accordance with FDA regulations. It is also known as human microdose approach in which small doses of drug are given to few people to quickly establish its effectiveness. This phase is generally conducted by pharmaceutical companies to determine which of their drug candidates has the best pharmacokinetic characteristics (Deore et al. 2019).

11.2.1.3.2 Phase 1: Safety

This phase of clinical research mainly focuses on the biological safety of a drug in human system. The general aim is to determine the drug's most obvious and serious adverse events and drug metabolism in the human body. Only a limited number of healthy volunteers participate in these trials (below 100) (https://clinicaltrials.gov/ct2/about-studies/glossary).

11.2.1.3.3 Phase 2: Efficacy

This phase involves the investigation of drug intervention in a large group of people/cohort (more than 100 people) to determine drug efficacy and further assessment of safety profiles (Zhang et al. 2020). This also includes comparing the efficacy in two

groups of people who have taken drug candidate and an inactive compound (called a placebo) or a different drug compound.

11.2.1.3.4 Phase 3

This phase mainly involves in generating detailed information on the efficacy and safety of drug by studying different populations, different doses, and also with other combination of drugs. These studies typically performed in longer duration with more number of volunteers. The results which come out of this phase are more plausible to determine long-term or specific side effects (https://clinicaltrials.gov/ct2/about-studies/glossary).

11.2.1.4 FDA Drug Review and Approval

A drug developer must mention all experimental studies, data, and detailed analysis about drug, from preclinical data to clinical phase 3 trial in the NDA form (New drug application) for its approval. As per the FDA, developers must include the following details along with the clinical results:

- Labeling usage
- Safety information
- Drug usage and abuse details
- Patent details
- Any prior data from studies conducted outside of the United States
- Institutional review board compliance information
- Directions for use

FDA's review team determines whether an NDA is complete or not. Incomplete NDA is eliminated and if it is completed, they extensively examine the submitted data for approval process. Generally, review team takes 6–10 months for deciding on drug approval. If FDA confirms that an investigational drug is safe and sound for its intended indication, the developer must create and update prescribing information. The process is known as "labeling." General information on what basis the drug has approved and drug usage details should be accurately mentioned on the label (FDA 2018).

11.2.1.5 Postmarket Drug Safety Monitoring (Phase 4)

This phase occurs after FDA approval of drug for marketing. It is a postmarketing surveillance involving pharmacovigilance and ongoing technical assistance. They collect more data in real-world situations concerning a drug's biological safety, effectiveness, optimum dose, and cost effectiveness. It may also be required by

regulatory authorities for labeling changes, risk management or for competitive purposes or other reasons (Deore et al. 2019).

Thus, the de novo drug development needs an ample amount of time, increased failure rate, more expensive, with limited approval rate (Sukhai et al. 2011). Given the sluggish success rate of *de nova* approaches, novel alternative strategies are therein required to find new therapeutic indication for plethora of existing approved drugs. One such strategy is drug repurposing that represents a viable alternative in clinical settings (Li and Jones 2012). In repurposing, patented or off-patent drugs with unknown anticancer activity can be proceeded into clinical trial for various cancer indications by investigating their pharmacokinetics (Sukhai et al. 2011).

11.3 Drug Repurposing/Repositioning

Drug repurposing is the technique of exploring and establishing new clinical applications for existing pharmaceutical drugs. It is also known by various names such as drug repositioning, drug retasking, drug reprofiling, drug rescuing, drug recycling, drug redirection, indication switching, and therapeutic switching (Gupta et al. 2013; Rudrapal et al. 2020). It involves discovering the novel therapeutic uses for nonprimary indications of old/existing/approved/investigational or discontinued drugs other than their intended/original medical indications. This method allows maximum utilization of therapeutic value of an existing drug. It is estimated that repurposing of approved drug takes an average timespan of 4–11 years at the expense of $40–80 million (Kale et al. 2021). Besides, the drug approval rate in repurposing approach is about ~30% when compared to ~10% by traditional approach (Ferreira and Andricopulo 2016).

Drug repurposing becomes a fascinating strategy in both biotech and pharmaceutical companies. Initial idea for drug repositioning came from serendipitous discovery in the 1920s. Due to technological advances, more novel approaches have been developed to escalate the process of drug repurposing. It utilizes the joint efforts of experimental and computational approaches to find new clinical applications of drug molecules on a logical basis. Since the drugs to be repurposed have defined profiles of biological safety, thermodynamic features, pharmacokinetics, optimum dose, adverse effects, mechanism of action, etc., the repurposing approach confers reduced risk of drug failure. In the case of repurposed drugs, they have already passed through in vitro and in vivo screening assays, optimization process with appropriate data and therefore these time consuming procedures can be bypassed (Ashburn and Thor 2004). Hence, it becomes rationale in discovering repurpose of existing drugs.

Some examples of repositioned drugs are sildenafil, thalidomide, minoxidil, dimethyl fumarate, aspirin, valproic acid, methotrexate, etc. A noted example, sildenafil (Viagra), a phosphodiesterase-5 (PDE5) inhibitor primarily indicated for hypertension and coronary artery disease (angina), manufactured by Pfizer (1980). They noted penile erection as side effects in phase I. Then, it has been repurposed to treat erectile dysfunction (Ashburn and Thor 2004). Metformin (Glucophage) is

intended as antidiabetic medication which has now been repurposed for oncology drug and now it is currently under phase II/phase III clinical trials (Rudrapal et al. 2020). Dimethyl fumarate which was earlier used in the treatment of psoriasis in Europe and later it was repurposed and approved to treat multiple sclerosis in 2013 (Parisi et al. 2020). Even a drug's side effects can be advantageous in the context of another application. For example, strong antiangiogenic activity of thalidomide found to be useful for the indication of multiple myeloma (Ashburn and Thor 2004). With the exploitation of omics data and system biology, the secondary targets of approved drugs can be scrutinized. Recent advances in computational tools such as the use of structure-based drug design (SBDD) and artificial intelligence (AI) technology have further promoted the drug repurposing process (Rudrapal et al. 2020) Also, the attrition rate for a repurposed drug before reaching the market is comparatively lower than for novel compounds (Scherman and Fetro 2020). Thus, it is an effective alternative to conventional drug synthesis and emerging approach in drug discovery and development process.

11.4 The Drug Repurposing Overview

Drug repositioning primarily involves four steps including drug compound identification, compound retrieval, development, and FDA postmarket surveillance. It begins with the identification of suitable drug molecules. Drugs constituting potential candidates for repurposing are categorized as generic drugs, failed drugs (have passed clinical Phase I but failed to exhibit efficacy in clinical Phase II for a particular disease), and patented drugs (Chakraborty and Trivedi 2015). Generic drugs are approved drugs that are no longer protected by patents. It is a common resource for academia, biotech, and pharmaceutical companies because they are available with well-documented safety profiles. They can be readily accessible for preclinical evaluation and subsequent clinical development (Chakraborty and Trivedi 2015). Failed drugs are drugs which failed the required criteria but may have passed some stages in clinical trials. Patented drugs are generally approved by FDA or in the investigational phase of later clinical studies (Chakraborty and Trivedi 2015). Among all people, the pharma companies have privilege in accession to information on drugs including both failed and patented drugs. Therefore, data accession facilities are favorable toward pharmaceutical companies which makes them primal partner in repurposing findings.

The drug repurposing process can either follow experimental or computational or mixed approaches. Once a secondary target is found to an existing drug, proof-of concept experimentation has to be conducted to investigate on the drug effects in new indication. In vitro, in vivo, and in silico studies are commonly employed to evaluate the repositioning. After hypothetical validation, the repurposed drug can pass directly into the phase II and III of clinical trials (Chakraborty and Trivedi 2015).

11.5 Profiles of Drug Repurposing

Two main profiles of drug repositioning are on-target and off-target. In on-target drug repositioning, pharmacological mechanism of a compound which has indicative use for new complication is known. Hence, the biological target of the particular drug molecule is same for the different indication (de Oliveira and Lang 2018). Repositioning of minoxidil (Rogaine) is the typical example of an on-target profile, since the drug has same target with two different therapeutic effects. Minoxidil was repurposed from an antihypertensive indication into preventing hair loss since it functions as vasodilators (Ashburn and Thor 2004) which is used to treat androgenic alopecia (Messenger and Rundegren 2004). The "off-target" strategy entails unknown pharmacokinetics and therefore, the target and the indications are novel (Ojezele et al. 2020). Both the compound's target and indication are different and out of the original scope. An instance of off-target profile is aspirin (Colsprin). Aspirin is a common NSAID to treat pain and inflammation. It also inhibits platelet activity, which decreases blood coagulation (antiplatelet drug). As a result, it is utilized to treat heart problems (Rudrapal et al. 2020). Another new application of aspirin as a promising candidate in the treatment of solid cancers has also been reported (Turanli et al. 2021).

11.6 Approaches of Drug Repurposing

Detection of novel drug–disease relationships is the foremost challenge in drug repurposing. A variety of approaches have been developed including the generally categorized empirical/experiment-based approach, computational/in silico-based approach, and mixed approach (Patel 2020; Chen et al. 2016).

11.6.1 Experimental Approach

The experiment-based approach is also known as activity-based repositioning which involves screening of old-intended drugs for new clinical indications based on experimental assays. This approach does not require prior data on protein structure. It employs target-based and phenotype-based analysis using functional experiments which can provide better understanding of connection between drugs and pathological condition. This approach is considered as more reliable and credible (Rudrapal et al. 2020). Cancer, diabetes, and infectious diseases are among the promising hits identified by this approach. Some potential anticancer drugs that have been explored through this method are itraconazole (antifungal), and digoxin (cardiac glycoside) (Turanli et al. 2018) (Table 11.1).

Table 11.1 Pros and cons of experimental-based drug repositioning (Shim and Liu 2014)

Pros	Cons
No limitation for target-centric and phenotype-centric screening assays	Labor and time-consuming process
Easy to validate "hits"	Needs an entire list of existing drugs
Low false-positive hits during the screening	Screening assay requires to be developed

Table 11.2 Pros and cons of computational drug repositioning (Chen et al. 2016; Shim and Liu 2014)

Pros	Cons
Time, labor, cost efficient, and low risk of failure	Requires precise/high-resolution structural information about drug targets
Entire list of existing drugs are not required	Disease based phenotypic or genotypic drug profiles are needed if the protein target is not found
Screening assays are not required	High degree of false-positive hits will be found

11.6.2 Computational Approaches

In silico drug repositioning is an effective strategy and has gained wide popularity with significant success in drug discovery process over the past few decades. It involves virtual analysis of databases and drug libraries (Chen et al. 2016) to accelerate the drug discovery process by using structural biology and omics technologies. By utilizing this approach, known biological targets, ligand molecules, biomarkers, or signal transduction pathways of a particular disease can be established (Jin and Wong 2014; Parvathaneni et al. 2019). Due to rapid advances, microarray techniques and all kinds of public databases are available and accessible (Xue et al. 2018). This knowledge and data further promoted the novel signature-based and network-based approaches (Table 11.2).

In silico approaches are broadly categorized into drug-based, target-based, therapy/disease-based, signature-based, and pathway- or network-based approaches.

11.6.2.1 Drug-Centric Repositioning

In the drug-oriented methodology, drug candidates are identified from serendipitous observations. The physiochemical properties of drug compounds, therapeutic effects, toxicities, and adverse events are assessed. This method is designed to assess the biological effectiveness of pharmacological molecules without having a thorough understanding of the biological targets. It is based on conventional pharmacology and drug discovery principles (Rudrapal et al. 2020). Novel target for a particular indication can be determined for a given drug using this approach (Parisi

et al. 2020). For instance, valproic acid is originally intended for bipolar disorder and seizures. It also have an off-target binding affinity with the histone deacetylase 2 (HDAC2), a protein which is involved in many types of cancers. Thus, it leads to repurposing for neoplastic conditions such as Familial Adenomatous Polyposis (FAP) (Huang and Guo 2006). Allopurinol, doxycycline, lidocaine, mazindol, valproic acid, and zidovudine are some of the drug candidates developed by drug-centric repositioning (Parisi et al. 2020).

11.6.2.2 Target-Centric Repositioning

The purpose of target-based methodology is to discover drugs based upon known target molecules. It involves in vitro and in vivo screening such as high-throughput screening (HTS) and/or high-content screening (HCS) of drugs for a protein or a biomarker of interest and also comprises in silico screening of drugs or compounds from drug libraries such as ligand-based screening or docking (Jin and Wong 2014). This method poses a significant success rate in drug discovery as compared to drug-oriented method because most targets link directly with the disease mechanisms (Swinney 2013). This method is based on establishing a novel relationship between a new indication and an established target. For example, the indication shift of tyrosine-protein kinase ABL from cancer to neuro-degeneration suggests the use of nilotinib inhibitor against the Parkinson syndrome (Karuppagounder et al. 2014). Sildenafil, tretinonin, histamine, propranolol, celecoxib, and azacitidine are some of the drugs which represent a case of target-centric repositioning (Parisi et al. 2020).

11.6.2.3 Disease-Centric Repositioning

The methodology of disease/therapy-oriented repositioning is used whenever there is more information on the disease model is available. The information is given by proteomics (disease-specific target proteins), genomics (disease-specific genetic data), metabolomics (disease-specific metabolic pathways/profile), and phenotypic data (off-target mechanism, pharmacological targets, disease pathways, pathogenesis, adverse events, etc.) concerning the disease process. As a result, the construction of specialized disease networks, the recognition of genetic expression, the consideration of critical targets, and the identification of disease-causing protein molecules which is involved in important cellular and metabolic pathways in the disease models will be identified (Rudrapal et al. 2020). This method is followed in successful drug development in the majority of cases (more than 60%). Remaining 30% of the cases use based on target-centric and less than 10% apply drug-centric approaches. Alitretinoin, arsenic trioxide, clofarabine, doxorubicin, floxuridine, idarubicin, lapatinib, nilotinib, and paclitaxel are some of the disease-centric repositioning cases (Parisi et al. 2020).

11.6.2.4 Signature-Based Approaches

This approach is based on classification of drugs based on their transcriptomic signatures. In this recent approach, signature reversion principal is followed so that if a drug-induced transcriptional signature is similar or dissimilar to a disease signature. Based on that, the drug may restore or reverse the disease phenotype. Disease-associated transcriptomics information are either self-generated in laboratories or readily available from public repositories such as the National Center for Biotechnology Information (NCBI), European Bioinformatics Institute (EBI), or the DNA Data Bank of Japan (DDBJ) (Shukla et al. 2021). It is effective in generating a connectivity map that links pathogenic conditions and drug actions. They are preferred to validate preclinical and clinical studies due to low false-positive rates compared to computational methods. Limitations include increased costs, time, and labor or dependent on specialized robotic equipment (Turanli et al. 2021).

11.6.2.5 Network-Based Approaches

In this approach, different essential associations between targets and drugs can be discovered via statistical and computational means from the data networks (Lotfi Shahreza et al. 2018). For example, in a metabolic network, the nodes represent chemical compounds and metabolites. Molecular networks such as drug–drug, drug–target, drug–disease, disease–disease, disease–gene, disease–drug, co-expression networks, protein–protein interactions, signaling networks, transcriptional regulatory networks, and metabolic networks can be utilized in systems pharmacology. Pathway/networks-based models are used for identifying molecular mechanisms and prognostic or diagnostic biomarkers in many diseases including wide variety of cancers (Turanli et al. 2021).

11.6.2.6 Mixed Approach

Recently, researchers have preferred combinational use of in silico and experimental approaches to find new therapeutic indications for existing drugs, called mixed approach. In the mixed approach, the result of computational methods is validated by preclinical biological experiments (in vitro and in vivo tests) and clinical studies (Xue et al. 2018). The simultaneous application of computational and experimental methodologies in a systematic manner provides a robust and rationale approach to the exploration of new indications, demonstrating a greater efficiency than the serendipitous findings (Rudrapal et al. 2020).

11.7 Drug Repurposing for Hematological Malignancies

Reaching the novel oncology drugs for malignancy into the marketplace is expensive and time consuming. Thus, in parallel with the design and synthesis of new therapeutic anticancer drugs, alternative strategies like repurposing of the large number of already approved both anticancer and non-anticancer drugs are also considered (Hernández-Lemus and Martínez-García 2021). There are several types of hematological malignancies which are further subtyped and grouped based on the underlying genetic aberrations that drive the disease or molecular signatures that underlie the progression of the disease. More than 70 types of lymphoma and 4 main types of leukemia are reported each warranting a specific drug treatment (Memorial Sloan Kettering Cancer Center 2022; Lymphoma Action 2022; American Cancer Society 2022; Cancer Treatment Centers of America 2021). Therefore, new drug entities to treat hematological malignancies are always in high demand. Considering the mundane process involved in developing drugs, huge amount of financial and manpower investment need to be dedicated to treat them. Since drugs for noncancer indications have prior preclinical data and partial or full clinical development, repurposing such drugs for hematological malignancies would cost much less, and drastically reduce the development time, which is evident in case of thalidomide (Kale et al. 2021). Even, academia and smaller biotechnology companies can involve in the field of drug discovery by utilizing drug repurposing methods mainly because of ensured "de-risk" in the early stages (Sukhai et al. 2011). The significant reduction in the cost of drugs will help pharmaceutical companies to deliver drugs to cancer patients earlier and at an affordable price.

Approved chemotherapeutic agent can be repurposed by testing its efficacy in other malignancies. In many cases, the evaluation of a newly approved chemotherapeutic agent for other indication is not based on extensive preclinical and mechanistic studies but rather analyzing the early signals in phase 1 clinical trials that include patients with diverse tumor types (Sukhai et al. 2011). For example, the first-in-class proteasome inhibitor bortezomib (Velcade) was effective for myeloma and therefore it was tested for repurposing. Currently, it is under phase 2 clinical trials for patients with diverse malignancies, including mantle cell lymphoma and acute myelogenous leukemia (AML) (Orlowski et al. 2002). Ultimately, these trials demonstrated that single-agent bortezomib produced response rates of 40–50% in patients with mantle cell lymphoma. Later, bortezomib was ultimately approved for this new indication. This kind of repurposing of an existing anticancer drug in a novel oncological setting is technically known as soft repurposing (Valli et al. 2020). Other chemotherapy drugs like clofarabine, cladribine, actinomycin D, azacytidine, melphalan, hydroxyurea, and arsenic trioxide can also be repurposed for AML (Valli et al. 2020).

Another way of repurposing is known as hard repurposing which involves the use of noncancer drugs as a repurposed oncotherapeutics. The rationale for evaluating the anticancer effects of noncancer drugs depends on their mechanisms of action, efficacy, and minimal side effects (Perez et al. 2021). The identification of

Table 11.3 Repurposed noncancer drugs for cancer (Kale et al. 2021; Gupta et al. 2013; Turanli et al. 2021)

Drug	Primary indication	New indication
Aspirin	Analgesic, antipyretic, anti-inflammatory	Colorectal cancer
Valproic acid	Antiepileptic	Solid tumors
Celecoxib	Osteoarthritis, rheumatoid arthritis	Colorectal cancer, lung cancer
Noscapine	Antimalarial, analgesic	Multiple cancer types
Nitroxoline	Antibiotic	Bladder, breast cancer
Thiocolchicoside	Muscle relaxant	Leukemia, multiple myeloma
Vesnarinone	Cardioprotective	Oral cancer, leukemia, lymphoma
Leflunomide	Rheumatoid arthritis	Prostate cancer
Minocycline	Acne	Ovarian cancer, glioma
Zoledronic acid	Antibone resorption	Prostate and breast cancer
Metformin	Antidiabetic	Breast, prostate, bladder, colorectal, endometrial, pancreatic, lung, thyroid, liver cancers
Statins	Myocardial infarction	Breast, prostate, hepatocellular, head, and neck cancers
Digoxin	Antiarrhythmic agent	Prostate, breast, renal, lung cancers, and melanoma
Disulfiram	Management of chronic alcoholism	Breast, colon, lung, thyroid, and uterine cancers
Itraconazole	Antifungal	Lung, prostate, esophageal, brain cancers
Niclosamide	Antihelminthic agent	Prostate cancer
Dexamethasone	Anti-inflammatory agent	Prostate cancer
Raloxifene	Osteoporosis	Breast cancer

noncancer-indicated compounds for its repurpose in hematological malignancies can be exploited by discovery of compounds being inhibitors of specific targets (i.e., receptors or enzymes deregulated in such malignancies) or through unbiased screening of "Hit" compounds (Valli et al. 2020).

The major obstacles being faced in chemotherapeutics are their side effects. They are primarily due to the drug's lack of selectivity for leukemic cells. Leukemic chemotherapeutics exhibit cardiotoxicity, because like cancer cells, cardiac muscle cells are also metabolically active. Further, leukemia treatments have also been associated with heart disease, long-term cognitive impairment, liver, and kidney damage (Perez et al. 2021). Thus, the need for selective drugs and the new treatment regimens for hematological malignancies can also be effectively developed through drug repurposing/repositioning. Some of the noncancer drugs which are identified as promising and potential drugs for repurposing in hematological malignancies and solid cancers are described in brief as follows (Tables 11.3 and 11.4).

Table 11.4 Repurposed noncancer drugs for hematological malignancies (Kale et al. 2021; Gupta et al. 2013; Wojcicki et al. 2020)

Drug	Primary indication	New indication
Thalidomide	Antiemetic	Multiple myeloma
Wortmannin	Antifungal	Leukemia
Zoledronic acid	Antibone resorption	Multiple myeloma
Statins	Myocardial infarction	Leukemia, lymphoma
Digoxin	Antiarrhythmic agent	Leukemia
Itraconazole	Antifungal	AML and ALL
Nelfinavir	Antiviral (HIV infection)	Multiple myeloma
Artesunate	Antimalarial	Leukemia
Aspirin	Anti-inflammatory	Multiple myeloma
Mebendazole	Antihelminthic	AML
Auranofin	Rheumatoid arthritis	CML and CLL
Leflunomide	Rheumatism	Multiple myeloma
Metformin	Antidiabetic (type 2 diabetes)	CML, B-cell lymphoma
Rapamycin	Antifungal	AML and CML
Bisphosphonates	Osteoporosis	Multiple myeloma
Lovastatin	Hypercholesterolemia	Refractory or relapsed AML
Thioridazine	Schizophrenia	Refractory or relapsed AML

11.8 Leukemia

Leukemia is the proliferation of immature, abnormal leucocytes in the bone marrow which limits the growth of normal blood cells. Based on the disease progression, it can be classified as acute or chronic leukemia and based on the type of cell involved, it is termed as myeloid and lymphoid leukemia (Lyengar and Shimanovsky 2021). It includes major types such as acute lymphoid leukemia (ALL), chronic lymphoid leukemia (CLL), acute myeloid leukemia (AML), and chronic myeloid leukemia (CML). Other rare forms of leukemia are hairy cell leukemia (HCL), prolymphocytic leukemia (PLL), myelodysplastic syndrome (MDS), and large granular lymphocytic (LGL) leukemia (Lyengar and Shimanovsky 2021).

11.8.1 Acute Lymphoid Leukemia (ALL)

Acute lymphoblastic leukemia (ALL) originates from proliferation of T (T-ALL) or B (B-ALL) cells progenitors. In B-ALL, induction therapy comprises of 4–6 weeks of a glucocorticoid (e.g., dexamethasone or prednisone), asparaginase, vincristine, and an anthracycline. This is followed by consolidation and maintenance therapies with the use of some induction agents such as 6-mercaptopurine or 6-thioguanine, or methotrexate for several months (Perez et al. 2021; Hunger and Mullighan 2015).

11.8.1.1 Tigecycline (TGC)

It is a first compound in glycylcycline class of antibiotics, derived from tetracyclines. It was approved by FDA in 2005 for skin, soft tissue, and intra-abdominal infections (Wexler 2015). It prevents amino acid elongation by binding to the 30S bacterial ribosomal subunit, thereby blocking entry of amino-acyl tRNA molecules to the A site (Greer 2006). Generally, the mechanism of bacterial resistance occurs by alterations in tetracycline efflux or ribosomal protection (Greer 2006). However, structurally modified novel TGC evades the Tet (A-E) efflux pumps and thus overcome Tet resistance in *Enterobacteriaceae* and Acinetobacter spp. (Livermore 2005). In ALL, characteristic elevated levels of OXPHOS in glucocorticoid (GC) resistance can be inhibited by TGC by suppressing the synthesis of mitochondria-encoded proteins, required for OXPHOS (Olivas-Aguirre et al. 2021). It is reported that TGC causes inhibition of mitochondrial respiration, triggering apoptosis and exhibit synergism with standard chemotherapeutic drugs vincristine and doxorubicin in multiple GC sensitive and GC resistant ALL cell lines (Fu et al. 2017). Besides, it is less cytotoxic to normal hematopoietic cells and effective against both newly diagnosed and for refractory treatment (Olivas-Aguirre et al. 2021).

11.8.1.2 Tamoxifen (TAM)

It is a gold-standard treatment indicated for the ER-positive breast cancers (Olivas-Aguirre et al. 2021; Yao et al. 2020). It is a nonsteroidal triphenylethylene derivative and has tissue specific-dual function of both estrogenic and antiestrogenic action. In breast cancer, it mediates its action by binding to the estrogen receptor and by blocking the proliferative actions of estrogen on mammary epithelium (Sporn and Lippman 2003). It is reported that TAM causes mitochondrial and lysosomal dysfunction and efficiently provokes autophagy, thereby inducing cell cycle arrest and reduces cell viability in GC-resistant Jurkat cells (Olivas-Aguirre et al. 2021). TAM suppresses protein kinase C and PI3K/Akt/mTOR signaling cascade pathways. It targets mitochondria by disrupting membrane fluidity and interacts with inner membrane pore proteins, electron transport chain proteins, and proteins of the Bcl-2 family, thereby triggering apoptosis (Olivas-Aguirre et al. 2021; Bogush et al. 2018). It causes activation of autophagy by increasing the permeability of the lysosomal membrane with the release of cathepsin D (Olivas-Aguirre et al. 2021; Bogush et al. 2018). Thus, it represents a favorable candidate for ALL treatments. There are also reports which showed that the differentiation-inducing effect of ATRA (all-trans retinoic acid) has significantly increased in acute promyelocytic leukemia when combined with Tamoxifen (Adachi et al. 2016).

11.8.1.3 Cannabidiol (CBD)

Cannabidiol is an active phytocannabinoid extracted from *Cannabis sativa* (Joca et al. 2021). The antidepressant effect of CBD is used as an adjunctive treatment for the management of seizures associated with Lennox–Gastaut syndrome and symptomatic relief of neuropathic pain or other painful conditions, like cancer (Drug Bank n.d.). It is demonstrated that CBD significantly impaired the viability and the migration of leukemic cells in T-ALL cell lines rather than the B-ALL. The primary target of CBD is mitochondria and mediates its action through MTP-driven necrosis. It is caused by direct interaction with VDAC channel in the outer mitochondrial membrane, resulting in Ca^{2+} overload and thus promoting the formation of the mitochondrial transition pore (MTP). CBD is found to be efficient in both GC-sensitive and GC-resistant cell lines (Olivas-Aguirre et al. 2021). The synergic effect of CBD was also studied in preclinical models of hematological malignancies. CBD was demonstrated to be effective along with ibrutinib in diffuse large B-cell lymphoma and mantle cell lymphoma (Strong et al. 2018). Importantly, CBD decreases the cytotoxicity of doxorubicin in ALL chemotherapy treatment (Hao et al. 2015). Thus, CBD can be included in the antileukemic treatments to improve the survival outcome of the patients.

11.8.2 Chronic Lymphoid Leukemia (CLL)

Chronic lymphocytic leukemia (CLL) is a B lymphoid malignancy which mainly depends on the microenvironment for disease pathogenesis (Gimenez et al. 2020). Initial chemo-immunotherapy consider as a gold treatment for CLL. It involves fludarabine, cyclophosphamide, and rituximab (FCR). It displays high emission rate but relapse and refractory becomes the major obstacle and it occurs especially in CLL with the deletion of chromosome 11q or 17p (Fiskus et al. 2014). Currently, new targeted therapies have been approved for CLL such as ibrutinib, a Bruton's tyrosine kinase (BTK) inhibitor which targets the B cell receptor (BCR) signaling, and venetoclax, a BCL2 inhibitor. These therapies significantly improve survival rate than chemo-immunotherapy (Gimenez et al. 2020; Bosch and Dalla-Favera 2019).

11.8.2.1 Simvastatin

Statins are the recent primary treatment for indication of hyperlipidemia. They can be broadly categorized as natural and synthetic. They differ in their ability to inhibit HMG-CoA reductase and their lipophilicity nature (Azemawah et al. 2019). Statins inhibit cholesterol biosynthesis and the byproducts of cholesterol provide survival for the cancer cells. Thus, the functionality of statins has been tested against a

number of cancer cells (Kale et al. 2021). The combined use of statin and aspirin increases the susceptibility of CLL patients to FCR immunochemotherapy (Podhorecka et al. 2010). Recent findings showed that the simvastatin was the most effective among statin compounds in reducing cell survival, proliferation, and cell adhesion in CLL (Gimenez et al. 2020). It causes apoptosis by inducing DNA damage (Gimenez et al. 2020). It is also synergistic along with the existing targeted therapies of venetoclax and ibrutinib (Gimenez et al. 2020).

11.8.2.2 Auranofin

Auranofin (Ridaura) is a gold containing compound indicated for the treatment of rheumatoid arthritis and approved by FDA in 1982 (Argüello-García et al. 2020). It has potent preclinical activity against CLL by inducing a lethal oxidative and endoplasmic reticulum stress response in cultured and primary CLL cells with deletion of chromosome 11q or 17p (Fiskus et al. 2014). It is also reported that synergistic effects were found with inhibitors of heme oxygenase-1 and glutamate-cysteine ligase against CLL cells (Fiskus et al. 2014). As a result, phase 1 and 2 clinical trials were evaluated for the safety and effectiveness of auranofin to treat patients with chronic lymphocytic leukemia (CLL), prolymphocytic lymphoma (PLL), or small lymphocytic lymphoma (SLL) (https://clinicaltrials.gov/ct2/show/NCT03829020).

11.8.3 Acute Myeloid Leukemia (AML)

Acute myeloid leukemia (AML) is a heterogeneous clonal myeloproliferative disorder characterized by proliferation of myeloblasts in the bone marrow and peripheral blood (Saultz and Garzon 2016). In acute leukemia, chemotherapeutic agents are used to induce remission of the disease by reducing the blast cells population and/or induce differentiation. The standard treatment of AML is the "7+3" drug regimen indicating the use of 7 days of cytarabine (AraC) and 3 days of anthracycline (e.g., daunorubicin, idarubicin, doxorubicin) (Valli et al. 2020). Relapse can occur often and remained to be the principal problem in leukemia. Therefore, the induction therapy is usually followed by consolidation therapy which comprises the use of high-dose AraC for treating refractory or relapsed AML (De Kouchkovsky and Abdul-Hay 2016).

11.8.3.1 Valproic Acid

Valproic acid is extracted from valeric acid which is naturally produced by the flowering plant valarian (*Valeriana officinalis*). In 1882, it was first synthesized by Beverly S. Burton. Clinically, it is used as long-term treatment of anticonvulsant in

epilepsy and mood stabilizing drug for bipolar disorder. It is orally administered and has a low toxicity profile. Later, its anticancer activity was found as a histone deacetylase (HDAC) inhibitor and importantly also found to induce differentiation and/or apoptosis of transformed hematopoietic stem cells and AML cells from patients (Andresen and Gjertsen 2017). Currently, it is involved in different anticancer clinical trials. Also, it exhibits synergistic effects with a numerous drugs. Emerging studies are focusing on novel development of low-toxic drug combinations with valproic acid.

11.8.3.2 Artesunate

Artesunate (Art) is a semi-synthetic derivative of artemisinin derivative from the plant *Artemisia annua* and indicated for the initial treatment of malaria in adult and pediatric patients. Artemisinins (ARTs) exhibited synergistic effect when combined with chemotherapeutics in AML cells in vitro by generating ROS, decreasing lysosomal integrity, and activating caspase and p38 mitogen-activated protein kinase (Wojcicki et al. 2020). Artesunate also causes inhibition of growth and stemness of transplanted tumors by suppressing MEK/ERK and PI3K/Akt pathway (Chen et al. 2020).

11.8.4 Chronic Myeloid Leukemia (CML)

Chronic myeloid leukemia (CML) is a clonal proliferative disease of myeloid origin, arises from the Philadelphia chromosome in single, pluripotent, hematopoietic stem cell. The cytogenetic lesion is associated with the reciprocal translocation t(9;22)(q34:11). Later, ABL (Abelson) proto-oncogene was found as being involved in this translocation (Frazer et al. 2007). An ideal drug target for CML treatment is BCR-ABL oncoprotein (Druker et al. 2001). The gold-standard treatment of CML is imatinib mesylate, a synthetic ATP inhibitor and approved by FDA in 2001 as first-line treatment (Frazer et al. 2007; Guilhot 2004). Second-generation ABL kinase inhibitors such as imatinib, dasatinib, and nilotinib have been in use for CML treatment in which imatinib serve as the first line of treatment. Other novel therapies like hommoharringtonine (HHT), repurposed arsenic trioxide, and proteasome inhibitors were also found to be effective against these cancers (Frazer et al. 2007).

11.8.4.1 Celecoxib

Celecoxib is approved by FDA in 1998 and marketed under the brand name Celebrex by Pfizer. It is clinically indicated for the relief of generalized pain, rheumatoid arthritis, osteoarthritis, and to reduce polyps in familial adenomatous

polyposis (Gupta et al. 2013). It is associated with antitumor activity because of its selective noncompetitive inhibition of pro-inflammatory cytokine, cyclooxygenase-2 (COX-2). COX-2 is overexpressed in hematological malignancies such as chronic lymphocytic leukemia, chronic myeloid leukemia, Hodgkin's lymphoma, non-Hodgkin's lymphoma, and multiple myeloma which promote cancer cell proliferation, survival, angiogenesis, and metastasis (Kale et al. 2021; Bernard et al. 2008). Celecoxib has shown inhibition of autophagy and enhances cytotoxicity of imatinib in imatinib-resistant CML cells (Lu et al. 2016). Thus, it holds a promising drug to be repurposed for hematological malignancies. Caution should be taken before prescribing NSAIDs in cancer therapy, as celecoxib leads to gastrointestinal, renal, and cardiotoxicity as adverse side effects (Drug Bank n.d.).

11.8.4.2 Pioglitazone

Pioglitazone is a PPAR-γ agonist (peroxisome proliferator-activated receptor gamma) drug approved by the FDA to treat diabetes. In CML, fused oncoprotein BCR-ABL1 positive progenitor cells are seen in mostly all patients and causing relapse after treatment with paradigm TKI (tyrosine kinase inhibitor) therapy such as imatinib, dasatinib, or nilotinib (Rousselot et al. 2017). Combinational regimen of pioglitazone with TKI therapy was found to be effective in controlling the residual CML stem cell pool (https://clinicaltrials.gov/ct2/show/NCT02730195). According to the proof of concept study, this drug regimen was found to be well tolerated and continued as long as the BCR-ABL1 signal remains detectable resulting in a favorable 56% rate of molecular response 4.5 (MR4.5) till 12 months (Rousselot et al. 2017).

11.9 Lymphoma

Lymphoma is characterized by the clonal proliferation of lymphocytes (B-cells and T-cells) in the lymph system and comprises about 5% of malignancies (Jamil and Mukkamalla 2021). It is broadly classified as Hodgkin's lymphoma (HL) and non-Hodgkin's lymphoma (NHL). In HL, there is a characteristic presence of abnormal, bi- or multinucleated Reed Sternberg cells (Shanbhag and Ambinder 2018) and accounts for about 10% of all lymphomas. The remaining 90% of lymphoma cases is from non-Hodgkin's, and it includes indolent follicular lymphoma, aggressive diffuse large B-cell, and Burkitt's lymphomas (Shankland et al. 2012).

11.9.1 Hodgkin's Lymphoma (HL)

In HL, the common regimens being used for first-line treatment are ABVD (Adriamycin (doxorubicin), bleomycin, vinblastine, dacarbazine), AAVD (similar to ABVD, but brentuximab vedotin (Adcetris) replaces bleomycin), BEACOPP (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine (Oncovin), procarbazine, prednisone) and gemcitabine along with other drugs. Drugs given as second-line treatment for HL recurrence are ICE (ifosfamide (Ifex), carboplatin etoposide), ESHAP (etoposide, methylprednisolone (Solu-Medrol), high-dose cytarabine, cisplatin) or DHAP (dexamethasone, high-dose cytarabine, cisplatin), GVD (gemcitabine, vinorelbine, doxorubicin), Gem-Ox (gemcitabine and oxaliplatin) or GDPF (gemcitabine, dexamethasone, cisplatin), brentuximab vedotin, and bendamustine (Cancer.Net n.d.). The emerging treatment of third-line Hodgkin lymphoma (HL) involves the use of repurposed drugs, targeted specific drugs, and chimeric antigen receptor (CAR) T-cell therapy (Fuerst 2018).

11.9.1.1 Verapamil (VRP)

VRP is an L-type calcium channel blocker, used as the medication for high blood pressure, angina, and supraventricular tachycardia by blocking voltage-dependent calcium (Cav) channels (Armando et al. 2020). Many evidences have shown that CaV channels are expressed in various cancers at the gene and protein levels (Yu et al. 2014). Phase I clinical trial studies are recruiting on the combined regimen of brentuximab vedotin with cyclosporine and verapamil hydrochloride in treating patients with relapsed/refractory Hodgkin lymphoma. Brentuximab vedotin is a targeted monoclonal antibody linked with vedotin toxin that targets CD30 positive cancer cells. The treatment is aided with immunosuppressive cyclosporine which improves bone marrow function. In this combination, verapamil hydrochloride is added as a synergistic compound by overcoming drug resistance (https://clinicaltrials.gov/ct2/show/NCT03013933).

11.9.2 Non-Hodgkin's Lymphoma (NHL)

NHL (non-Hodgkin Lymphoma) is a heterogeneous group of lymphoproliferative malignancies arising at both nodal and extranodal locations (25% of NHL cases) (Al-Naeeb et al. 2018). Extranodal sites for NHL are found as Waldeyer's ring (i.e., the pharynx, tonsils, and base of the tongue), thyroid glands, orbit, paranasal sinuses, and salivary glands (Singh et al. 2020). The most common NHL subtypes are diffuse large B-cell lymphoma (about 30%), follicular lymphoma (about 20%), and other subtypes each having a frequency of less than 10% (Singh et al. 2020). In terms of aggressiveness, indolent B-cell lymphomas constitute about 35–40% of the NHL in

which survival is generally measured in years. The most common aggressive subtypes comprise about 5% of the NHL and patient has few weeks survival if left untreated (Crisci et al. 2019). For instance, relapsed or refractory mantle cell lymphoma (MCL) and diffuse large B-cell lymphoma (DLBCL) are aggressive subtypes. The most common chemotherapy combination for the first-line treatment of aggressive NHL is called CHOP which comprises of cyclophosphamide, doxorubicin (hydroxydaunorubicin), prednisone, and vincristine (Oncovin). For B-cell lymphoma, anti-CD20 monoclonal antibody, such as rituximab (Rituxan) or obinutuzumab (Gazyva) are prescribed along with CHOP medications (Cancer.Net n.d.).

11.9.3 Aggressive Diffuse Large B-Cell Lymphoma

Diffuse large B-cell lymphoma (DLBCL) is the most commonly diagnosed non-Hodgkin lymphoma. It achieves 40–60% complete response (CR) rate after frontline R-CHOP regimen (rituximab, cyclophosphamide, doxorubicin hydrochloride (hydroxydaunorubicin), vincristine sulfate (Oncovin), and prednisone) (Xiang et al. 2021). In DLBCL, gene rearrangement in MYC (Single hit), BCL2, BCL6, or both (double-hit) are seen in few cases and they are resistant to R-CHOP modality. For this, EPOCH-R regimen (etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, and rituximab) produced considerable remission in patients with MYC-rearranged aggressive B-cell lymphomas (Dunleavy et al. 2018).

11.9.3.1 Auranofin

The BTK (Bruton's tyrosine kinase) inhibitor, ibrutinib resistance generally develops in mantle cell lymphoma (MCL) and diffuse large B-cell lymphoma (DLBCL). It is due to that specific tumor suppressor gene defects including TP53 and CDKN2A are correlated with relapsed/refractory feature and leading to poor clinical outcomes (Eskelund et al. 2017). As auranofin is being repurposed for refractory hematological malignancies with proven safety profile, its efficacy was also demonstrated in DLBCL and MCL cell lines and found it targets thioredoxin reductase-1 (Txnrd1) effectively by causing DNA damage, reactive oxygen species (ROS) production, cell growth inhibition, and apoptosis in aggressive B-cell lymphomas, especially in *TP53*-mutated or *PTEN*-deleted lymphomas (Wang et al. 2019).

11.9.4 Multiple Myeloma (MM)

Multiple myeloma is a malignancy that occurs in the subset of B-cells known as plasma cells (Bird and Boyd 2019). The current treatment algorithms include the use of several combinations of alkylating agents (cyclophosphamide, melphalan), corticosteroids (dexamethasone, prednisone), immunomodulatory drugs (thalidomide, lenalidomide, pomalidomide), proteasome inhibitors (bortezomib, carfilzomib, ixazomib), and monoclonal antibodies (daratumumab and isatuximab) targeting CD38 (Rajkumar & Kumar, 2020). Generally, multiple myeloma patients are treated with corticosteroids like dexamethasone and prednisone combined with other drugs such as cyclophosphamide (Cytoxan), etoposide (VP-16), doxorubicin (Adriamycin), liposomal doxorubicin (Doxil), melphalan and bendamustine (Treanda). Bortezomib (Velcade) was the first protesome inhibitors approved by FDA for MM malignancies (American Cancer Society n.d.).

11.9.4.1 Thalidomide

Thalidomide was developed in the 1950s as a sedative and used for treating pregnancy-related nausea. However, it was withdrawn from the market due to its toxic effects of causing congenital defects. It was estimated that at least 10,000 infants were born with malformations in limbs and other body extremities in over 46 countries (McCabe et al. 2015). Later, researchers, such as pharmaceutical company Celgene, looked for its therapeutic effects. Thalidomide significantly reduces neo-vascularization and eventually found the drug's ability to inhibit TNF-alpha in patients with refractory multiple myeloma. Finally, FDA approved thalidomide in 2006 as a treatment for multiple myeloma in combination with dexamethasone.

11.9.4.2 Nelfinavir

It is an FDA-approved drug in the year 1997 as protease inhibitors and used against HIV-1 and HIV-22 (Allegra et al. 2020). The common side effects recorded are insulin resistance, hyperglycemia, and lipodystrophy (Shim and Liu 2014). The antimyeloma activity of nelfinavir was evident by the findings of protease inhibitors such as ritonavir, saquinavir, and nelfinavir, which caused induction of growth arrest and apoptosis in the human MM cell lines associated with downregulation of antiapoptotic protein Mcl-1 (Ikezoe et al. 2004). These research findings directed to clinical trials on the combinational drug use of metformin, nelfinavir, and bortezomib in treating patients with relapsed and/or refractory multiple myeloma (https://clinicaltrials.gov/ct2/show/NCT03829020). Besides, an active oral drug combination of nelfinavir–lenalidomide–dexamethasone is effective in lenalidomide-refractory MM (Hitz et al. 2019).

11.10 Status of Drug Repurposing in Hematological Malignancies

In total, 32 compounds are considered for possessing repurposing potential in the case of AML. Among them, 27 were successfully validated in the preclinical setting in the context of leukemia elimination and 13 are under investigation in clinical trials as per the data entered into the clinicaltrials.gov database. The clinical validation of these compounds has been disappointing (Valli et al. 2020). As a result, FDA or EMA approval has not given to any of the repurposed compounds so far and hence, they have not been implemented into AML treatment. However, some of the most promising combinations resulting in potential responses are VPA with 5-azacytidine and ATRA, plerixafor with decitabine or plus fludarabine, idarubicin, cytarabine, and G-CSF, pioglitazone with low-dose 5-azacytidine or with standard treatment, and pravastatin with standard treatment (Valli et al. 2020).

The toxicity of the repurposed drugs is also an aspect of these limitations in drug repurposing strategy. For instance, regimen of VPA and decitabine is associated with distinct VPA-dependent encephalopathy (Lübbert et al. 2016). Pravastatin together with idarubicin and cytarabine leads to multiorgan failure (Shadman et al. 2015). Further, when compared to other tumors, drug repurposing approaches in hematological malignancies have failed to speed up the development process. Hence, a major limitation in drug repurposing for hematological malignancies are due to long duration to generate relevant supporting data and suspension of clinical trials mid-way due to lack of efficacy and toxicity.

11.11 Intellectual Property and Regulatory Issues in Drug Repurposing

Drug repurposing is a complex process that involves multiple factors including technology, commercial models, patents, investments, and market demands. The main challenge in the drug repositioning is its weak intellectual property (IP) protection as per the IP and patent laws. As the drug about to be repurposed has already been patented as a new chemical entity, it discourages companies from investing and developing repurposed drugs. Thereby it prevents entering of repurposed drugs entering into market. Providing secondary patents gives a chance for researchers to find new indications for existing drugs. When the IP problem solved, many repositioning projects have been developed with low budget (Xue et al. 2018). Some regulatory issues are also commonly encountered in drug repositioning. As per regulatory guidelines of FDA or EMA, if the existing data for the compounds are not satisfactory and do not comply with the requirements, there is a requirement of carrying out preclinical and/or clinical research again. There is a wide availability of computational tools and databases and selecting the appropriate approach for repositioning is important and a challenge due to regulatory

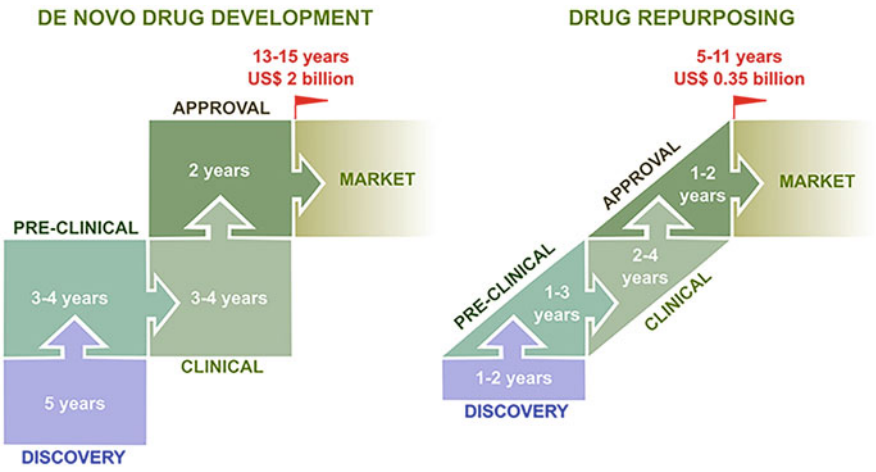


Fig. 11.1 From the bench to the bedside: comparison between de novo drug development and drug repurposing (Pizzorno et al. 2019)

issues (Rudrapal et al. 2020). Hence, it is of utmost importance to adhere strictly to the standard regulatory guidelines for repurposed drug discovery (Fig. 11.1).

11.12 Conclusion

In this modern era, drug repositioning is embarked as a new avenue in the development of new therapies based upon existing/approved medicines with rationale and innovational approach. It has received increasing attention, as it offers significant reduction in R&D costs, more probable chances of success, shorter development time, lower investment risk, less attrition rate, and thereby gained increasing market demands. These advantages are beneficial for academicians, discovery scientists, drug researchers, consumers, and pharmaceutical companies by enabling the application of novel, effective, alternate approaches of repositioning strategy in the drug discovery program for almost all human diseases (Sleire et al. 2017). Given the high cost of traditional drug discovery pathways, drug repurposing approach is highly appealing to biotechnology companies as well as academic groups. Mainly, in the era of precision medicine, this strategy has become very much useful to establish the unknown mechanism of action of drugs through exploration of novel disease/metabolic/signaling pathways, or off-targets and target-specific mechanisms/genetic expression profile for even genetic disorders (Rudrapal et al. 2020). To ensure high success rates of repositioned drugs, challenges are needed to be met, and more in-depth understanding must be executed with mixed approaches. Therefore, increasing the number of drug repositioning opportunities will be achieved by

extending the collaborative networks between academia, industry, and charitable organizations.

To conclude, in the context of a globalized world facing major uncertainties including population dynamics, climate change, and the multiple emergence/re-emergence of new diseases, the effectiveness of the classic de novo development of anticancer, antivirals, and other drugs remains a challenging part. Despite inherent limits, drug repurposing offers huge number of possibilities to rapidly and efficiently find new effective anticancer drugs against various human diseases.

Acknowledgments Department of Biotechnology (DBT), Ramalingaswami Re-entry Fellowship Program for supporting the research of Dr. Thirunavukkarasu Velusamy is gratefully acknowledged. RUSA2.0-BCTRC (Bharathiar Cancer and Theranostics Research Centre) student fellowship for supporting Ms. Pavithra Balakrishnan is highly acknowledged.

References

Adachi K, Honma Y, Miyake T, Kawakami K, Takahashi T, Suzumiya J (2016) Tamoxifen enhances the differentiation-inducing and growth-inhibitory effects of all-trans retinoic acid in acute promyelocytic leukemia cells. Int J Oncol 48(3):1095–1102

Allegra A, Innao V, Allegra AG, Pulvirenti N, Pugliese M, Musolino C (2020) Antitumorigenic action of nelfinavir: effects on multiple myeloma and hematologic malignancies. Oncol Rep 43(6):1729–1736

Al-Naeeb AB, Ajithkumar T, Behan S, Hodson DJ (2018) Non-Hodgkin lymphoma. BMJ 362: k3204

American Cancer Society (2022). https://www.cancer.org/cancer/leukemia.html

American Cancer Society. https://www.cancer.org/cancer/multiple-myeloma/treating/chemotherapy.html

Andresen V, Gjertsen BT (2017) Drug repurposing for the treatment of acute myeloid leukemia. Front Med 4:211

Argüello-García R, Leitsch D, Skinner-Adams T, Ortega-Pierres MG (2020) Drug resistance in giardia: mechanisms and alternative treatments for giardiasis. Adv Parasitol 107:201–282

Armando RG, Mengual Gómez DL, Gomez DE (2020) New drugs are not enough-drug repositioning in oncology: an update. Int J Oncol 56(3):651–684

Ashburn TT, Thor KB (2004) Drug repositioning: identifying and developing new uses for existing drugs. Nat Rev Drug Discov 3(8):673–683

Azemawah V, Movahed MR, Centuori P, Penaflor R, Riel PL, Situ S, Shadmehr M, Hashemzadeh M (2019) State of the art comprehensive review of individual statins, their differences, pharmacology, and clinical implications. Cardiovasc Drugs Ther 33(5):625–639

Bernard MP, Bancos S, Sime PJ, Phipps RP (2008) Targeting cyclooxygenase-2 in hematological malignancies: rationale and promise. Curr Pharm Des 14(21):2051–2060

Bird SA, Boyd K (2019) Multiple myeloma: an overview of management. Palliat Care Social Pract 13:1178224219868235

Bogush TA, Polezhaev BB, Mamichev IA, Bogush EA, Polotsky BE, Tjulandin SA, Ryabov AB (2018) Tamoxifen never ceases to amaze: new findings on non-estrogen receptor molecular targets and mediated effects. Cancer Invest 36(4):211–220

Bosch F, Dalla-Favera R (2019) Chronic lymphocytic leukaemia: from genetics to treatment. Nat Rev Clin Oncol 16(11):684–701

Cancer Treatment Centers of America (2021). https://www.cancercenter.com/cancer-types/leukemia/types

Cancer.Net. www.cancer.net/cancer-types/lymphoma-non-hodgkin/types-treatment
Chakraborty A, Trivedi V (2015) Streamlining the drug discovery process through repurposing of clinically approved drugs. Austin J Biotechnol Bioeng 2:3–5
Chen X, Yan CC, Zhang X, Zhang X, Dai F, Yin J, Zhang Y (2016) Drug–target interaction prediction: databases, web servers and computational models. Brief Bioinform 17(4):696–712
Chen S, Gan S, Han L, Li X, Xie X, Zou D, Sun H (2020) Artesunate induces apoptosis and inhibits the proliferation, stemness, and tumorigenesis of leukemia. Ann Transl Med 8(12):767
Clinicaltrails.gov. https://clinicaltrials.gov/ct2/show/NCT02730195
Clinicaltrials.gov. https://clinicaltrials.gov/ct2/about-studies/glossary
Clinicaltrials.gov. https://clinicaltrials.gov/ct2/show/NCT03829020
Clinicaltrials.gov. https://clinicaltrials.gov/ct2/show/NCT03013933
Crisci S, Di Francia R, Mele S, Vitale P, Ronga G, De Filippi R, Berretta M, Rossi P, Pinto A (2019) Overview of targeted drugs for mature B-cell non-Hodgkin lymphomas. Front Oncol 9:443
Croston GE (2017) The utility of target-based discovery. Expert Opin Drug Discovery 12(5): 427–429
De Kouchkovsky I, Abdul-Hay M (2016) Acute myeloid leukemia: a comprehensive review and 2016 update. Blood Cancer J 6(7):e441
de Oliveira EAM, Lang KL (2018) Drug repositioning: concept, classification, methodology, and importance in rare/orphans and neglected diseases. J Appl Pharma Sci 8(8):157–165
Deore AB, Dhumane JR, Wagh R, Sonawane R (2019) The stages of drug discovery and development process. Asian J Pharma Res Dev 7(6):62–67
DiMasi JA, Grabowski HG, Hansen RW (2016) Innovation in the pharmaceutical industry: new estimates of R&D costs. J Health Econ 47:20–33
Drug Bank (n.d.). https://go.drugbank.com
Druker BJ, Sawyers CL, Capdeville R, Ford JM, Baccarani M, Goldman JM (2001) Chronic myelogenous leukemia. ASH Educ Program Book 2001(1):87–112
Dunleavy K, Fanale MA, Abramson JS, Noy A, Caimi PF, Pittaluga S, Parekh S et al (2018) Dose-adjusted EPOCH-R (etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, and rituximab) in untreated aggressive diffuse large B-cell lymphoma with MYC rearrangement: a prospective, multicentre, single-arm phase 2 study. Lancet Haematol 5(12):e609–e617
Eskelund CW, Dahl C, Hansen JW, Westman M, Kolstad A, Pedersen LB, Montano-Almendras CP, Husby S, Freiburghaus C, Ek S, Pedersen A (2017) TP53 mutations identify younger mantle cell lymphoma patients who do not benefit from intensive chemoimmunotherapy. Blood 130(17):1903–1910
FDA (2018) The drug development process. www.fda.gov/patients/learn-about-drug-and-device-approvals/drug-development-process
FDA (2021) Novel drug approvals for 2021. https://www.fda.gov/drugs/new-drugs-fda-cders-new-molecular-entities-and-new-therapeutic-biological-products/novel-drug-approvals-2021
Ferreira LG, Andricopulo AD (2016) Drug repositioning approaches to parasitic diseases: a medicinal chemistry perspective. Drug Discov Today 21(10):1699–1710
Fiskus W, Saba N, Shen M, Ghias M, Liu J, Gupta SD, Chauhan L, Rao R, Gunewardena S, Schorno K, Austin CP (2014) Auranofin induces lethal oxidative and endoplasmic reticulum stress and exerts potent preclinical activity against chronic lymphocytic leukemia. Cancer Res 74(9):2520–2532
Frazer R, Irvine AE, McMullin MF (2007) Chronic myeloid leukaemia in the 21st century. Ulster Med J 76(1):8
Fu X, Liu W, Huang Q, Wang Y, Li H, Xiong Y (2017) Targeting mitochondrial respiration selectively sensitizes pediatric acute lymphoblastic leukemia cell lines and patient samples to standard chemotherapy. Am J Cancer Res 7(12):2395
Fuerst ML (2018) Options emerge for relapsed/refractory Hodgkin lymphoma. Oncol Times 40:7
Gimenez N, Tripathi R, Giró A, Rosich L, López-Guerra M, Lopez-Oreja I, Playa-Albinyana H, Arenas F, Mas JM, Perez-Galan P, Delgado J (2020) Network-based drug discovery identifies

statins as a repurposing agent to improve activity of current therapies in chronic lymphocytic leukemia
Greer ND (2006) Tigecycline (Tygacil): the first in the glycylcycline class of antibiotics. In: Baylor University Medical Center proceedings, vol. 19, no. 2. Taylor & Francis, pp 155–161
Guilhot F (2004) Indications for imatinib mesylate therapy and clinical management. Oncologist 9(3):271–281
Gupta SC, Sung B, Prasad S, Webb LJ, Aggarwal BB (2013) Cancer drug discovery by repurposing: teaching new tricks to old dogs. Trends Pharmacol Sci 34(9):508–517
Hao E, Mukhopadhyay P, Cao Z, Erdélyi K, Holovac E, Liaudet L, Lee WS, Haskó G, Mechoulam R, Pacher P (2015) Cannabidiol protects against doxorubicin-induced cardiomyopathy by modulating mitochondrial function and biogenesis. Mol Med 21(1):38–45
Hay M, Thomas DW, Craighead JL, Economides C, Rosenthal J (2014) Clinical development success rates for investigational drugs. Nat Biotechnol 32(1):40–51
Hernández-Lemus E, Martínez-García M (2021) Pathway-based drug-repurposing schemes in cancer: the role of translational bioinformatics. Front Oncol 10:2996
Hitz F, Kraus M, Pabst T, Hess D, Besse L, Silzle T, Novak U, Seipel K, Rondeau S, Stüdeli S, Vilei SB (2019) Nelfinavir and lenalidomide/dexamethasone in patients with lenalidomide-refractory multiple myeloma. A phase I/II trial (SAKK 39/10). Blood Cancer J 9(9):1–8
Honek J (2017) Preclinical research in drug development. Med Writing 26:5–8
Huang X, Guo B (2006) Adenomatous polyposis coli determines sensitivity to histone deacetylase inhibitor–induced apoptosis in colon cancer cells. Cancer Res 66(18):9245–9251
Hughes JP, Rees S, Kalindjian SB, Philpott KL (2011) Principles of early drug discovery. Br J Pharmacol 162(6):1239–1249
Hunger SP, Mullighan CG (2015) Acute lymphoblastic leukemia in children. N Engl J Med 373(16):1541–1552
Ikezoe T, Saito T, Bandobashi K, Yang Y, Koeffler HP, Taguchi H (2004) HIV-1 protease inhibitor induces growth arrest and apoptosis of human multiple myeloma cells via inactivation of signal transducer and activator of transcription 3 and extracellular signal-regulated kinase 1/2. Mol Cancer Ther 3(4):473–479
Jamil A, Mukkamalla SKR (2021) Lymphoma. StatPearls [Internet]
Jin G, Wong ST (2014) Toward better drug repositioning: prioritizing and integrating existing methods into efficient pipelines. Drug Discov Today 19(5):637–644
Joca S, Silote GP, Sartim A, Sales A, Guimarães F, Wegener G (2021) Putative effects of cannabidiol in depression and synaptic plasticity. In: The neuroscience of depression, pp 459–467
Jorgensen WL (2009) Efficient drug lead discovery and optimization. Acc Chem Res 42(6): 724–733
Jurlander J (2011) Hematological malignancies, leukemias and lymphomas. In: Encyclopedia of cancer, pp 1640–1644
Kale VP, Habib H, Chitren R, Patel M, Pramanik KC, Jonnalagadda SC, . . ., Pandey MK (2021) Old drugs, new uses: drug repurposing in hematological malignancies. In: Seminars in cancer biology, vol. 68. Academic, pp 242–248
Karuppagounder SS, Brahmachari S, Lee Y, Dawson VL, Dawson TM, Ko HS (2014) The c-Abl inhibitor, nilotinib, protects dopaminergic neurons in a preclinical animal model of Parkinson's disease. Sci Rep 4(1):1–8
Kubota K, Funabashi M, Ogura Y (2019) Target deconvolution from phenotype-based drug discovery by using chemical proteomics approaches. Biochim Biophys Acta 1867(1):22–27
Li YY, Jones SJ (2012) Drug repositioning for personalized medicine. Genome Med 4(3):1–14
Livermore DM (2005) Tigecycline: what is it, and where should it be used? J Antimicrob Chemother 56(4):611–614
Lotfi Shahreza M, Ghadiri N, Mousavi SR, Varshosaz J, Green JR (2018) A review of network-based approaches to drug repositioning. Brief Bioinform 19(5):878–892

Lu Y, Liu LL, Liu SS, Fang ZG, Zou Y, Deng XB, Long ZJ, Liu Q, Lin DJ (2016) Celecoxib suppresses autophagy and enhances cytotoxicity of imatinib in imatinib-resistant chronic myeloid leukemia cells. J Transl Med 14(1):1–13

Lübbert M, Grishina O, Schmoor C, Schlenk RF, Jost E, Krauter J, Heuser M, Thol F, Schittenhelm MM, Salih HR, Kündgen A (2016) Results of the randomized phase II study decider (AMLSG 14-09) comparing decitabine (DAC) with or without valproic acid (VPA) and with or without all-trans retinoic acid (ATRA) add-on in newly diagnosed elderly non-fit AML patients. Blood 128(22):589

Lyengar V, Shimanovsky A (2021) Continuing education activity

Lymphoma Action (2022). https://lymphoma-action.org.uk/types-lymphoma

McCabe B, Liberante F, Mills KI (2015) Repurposing medicinal compounds for blood cancer treatment. Ann Hematol 94(8):1267–1276

Memorial Sloan Kettering Cancer Center (2022). https://www.mskcc.org/cancer-care/types/lymphoma/types

Messenger AG, Rundegren J (2004) Minoxidil: mechanisms of action on hair growth. Br J Dermatol 150(2):186–194

Moffat JG, Vincent F, Lee JA, Eder J, Prunotto M (2017) Opportunities and challenges in phenotypic drug discovery: an industry perspective. Nat Rev Drug Discov 16(8):531–543

Mullard A (2018) 2017 FDA drug approvals. Nat Rev Drug Discov 17(2):81–86

National Institutes of Health (US) (2007) Biological sciences curriculum study. Bethesda

Ojezele MO, Mordi J, Adedapo EA (2020) Drug repurposing: cost effectiveness and impact on emerging and neglected diseases. J Cameroon Acad Sci 16(1):3–17

Olivas-Aguirre M, Torres-López L, Pottosin I, Dobrovinskaya O (2021) Overcoming glucocorticoid resistance in acute lymphoblastic leukemia: repurposed drugs can improve the protocol. Front Oncol 11:647

Orlowski RZ, Stinchcombe TE, Mitchell BS, Shea TC, Baldwin AS, Stahl S, Adams J, Esseltine DL, Elliott PJ, Pien CS, Guerciolini R (2002) Phase I trial of the proteasome inhibitor PS-341 in patients with refractory hematologic malignancies. J Clin Oncol 20(22):4420–4427

Parisi D, Adasme MF, Sveshnikova A, Bolz SN, Moreau Y, Schroeder M (2020) Drug repositioning or target repositioning: a structural perspective of drug-target-indication relationship for available repurposed drugs. Comput Struct Biotechnol J 18:1043–1055

Parvathaneni V, Kulkarni NS, Muth A, Gupta V (2019) Drug repurposing: a promising tool to accelerate the drug discovery process. Drug Discov Today 24(10):2076–2085

Patel A (2020) Drug repurposing in oncotherapeutics. In: Drug repurposing: hypothesis, molecular aspects and therapeutic applications, p 121

Paul SM, Mytelka DS, Dunwiddie CT, Persinger CC, Munos BH, Lindborg SR, Schacht AL (2010) How to improve R&D productivity: the pharmaceutical industry's grand challenge. Nat Rev Drug Discov 9(3):203–214

Perez DR, Sklar LA, Chigaev A, Matlawska-Wasowska K (2021) Drug repurposing for targeting cyclic nucleotide transporters in acute leukemias—a missed opportunity. In: Seminars in cancer biology, vol. 68. Academic, pp 199–208

Pizzorno A, Padey B, Terrier O, Rosa-Calatrava M (2019) Drug repurposing approaches for the treatment of influenza viral infection: reviving old drugs to fight against a long-lived enemy. Front Immunol 10:531

Podhorecka M, Halicka D, Klimek P, Kowal M, Chocholska S, Dmoszynska A (2010) Simvastatin and purine analogs have a synergic effect on apoptosis of chronic lymphocytic leukemia cells. Ann Hematol 89(11):1115–1124

Rajkumar SV, Kumar S (2020) Multiple myeloma current treatment algorithms. Blood Cancer J 10(9):1–10

Rao VS, Srinivas K (2011) Modern drug discovery process: an in silico approach. J Bioinform Seq Anal 3(5):89–94

Rousselot P, Prost S, Guilhot J, Roy L, Etienne G, Legros L, Charbonnier A, Coiteux V, Cony-Makhoul P, Huguet F, Cayssials E (2017) Pioglitazone together with imatinib in chronic myeloid leukemia: a proof of concept study. Cancer 123(10):1791–1799

Rudrapal M, Khairnar JS, Jadhav GA (2020) Drug repurposing (DR): an emerging approach in drug discovery. Drug repurposing hypothesis. Mol Aspects Ther Appl

Salazar DE, Gormley G (2017) Modern drug discovery and development. In: Clinical and translational science. Academic, pp 719–743

Saultz JN, Garzon R (2016) Acute myeloid leukemia: a concise review. J Clin Med 5(3):33

Scherman D, Fetro C (2020) Drug repositioning for rare diseases: knowledge-based success stories. Therapies 75(2):161–167

Shadman M, Mawad R, Dean C, Chen TL, Shannon-Dorcy K, Sandhu V, Hendrie PC, Scott BL, Walter RB, Becker PS, Pagel JM (2015) Idarubicin, cytarabine, and pravastatin as induction therapy for untreated acute myeloid leukemia and high-risk myelodysplastic syndrome. Am J Hematol 90(6):483–486

Shanbhag S, Ambinder RF (2018) Hodgkin lymphoma: a review and update on recent progress. CA Cancer J Clin 68(2):116–132

Shankland KR, Armitage JO, Hancock BW (2012) Non-Hodgkin lymphoma. Lancet 380(9844): 848–857

Shim JS, Liu JO (2014) Recent advances in drug repositioning for the discovery of new anticancer drugs. Int J Biol Sci 10(7):654

Shukla R, Henkel ND, Alganem K, Hamoud AR, Reigle J, Alnafisah RS, Eby HM, Imami AS, Creeden JF, Miruzzi SA, Meller J (2021) Signature-based approaches for informed drug repurposing: targeting CNS disorders. Neuropsychopharmacology 46(1):116–130

Singh R, Shaik S, Negi BS, Rajguru JP, Patil PB, Parihar AS, Sharma U (2020) Non-Hodgkin's lymphoma: a review. J Fam Med Prim Care 9(4):1834

Sleire L, Førde HE, Netland IA, Leiss L, Skeie BS, Enger PØ (2017) Drug repurposing in cancer. Pharmacol Res 124:74–91

Sporn MB, Lippman SM (2003) Agents for chemoprevention and their mechanism of action. Holland-Frei cancer medicine, 6th edn. BC Decker, Hamilton

Steinmetz KL, Spack EG (2009) The basics of preclinical drug development for neurodegenerative disease indications. BMC Neurol 9(1):1–13

Strong T, Rauvolfova J, Jackson E, Pham LV, Bryant J (2018) Synergistic effect of Cannabidiol with conventional chemotherapy treatment. Blood 132:5382

Sukhai MA, Spagnuolo PA, Weir S, Kasper J, Patton L, Schimmer AD (2011) New sources of drugs for hematologic malignancies. Blood 117(25):6747–6755

Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F (2021) Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71(3):209–249

Swinney DC (2013) Phenotypic vs. target-based drug discovery for first-in-class medicines. Clin Pharmacol Therap 93(4):299–301

Swinney DC, Anthony J (2011) How were new medicines discovered? Nat Rev Drug Discov 10(7): 507–519

Terstappen GC, Schlüpen C, Raggiaschi R, Gaviraghi G (2007) Target deconvolution strategies in drug discovery. Nat Rev Drug Discov 6(11):891–903

Turanli B, Grøtli M, Boren J, Nielsen J, Uhlen M, Arga KY, Mardinoglu A (2018) Drug repositioning for effective prostate cancer treatment. Front Physiol 9:500

Turanli B, Altay O, Borén J, Turkez H, Nielsen J, Uhlen M, Arga KY, Mardinoglu A (2021) Systems biology based drug repositioning for development of cancer therapy. In: Seminars in cancer biology, vol. 68. Academic, pp 47–58

Valli D, Gruszka AM, Alcalay M (2020) Has drug repurposing fulfilled its promise in acute myeloid leukaemia? J Clin Med 9(6):1892

Wang J, Wang J, Lopez E, Guo H, Zhang H, Liu Y, Chen Z, Huang S, Zhou S, Leeming A, Zhang RJ (2019) Repurposing auranofin to treat TP53-mutated or PTEN-deleted refractory B-cell lymphoma. Blood Cancer J 9(12):1–6

Wexler HM (2015) Anaerobic infections: a clinical overview. In: Molecular medical microbiology, pp 875–897

Wojcicki AV, Kadapakkam M, Frymoyer A, Lacayo N, Chae HD, Sakamoto KM (2020) Repurposing drugs for acute myeloid leukemia: a worthy cause or a futile pursuit? Cancers 12(2):441

World Health Organization (WHO) (2021)

Xiang C, Ni H, Wang Z, Ji B, Wang B, Shi X, Wu W, Liu N, Gu Y, Ma D, Liu H (2021) Agent repurposing for the treatment of advanced stage diffuse large B-cell lymphoma based on gene expression and network perturbation analysis. Front Genet 12:1972

Xue H, Li J, Xie H, Wang Y (2018) Review of drug repositioning approaches and resources. Int J Biol Sci 14(10):1232

Yao J, Deng K, Huang J, Zeng R, Zuo J (2020) Progress in the understanding of the mechanism of tamoxifen resistance in breast cancer. Front Pharmacol 11:592912

Yu W, Wang P, Ma H, Zhang G, Yulin Z, Lu B, Wang H, Dong M (2014) Suppression of T-type Ca2+ channels inhibited human laryngeal squamous cell carcinoma cell proliferation running title: roles of T-type Ca2+ channels in LSCC cell proliferation. Clin Lab 60(4):621–628

Zhang Z, Zhou L, Xie N, Nice EC, Zhang T, Cui Y, Huang C (2020) Overcoming cancer therapeutic bottleneck by drug repurposing. Signal Transduct Target Ther 5(1):1–25

Chapter 12
Drug Repurposing for, ENT and Head and Neck, Infectious and Oncologic Diseases: Current Practices and Future Possibilities

Sourabha Kumar Patro, Naresh Kumar Panda, and Maryada Sharma

Abstract The specialty of otolaryngology and head and neck surgery involves various subspecialties, encompassing clinical conditions ranging from medical to surgical issues in infections, noninfectious benign conditions and various benign and malignant tumors. Drug repurposing has proven to be significant in multiple fields and is still investigational in many promising possible solutions to different clinical challenges in this specialty. We discuss some classes of drugs that have been successfully repurposed for ENT pathologies. We also discuss the novel research goals that are being pursued in our department in the context of drug repurposing for airway infectious diseases including COVID-10 and mucormycosis.

There has been a silent and underappreciated rise in drug-resistant invasive fungal infections (IFIs). Emerging Mucorales are difficult to diagnose and tolerant to many of the frontline antifungal therapies. There is an urgent need to combat these emerging pathogens and investigate the molecular mechanisms underlying their potentiated virulence traits to identify potential therapeutic targets susceptible to anti-fungal compounds. The drug development process for IFIs remains largely expensive, and is inherently risky. These challenges declare an urgent need for discovery of new antifungal drugs and encourage drug repurposing as alternative approach to fungal control. The understanding of molecular underpinnings behind fungi and human host continue to grow, however, further research endeavors are underway to fully explore the fungal pathogenesis, (including the role of iron) to gather new insights to achieve improved therapeutics. Above all, creative screening tools and out-of-the-box ideas aimed at increasing the possibility of identifying potential first-in-class antifungals are highly encouraged. The recently emerging fungal co-infections in the COVID-19 disease patients has revived the interest in the pathophysiology and clinical management of the IFIs, and identification of potential druggable nodes in olfactory niche to inhibit the spread of COVID-19

S. K. Patro (✉) · N. K. Panda · M. Sharma (✉)
Department of Otolaryngology and Head and Neck Surgery, Postgraduate Institute of Medical Education and Research, Chandigarh, India
e-mail: sharma.maryada@pgimer.edu.in

R. C. Sobti et al. (eds.), *Drug Repurposing for Emerging Infectious Diseases and Cancer*, https://doi.org/10.1007/978-981-19-5399-6_12

and associated co-infections by leveraging in vitro-disease models of host-pathogen interaction. We employed our recently established COVID-19 disease model to decipher potential anti-metabolic molecules that can be repurposed as novel bilateral drugs having anti-fungal and host-directed features with extended applicability in diabetes, COVID-19, and mucormycosis with and without COVID-19.

Keywords COVID-19 · CAM · Mucormycosis · Intranasal spray · Drug repurposing

12.1 Section A: Repurposing Novel Antimetabolic Imidazole Drug for Infectious Airways Diseases with Implications in Development of Intranasal Spray Prototype

12.1.1 Introduction

Fungi are associated with nearly 1.5 million deaths yearly resulting in enormous economic load, despite that the fungal infections are underestimated and underappreciated in their role as pathogenic microorganisms compared to bacteria and viruses. The lack of development of new antifungals with enhanced activity has resulted in emergence of life-threatening multidrug-resistant fungus community including the *Candida auris*, *Aspergillus, Cryptococcus spp.* (Peyclit et al. 2021), and notably in vitro antifungal drug resistance profiles are alarming for the clinically relevant members of Mucorales, specifically for the newer triazole drugs (Borman et al. 2021). The expansion and advancements in treatment regimens involving immunomodulation-based therapies for improvement in patient outcomes in oncology, hematology, transplantation, and intensive care medicine, along with burgeoning diabetic population have resulted in increasing numbers of immunocompromised individuals with heightened risk of developing invasive fungal infections (IFIs), as immunosuppressive conditions provide optimal ground for emergence of opportunistic fungal infections concomitant to development of antifungal resistance. The selective drug-pressure and fungal-host factors have counteracted the limited capacity of antifungal armamentarium resulting in the emergence of new epidemiological and pathological landscape of the IFIs. The recently emerging fungal co-infections in the COVID-19 disease patients have revived the interest in the pathophysiology and clinical management of the IFIs (Treviño-Rangel et al. 2021). Further, the antifungal armamentarium is limited with narrow spectrum, drug tolerance and resistance, and associated toxicities (Nivoix et al. 2020). Recent reports have linked the increased rate of co-infections during COVID-19 pandemic, in particular the fungal infections secondary to COVID-19 disease are on rise, and COVID-19 patients with ARDS were reported to had developed IFIs including pulmonary aspergillosis and candidiasis that complicated the clinical course of the disease. Recently, there has also been an unprecedented

surge in case reports of life-threatening IFI mucormycosis in Indian patients infected with COVID-19 (Silva et al. 2021; García-Vidal et al. 2020; Arastehfar et al. 2020a, b, c; White et al. 2021; Al-Hatmi et al. 2020; Antinori et al. 2020; Chowdhary et al. 2020; Mastrangelo et al. 2020; Heard et al. 2020; Moser et al. 2021; Garg et al. 2021; Singh et al. 2022; Sharma et al. 2022). Our health care center had recently reported a statistically significant increase in the number of mucormycosis infections during COVID-19 second wave (Muraleedharan et al. 2022). Despite the fact that worldwide most IFIs are associated with unacceptably high mortality rate that may go over 50%, the public health organizations including World Health Organization lack a dedicated fungal surveillance program (Brown et al. 2012; Denning et al. 2017). As the emerging variants/mutations in SARS-CoV-2 spike protein are on the rise, it might make vaccine strategies a challenge worldwide, triggering need for booster shots. Therefore, the identification of host-directed targets and subsequent development of intranasal sprays targeting potential druggable targets via the nasal route, is a promising approach for future therapeutics. The development of prophylactic intranasal drugs will also help in the early intervention in even asymptomatic COVID-positive patients who are high-risk spreaders and thus help preventing infection and IFIs co-infection spread and mortality in immunocompromised patients. We discuss the importance of our preliminary findings resulting in identification of potential antimetabolic olfactory therapeutic nodes that can be targeted to prevent the spread of COVID-19 and associated fungal co-infections like mucormycosis by employing novel intranasal spray formulations.

12.1.2 Novel Olfactory Druggable Targets for Clinical Management of COVID-19 and COVID-19-Associated Mucormycosis (CAM)

Olfactory niche is a preferred route of entry and spread for major airway infectious pathologies; however, emerging studies are reflecting that nasal tissue is not just an inert portal of entry as neurosensory olfactory deficit marked by anosmia (loss of smell) or olfactory dysfunction has emerged as a hallmark and consistent neurological symptom of COVID-19/Long-COVID-19. Importantly, the mechanistic detailing of anosmia remains largely unexplored (primarily in highly transmissible diseases) due to infection control concerns in disease-active patients, as the nasal area is the main route of disease transmission and spread, therefore, making it less available for doing clinical research. We recently attempted to overcome this limitation by establishing a *novel* virus-free cellular model (COVID-19 disease model) that displays COVID-19-like disease signatures including maturity-onset diabetes, complement–coagulation, and olfactory transduction pathways in disease-competent monocytic immune cells (https://doi.org/10.3390/diabetology3010013, https://doi.org/10.3390/diabetology3020017). The pathway

networks that emerged from extensive analysis of technologically advanced unbiased high-throughput transcriptomic data generated from the above model have converged on neurosensory and inflammatory nodes that recapitulate inflammatory diseases like diabetes, COVID-19, CAM, alcoholic liver disease, autoimmune inflammatory diseases like rheumatoid arthritis, neurosensory/neurodegenerative and neuropsychiatric diseases like Alzheimer's, and Parkinson's, with early signs of anosmia.

Based on our robust findings emanating from in vitro COVID-19 disease modeling, we have been able to establish a novel cell-autonomous role of COVID-19-associated serine proteases in olfactory dysfunction that could be important in predicting neurological sequelae in CNS and suggest a new paradigm that could involve olfactory transduction-mediated neuroinflammation in the respiratory tract and peripheral tissues with resident macrophages expressing neuropeptide receptors. However, assessment of the associated growth-factor responsiveness in addition to olfactory transduction pathways and correlating these with the inflammatory targets in real-world patient samples would help translate the key findings of our in vitro study for diagnostics and therapeutics intervention in COVID-19 and CAM. Merging studies worldwide have a consensus on the role of uncontrolled diabetes as a major comorbid risk factor for COVID-19 and CAM, intriguingly both having nasal route of entry. Therefore, we propose to now extend unbiased multiomic approach of target profiling in COVID-19, mucor, and CAM patient samples to screen the potential diabetes-associated cellular stressors in the blood and nasal route that could help better understand the pathophysiology of COVID-19 and CAM and facilitate development of novel superior intranasal therapeutic interventions to control diabetes, COVID-19, and CAM. With the mutations in SARS-CoV-2 spike protein on the rise, it might make vaccine strategies a challenge worldwide, triggering need for booster shots. It is challenges like the above what makes the findings of our COVID-19 model (Sharma et al. 2022) both attractive and of therapeutic significance, which might hold the key to developing and treating people with a potential L-carnosine based drug via the nasal route that is not just safe, self-administrable, and efficacious, but also affordable. This is significant particularly, at an early asymptomatic stage of COVID-19 positivity to reign the pandemic and prevent avoidable deaths.

We have recently proposed L-carnosine for repurposing in mucormycosis, and CAM following some preliminary findings that suggested its novel anti-Mucorales actions under in vitro growth inhibition assays. Our study comes during the grim milestone that United States has reached a staggering 979,870 deaths nationwide, which put the COVID-19 deaths at 6.1 million worldwide. Though the vaccine development was undertaken and completed at an unprecedented speed, on-going challenges with the pandemic, such as the rapid spread of disease via asymptomatic patients, slow progress of vaccination across the world owing to the logistics, and nonavailability of raw materials impacting the production have been largely responsible for the astronomical rise in death tolls, a classic case in point being the chaos

that unfolded last summer in India with the delta breakout. The emergence and rapid spread of new and potent variants that are more contagious and/or deadly, indicating the possibility of loss of vaccine efficacy against the disease, is a major concern, before herd immunity could be achieved. The presymptomatic or acute phase of COVID-19 is marked by high viral loads/replication in the nasal/olfactory epithelium, which makes it a potential target to inhibit the intensification of infection by limiting the spread of the infection and associated fungal co-infections using intranasal sprays. The prophylactic/therapeutic targeting of the nasal epithelium by employing intranasal sprays could be a potential approach to prevent diabetes-associated COVID-19 and CAM.

12.1.3 Scope of Intranasal Sprays for Treating Infectious Airway Diseases

Based on our cardinal findings, we are now encouraged to carry a preclinical observational study aimed at exploring diabetes-associated diagnostic and therapeutic targets in diabetic COVID-19, mucormycosis, COVID-19-associated mucormycosis (CAM), post-COVID Mucor/CAM patients, and healthy controls. We are trying to validate the findings obtained from our novel in vitro COVID-19 disease model in plasma, serum, blood cells, and nasopharyngeal swab samples from delta and omicron variants of SARS-CoV-2 and mucormycosis-infected COVID-19 patients to verify our novel druggable screens in blood and nasal niche. The findings emerging from our on-going study may facilitate to establish (1) a novel blood-based diagnostic test for COVID-19 variants, (2) predict the disease-severity based on these blood tests, and (3) also build a prototype for prophylactic antidiabetic intranasal sprays with implications in controlling diabetes and diabetes-associated viral and invasive fungal infections (IFIs) in near future.

12.1.4 Literature in Support of Implication of our Our Preliminary Findings for Developing Anti-IFI Intranasal Sprays

There is an increase trend of diabetes mellitus (DM) prevalence worldwide, and the situation in India is alarmingly high as nearly 8% of adults aged <20 years have DM. Uncontrolled DM stands as the major comorbid risk factor for COVID-19 and COVID-19-associated mucormycosis (CAM), both having nasal route of entry. A plethora of serine proteases (SPs) like furin, TMPRSS2, furin-like PCs, and trypsin-like proteases in the nasal microenvironment facilitate heightened transmissibility of emerging SARS-CoV-2 variants that have enhanced susceptibility to SP-cleavable

polybasic amino acids (P681R in delta variant, P681H in omicron) in spike protein, which is the first indispensable step for viral entry (Baggen et al. 2021). These emerging COVID-19 variants of concern have further resulted in recent breakthrough infections in ChAdOx1 health care vaccines in India, suggestive of reduced vaccine effectiveness against VOCs (Mlcochova et al. 2021).

We successfully carried out rigorous in vitro disease modeling of COVID-19 and CAM by establishing a novel model that involved treatment of monocytic cells with serine protease (SP) trypsin, followed by extensive characterization using transcriptomics and proteomics. We identified the potential SP-interactome that displayed pathological maturity onset diabetes, complement-coagulation, olfactory transduction (OT), and COVID-19 disease pathways. Importantly, downregulated OT pathway correlated with suppression of RNAi and TCR complex, and upregulation COVID-19 disease pathway. Hence, we proposed rescuing olfactory epithelium health with 2DGLC (antimetabolic 2-Deoxy glucose, 2DG and amino acid L-carnosine, LC) that were indicated as potential repurposing drugs from in vitro omics data. 2DGLC also showed antifungal activity (growth inhibition assay) against group of clinically relevant Mucorale species, a novel antifungal action observed by us (Sharma et al. 2022). Both drugs have proven antidiabetic and potential in-silico anti-COVID-19 effect.

The intranasal 2DGLC stands different from other topical COVID-9 therapies in market. 2DGLC is primarily targeted toward diabetes and associated viral/fungal infections in contrast to sprays such as Sanotize nasal spray (nitric oxide spray) with a said role to reduce COVID-19 viral load and Viruprotect oral spray (trypsin-based oral spray with moderate reduction in viral load and with many side effects). We propose an antiproteolytic action of 2DGLC as opposed to proteolytic Viruprotect (Huijghebaert et al. 2021). To best of our knowledge, no antidiabetic nasal spray with potential antiviral/fungal action is available till date, despite the fact that DM is a strong comorbidity for COVID-19 and CAM, and both have nasal route of entry. 2DGLC is expected to have a higher anticipated compliance due to topical mode of delivery and less side effects due to less systemic absorption compared to the systemic modes of delivery. About 7–14% of COVID-19 patients have DM as predominant comorbidity, reciprocally pandemic induced hyperglycemia is paramount to half of hospitalized patients, lastly diabetes and COVID-19 (delta variant) resulted in rise to over 2.5 times (average) case of CAM with more than 47,000 CAM reported from Indian subcontinent alone in whooping 3 months (May–July 2021) duration. Predominant presentation of CAM was rhino-orbital-cerebral-mucormycosis (ROCM) with massive olfactory-orbit damage, which was 89% in India and 64% globally. Nasal spray formulations of various drugs including steroid formulations are produced and marketed by various companies such as Cipla, Ranbaxy, GSK, Bayer, Neilmed, etc. We are currently validating the potential metabolic targets in the blood and nasopharyngeal samples of COVID-19, mucor, and CAM patients to translate the findings toward real-world ramifications extending to build intranasal sprays for infectious airway diseases.

12.1.5 Challenges in the COVID-19 and CAM Therapeutics

There have been endless efforts to come up with host-directed (human proteins) rather than viral-directed therapeutics as the virus is rapidly evolving and mutating resulting in emergence of variants of concern and loss of vaccine effectiveness that led to breakthrough infection as seen with omicron variant recently. The rapidly mutating viral spike protein (against which most of the vaccines have been made) makes it difficult to have faithful vaccines that can target multiple variants of SARS-CoV-2. The trinity of anosmia (loss of smell), thrombosis (coagulopathy), and diabetes has emerged as the most common correlate of COVID-19 disease; however, no mechanistic insights have been established between these three pathways in context of COVID-19 disease till date. Ours' is the first mechanistic study done under laboratory conditions using disease-competent blood cell line. Importantly, our study identifies serine-protease protease-activated receptor(s) (PARs) as the potential druggable upstream hierarchical node that resulted in upregulation of olfactory transduction (neurosensory) and complement-coagulation (inflammatory) pathways along with co-upregulation of new-onset /maturity onset-diabetes pathway. We have identified additional important functions of serine proteases besides facilitating viral entry into the human cells. We delineated how the serine proteases that are activated following the viral entry, collaterally deregulate metabolic, neurosensory, and inflammatory pathways and thereby can act as potential cahoots to virus in exacerbating the COVID-19 disease. Interestingly, recent studies are supporting the emerging role of nasal serine proteases in VOCs' emergence and transmission, a recent study re-emphasizes the need for development of host-directed serine protease TMPRSS2 inhibitors as pan-SARS-CoV-2 therapeutics and prophylactics to limit resistance to VOCs (Shapira et al. 2022). Serine proteases have been so far predominantly linked to cleavage of viral spike protein to facilitate viral entry in human cells. However, a cell-autonomous (acting on host cells as opposed to viral proteins) role for serine proteases has not been explored in context of COVID-19. Our study establishes a novel association between proteolytic-stress triggered metabolic reshaping of host cells and makes an attempt to address the link between COVID-19-associated serine protease-mediated new-onset diabetes that could intensify COVID-19 and CAM pathophysiology. The deregulated histidine (amino acid with imidazole ring) amino acid metabolic pathway and ferroptosis (iron-mediated cell death) in our in vitro screen encouraged us to propose L-carnosine (naturally occurring dipeptide β-alanyl-L-histidine) dipeptide with an inherent imidazole ring toward repurposing for COVID-19 and CAM therapeutics. Carnosine is known to have antioxidant, anti-inflammatory, pH buffering, and metal (iron) chelating properties. It is a naturally occurring imidazole dipeptide that has been associated with antidiabetic effects. Recently, it was shown to possess anti-inhibitory action on ACE2, an obligatory SARS-CoV-2 entry receptor. Therefore, we discuss the potential beneficial effects of carnosine as an adjuvant therapy in COVID-19-associated mucormycosis patients with underlying T2DM, its pH buffering features may

enhance the bioavailability of Posaconazole, and its (L-carnosine) inherent imidazole ring may offer antifungal activity.

12.1.6 Repurposing Natural Azoles in COVID-19-Associated Mucormycosis

12.1.6.1 Mucorales and Antifungal Resistance

There has been a silent and underappreciated rise in drug-resistant invasive fungal infections (Arastehfar et al. 2020a, b, c; Carolus et al. 2021). Emerging Mucorales are difficult to diagnose and tolerant to many of the frontline antifungal therapies. Mucorales are emerging as pathogens associated with high mortality due to lack of information on their virulence determinants that could be possibly repurposed as potential targets for drug therapy. Moreover, the Mucorales are inherently resistant to most of the current antifungal compounds attributed to the redundancy in their iron assimilation metabolism and presence of noncanonical RNA interference pathway (NCRIP). Current antifungals used in clinical setting of mucormycosis treatment include liposomal amphoteric B formulation, which has its limitations related to nephrotoxicity in prolonged use in the patients. Therefore, the mortality rate in mucormycosis could approach over 90% following dissemination via blood vessels. There is an urgent need to combat these emerging pathogens and investigate the molecular mechanisms underlying their potentiated virulence traits to identify potential therapeutic targets susceptible to antifungal compounds (Prakash and Chakrabarti 2019; Prakash et al. 2017; Jeong et al. 2019; Hassan and Voigt 2019; Caetano et al. 2019; Maurer et al. 2015; Cornely et al. 2019; Gebremariam et al. 2019; Lax et al. 2020; Cánovas-Márquez et al. 2021; Pérez-Arques et al. 2019, 2020, 2021).

Mucorales have inherent resistance to azole drugs that is partly linked to the deregulated iron metabolism due to the role of iron-containing cofactors in ergosterol synthesis pathway. Therefore, combination therapy of iron chelators and antifungal azole drugs has been encouraged in treatment of mucormycosis. Azole drug Posaconazole used in treatment of mucormycosis is reported to have erratic absorption profiles due to altered gastrointestinal pH resulting in low Posaconazole plasma concentrations in large groups of patients. A unique predisposition for mucormycosis is diabetic ketoacidosis that limits chelation of iron by host transferrin; therefore, the nontransferrin bound iron and high glucose are linked to rapid spread of mucormycosis. The natural occurrence of carnosine azoles in human hosts, and its iron-chelating features might reduce the chances of drug resistance in fungi if it happens to have bilateral (antihost) and novel anti-fungal effects. Carnosine may exhibit beneficial effects of mitigating ferroptosis, promote iron-homeostasis and facilitate management of CAM due to its antioxidant and antichelating properties.

12.1.6.2 L Carnosine/Anserine Azoles with Antidiabetic and Anti-COVID (Host Targeting) Potential for Repurposing in CAM

Novel azoles-imidazole and triazoles, such as based on zeolitic imidazole frameworks (Morcoss et al. 2020; Jiao et al. 2019), selenium containing miconazole (Xu et al. 2020a, b), azole with fused triazinone scaffold (Montoir et al. 2020) have recently been proposed. Cyp51B novel mutation has been reported to underlie azole resistance in *A. fumigatus* (Gonzalez-Jimenez et al. 2020). Novel ergosterol biosynthesis pathway intermediates and modifiers are also reported (Sun et al. 2019; Sinha et al. 2020; Yakobov et al. 2020). Azoles are being investigated for their potential as antidiabetic drugs/ α-glucosidase inhibitors (Sari et al. 2021). However, naturally occurring azoles like L-carnosine and its methylated derivative anserine have not been explored for their antifungal effects.

Carnosine (naturally occurring dipeptide β-alanyl-L-histidine), and anserine (-β-alanyl-3-methy-L-histidine) are present in many human tissues including skeletal muscles, heart, brain, lung, and kidney; it has an important role in alleviating metabolic, cardiovascular, kidney, lung, and inflammatory diseases (Tanaka and Kawahara 2020; Menini et al. 2020; Kilis-Pstrusinska 2020; Caruso et al. 2021; Peters et al. 2020). Data from recent human intervention trials from worldwide have proved the efficacy of carnosine and anserine (imidazole dipeptides) daily supplementation in lowering the risk of developing Alzheimer disease and dementia, which are enhanced due to lifestyle related diseases like diabetes, hyperlipidemia, and hypertension (Masuoka et al. 2021). Carnosine is a common imidazole dipeptide along with other known dipeptides like anserine, ophidine/balerine, homocarnosine, acetyl-carnosine, and carnitine that have health benefits. It is available as an over-the-counter food supplement, with remarkable antioxidative, anti-inflammatory, and neuroprotective properties (Caruso et al. 2019). It has been suggested as a promising nutraceutical for the prevention and treatment of diverse diseases. Several pilot clinical trials with carnosine supplementation/intervention have demonstrated its therapeutic potential with no prominent side effects. The presence of human serum carnosinase in blood is a limitation to carnosine supplementation as it degrades carnosine; however, anserine, which is a methylated carnosine analog, is more resistant to carnosinase-mediated degradation. Hence L-carnosine/anserine combination strategy is believed to have more promising outcomes. Several double-blind, placebo-controlled, randomized controlled trials have been reported with imidazole dipeptide containing chicken derived foods (Szcześniak et al. 2014; Rokicki et al. 2015; Hisatsune et al. 2016; Katakura et al. 2017; Ding et al. 2018; Masuoka et al. 2019).

Importantly, carnosine has been suggested to have protective effects in COVID-19 disease as it has proven anti-inflammatory effects particularly in the elderly, and it has been shown to have inhibitory effects on SARS-CoV-2 entry receptor ACE2 (Hipkiss 2020; Saadah et al. 2020). Carnosine has been associated with antioxidant

and antiglycating effects (Aydın et al. 2017; Bingül et al. 2017; Wang-Eckhardt et al. 2020); unique pH buffering properties due to the imidazole ring (Smith 1938; Davey 1960); reduced infectivity of RNA viruses like Zika, Dengue, and influenza (Xu and Wang 2015; Rothan et al. 2019); inhibitory action towards CD26/DPP4, a receptor for SARS-CoV-2 (Gallego et al. 2014; Vahdatpour et al. 2019); metal chelating effects including transition metals iron and zinc (Mozdzan et al. 2005; Hewlings and Kalman 2020). Carnosine supplementation was shown to reduce the plasma soluble transferrin receptor in healthy overweight or obese individuals in a pilot randomized trial (Naderpoor et al. 2018). Iron is known to affect the clinical course of type 2 diabetes (T2DM) as accompanying increased intracellular accumulation of iron may provide an alternative source for reactive oxygen species (ROS). L-carnosine prevented toxicity of high glucose in a dose-dependent manner, during the $FeCl_3$ challenge suggesting a protective effect mediated by chelation of iron (Zhang et al. 2016). Most notably, a recent study has demonstrated a remarkable role of diamino benzimidazole compound in activating the viral-sensing pathway via acting as a STING agonist that resulted in transient pro-inflammatory cytokine production and lymphocyte activation in lungs and resulted in SARS-CoV-2 replication inhibition. Intranasal administration was also found to be protective against SARS-CoV-2 infection (Humphries et al. 2021), encouraging the development of intranasal formulations of imidazole compounds for airway infectious diseases. The diamino benzimidazole compounds have been reported to possess potent STING activating property, and enhanced tissue penetrance (Ramanjulu et al. 2018).

12.1.7 Iron Metabolism and Homeostasis in Fungal Infections

The iron-sensing fungal transcription factors are known to be dependent on iron-sulfur cluster signaling, and iron-overload oxidative stress regulators/antioxidant glutaredoxins also have a crucial place in relaying intracellular iron status to these transcription factors as has been recently reported for *Candida* and *Aspergillus* species (Gupta and Outten 2020), and iron deficiency is also linked to impairment of translation mediated by Gcn2-eIF2α (Romero et al. 2020). Iron-dependent cell death or ferroptosis had not been reported in any of the microbial system (bacteria, archaea, fungi) up until recently when a study demonstrated ferroptosis in rice blast fungus *Magnaporthe oryzae*, which was correlated to the associated virulence of the fungus (Shen et al. 2020). The iron bioavailability in host–pathogen interactions is integral to microbial pathogenesis; however, demonstration of ferroptosis in a pathogenic fungus clearly reinforces the role of iron metabolism in fungal infections (Shen and Naqvi 2021).

12.1.7.1 Mitochondrial-Driven Iron Metabolism in Azole Drug Resistance

Azoles are first-line antifungals employed in the clinic to inhibit fungal cell membrane ergosterol synthesis by inhibiting cyp51A/Erg11; however, owing to their fungistatic nature and extensive use there has been rapid and constant drug emergence that calls for an urgent need to investigate the molecular mechanisms underlying pan-azole resistance that is intimately linked to fungal virulence. Azoles target the mitochondrial cytochrome P450 enzyme Erg11; therefore, mitochondrial processes and components are implicated in azole drug resistance. Moreover, mitochondria integrate multiple metabolic pathways including lipid metabolism, iron metabolism and bioenergetics, and also cell wall synthesis (Thomas et al. 2013; Dagley et al. 2011; Vincent et al. 2017; Long et al. 2016; Demuyser et al. 2017). Disruption of mitochondrial functions relating to electron transport chain, calcium homeostasis, mitochondrial transcription can result in azole drug resistance or susceptibility in IFIs (Duvenage et al. 2019; Li et al. 2019; Bowyer et al. 2020; Neubauer et al. 2015; Li et al. 2019; Xu et al. 2020a, b; Truong et al. 2019; Sturm et al. 2020). The interconnection between mitochondrial function, cellular iron homeostasis, and ergosterol biosynthesis has been suggested to determine azole resistance (Song et al. 2020). Detailed mechanistic studies are warranted to delineate the precise mechanism of mitochondrial involvement in azole resistance (Song et al. 2016; Horianopoulos and Kronstad 2019; Song et al. 2020). Recently, a mitochondrial citrate transporter (MCT) was proposed as a link between carbohydrate and lipid metabolism, the expression and role of MCT has recently been reported in *Mucor circinelloides,* which has high lipid accumulating propensity (Yang et al. 2022).

Azole agriculture fungicides like propiconazole, and tebuconazole work by similar action mechanism as clinical azoles, thus facilitating environmental-selection pressure for emergence of pan-azole resistance. The advancements in new classes of antifungal drugs are also impeded by the close evolutionary relationship of fungi with the human hosts and conservation of several potential druggable biochemical pathways, which cannot be inhibited right away without prior understanding of the molecular mechanisms of pathway intermediates that can have potential tractable nodes nonconvergent with human hosts. The emerging high-resolution atomic structures determined by X-ray crystallography and cryo-EM of mammalian ABC transporters are being leveraged to recreate comparative structural models of mammalian ABCG and structurally conserved fungal PDRs (pleiotropic drug transporters) to come up with new therapeutic catalytic targets in fungal PDRs to prevent multidrug resistance in infectious diseases (Khunweeraphong and Kuchler 2021). Given the above challenges, primarily the azole resistance is persistently chased with investigations for new azole drug candidates (Rosam et al. 2020; Han et al. 2020; Shafiei et al. 2020; Ellsworth and Ostrosky-Zeichner 2020; Pagano et al. 2020; Nagy et al. 2021; Arai et al. 2021; Furukawa et al. 2020). The potential antifungal and nonantifungal drugs with antifungal activities are being actively

explored and proposed for repurposing (Espinel-Ingroff et al. 2021; Peyclit et al. 2021; Arastehfar et al. 2020a, b, c). The drug development process for IFIs remains largely expensive, and is inherently risky. These challenges declare an urgent need for discovery of new antifungal drugs and encourage drug repurposing as alternative approach to fungal control (Kim and Eom 2021).

Antifungal combinations in Mucorales have also been recently proposed to evaluate if combination therapy works better in unacceptably high mortality causing mucormycosis infection. In patients with rhino-orbital cerebral mucormycosis with underlying ketoacidosis, combination of caspofungin and polyene was synergistic, adjuvant therapy with iron chelator deferasirox was unfavorable (Schwarz et al. 2019). Few fungal pathogens like Mucorales are inherently resistant to antifungals and hence difficult to manage clinically, therefore, a new class of antifungals that belong to antimicrobial peptides (AMPs) with fungal cell membrane, cell wall and nucleic acid inhibiting properties have also been proposed (Buda De Cesare et al. 2020). Sumoylation is a post-translational modification that is becoming linked to fungal virulence and hence represents another potential target (Gupta et al. 2020). Above all, creative screening tools and out-of-the-box ideas aimed at increasing the possibility of identifying potential first-in-class antifungals are highly encouraged (Beattie and Krysan 2020).

12.1.8 Novel Azoles Targeting Host–Fungal Interactions for Drug Repurposing in CAM

The understanding of molecular underpinnings behind fungi and human host continue to grow, however, further research endeavors are underway to fully explore the fungal pathogenesis, (including the role of iron) to gather new insights to achieve improved therapeutics (Paulovičová et al. 2019; Bruno et al. 2020; Badjatia et al. 2021; Ramos-Alonso et al. 2020; Barba-Aliaga et al. 2021; Duval et al. 2020; Tripathi et al. 2020; Soliman et al. 2021; Chaturvedi and de Hoog 2020; Yan and Khan 2021; O'Brien et al. 2020; Kozłowska et al. 2020; Forgács et al. 2020; Vianna et al. 2020). Fungal pathogens infect and spread in the host by evading the host immune evasion by shielding the immunomodulatory cell wall moieties; binding and degrading host immune factors like complement or phagocytic lysosomes; detoxifying host neutrophil generated oxidative stress by employing antioxidant arsenal of glutathione reductase, thioredoxin, and super dismutases; and modulating the host cell death pathways for benefitting infection (König et al. 2021; d'Enfert et al. 2021). There is an increasing recognition of the regulated cell death pathways like apoptosis, pyroptosis (pro-inflammatory inflammasome), and necroptosis employed by the pathogenic fungi to induce killing of the host cells and subvert the immune response toward its survival (Williams et al. 2021). Amino acids are source of carbon and nitrogen in fungal growth; however, the amino acid metabolic pathways are complex and under extensive investigation to offer druggable nodes (Garbe and Vylkova

2019; Idrees et al. 2020). A sterol-response pathway mediated alkaline pH (abiotic stress) survival in human lungs was recently reported in *Cryptococcus neoformans* (Brown et al. 2020). Iron–sulfur cluster signaling are also highly implicated in fungal pathogenesis in IFIs as iron homeostasis is central to fungal virulence (Gupta and Outten 2020).

12.1.8.1 Novelty of Repurposing L-Carnosine in Mucormycosis and CAM

We propose a potential (yet completely unexplored) bilateral (antifungal and host-directed) role of natural azoles L-carnosine/anserine with antidiabetic and anti-COVID (host targeting) potential for repurposing in mucormycosis with and without COVID-19 in type 2 diabetic patients (Fig. 12.1). The natural occurrence of carnosine azoles in human hosts, and its iron-chelating features might reduce the chances of drug resistance in fungi if it happens to have bilateral (antihost) and novel potential antifungal (due to inherent imidazole ring) effects. Carnosine may exhibit beneficial effects of mitigating ferroptosis, promote iron-homeostasis, and facilitate management of COVID-19 and CAM due to its antidiabetic, anti-inflammatory, antioxidant, and antichelating properties (Silva et al. 2021; García-Vidal et al. 2020; Arastehfar et al. 2020a, b, c; White et al. 2021; Al-Hatmi et al. 2020; Antinori et al. 2020; Chowdhary et al. 2020; Mastrangelo et al. 2020; Heard et al. 2020; Moser et al. 2021; Garg et al. 2021; Singh et al. 2022; Sharma et al. 2022). Novel synthetic azoles are being investigated for their potential as antidiabetic drugs/ α-glucosidase inhibitors (Sari et al. 2021). However, naturally occurring azoles with an inherent imidazole ring like L-carnosine/anserine have not been explored for their antifungal effects till date.

12.2 Section B: Current Use and Evidence in Otolaryngology and Head and Neck Surgery (ENT)

12.2.1 Introduction

Repurposing an existing drug in drug research for an established and marketed drug is a savior in difficult times, saving colossal time, energy, and finances for humanity in solving impending clinical problems (Drug repurposing and repositioning: workshop summary 2014). The field of ENT has also seen similar management changes and significant clinical discoveries with the help of the repurposing of existing drugs for clinical scenarios (Choo et al. 2019). The specialty of otolaryngology and head and neck surgery involves various subspecialties, encompassing clinical conditions ranging from medical to surgical issues in infections, noninfectious benign conditions, and various benign and malignant tumors. Drug repurposing has proven to be

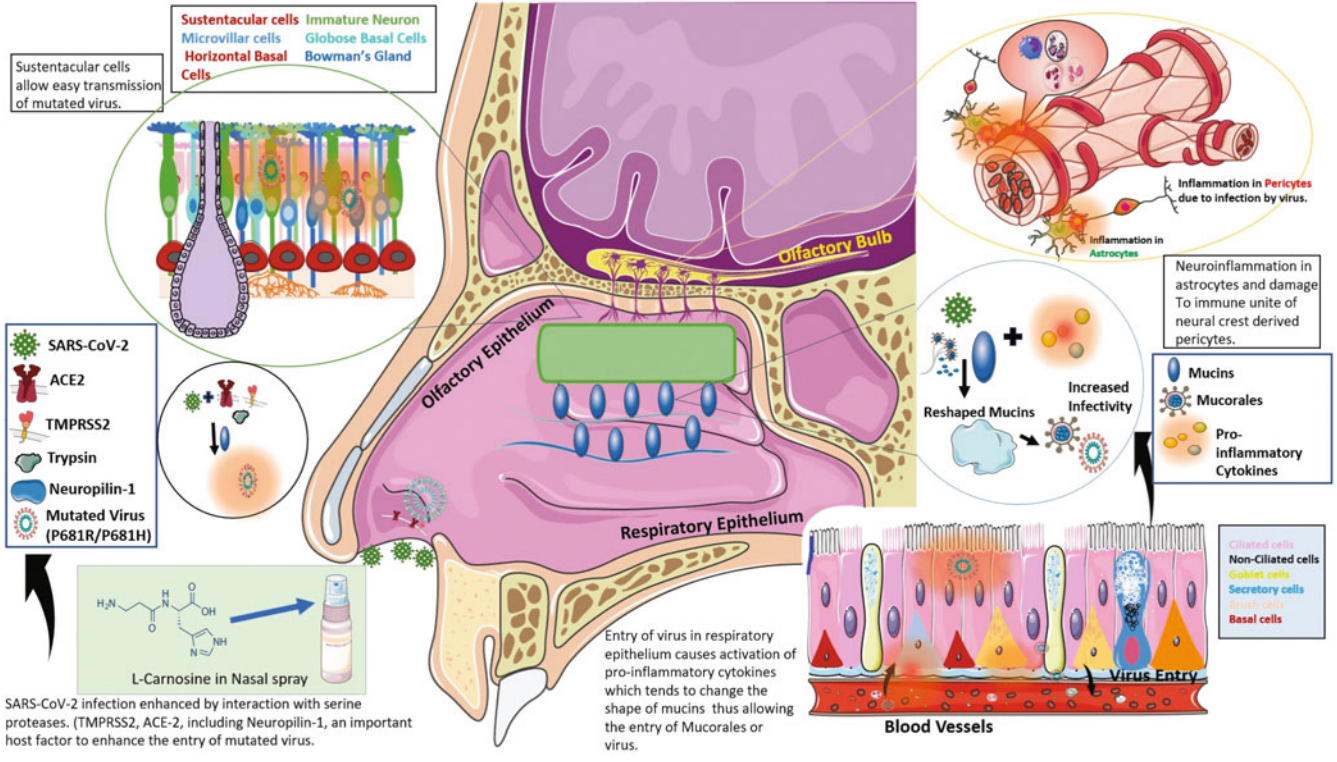


Fig. 12.1 Olfactory niche serine protease TMPRSS2 is an obligatory host receptor that supports infection and spread of SARS-CoV-2. We recently proposed trypsin-like proteases in olfactory niche (FVIIa and FXa complement coagulation serine proteases) as additional players in spread of infection resulting in neuro-inflammatory damage to the host independently of viral loads (Sharma et al. 2022). This process is mediated by metabolic deregulation resulting in altered histidine metabolism, hence we proposed L-carnosine, a nutraceutical drug that is a physiological histidine/imidazole ring containing molecule to be repurposed for intranasal sprays to inhibit the spread of COVID-19 and CAM

significant in multiple fields and is still investigational in many promising possible solutions to different clinical challenges in the specialty.

12.2.1.1 Sinonasal and Airway Diseases

Cimetidine is one of the most prominent examples of drug repurposing in ENT. It was initially used and approved as an H_2-receptor antagonist for acid peptic disease and peptic ulcer (Brogden et al. 1978). Due to its antisecretory properties, it was later considered for Zollinger–Ellison syndrome in high doses (Malagelada and Cortot 1978; Johnston and Wright 1990). This high dose therapy showed immunomodulatory properties of cimetidine (Ershler et al. 1983; Sahasrabudhe et al. 1987; Kumar 1990; Jafarzadeh et al. 2019) leading to the repurposing of cimetidine for various other conditions such as hypogammaglobulinemia (White and Ballow 1985), erythema multiforme prevention (Kurkcuoglu et al. 1993), Herpes zoster (Miller et al. 1989; Komlos et al. 1994), psoriasis (Stashower et al. 1993; Urrea et al. 1995), different recalcitrant viral warts in adults (Glass and Solomon 1996), and children (Saenz-Santamaria and Gilaberte 1997; Das et al. 2018). Their potential role in warts led to further repurposing of the drug in cases of Juvenile onset recurrent respiratory papillomatosis (JORRP or RRP) (Harcourt et al. 1999), a condition caused by respiratory region (larynx–trachea–bronchi) papillomatosis caused by HPV 6 and 11 types, proving as a promising therapy in recalcitrant JORRP. Similarly, due to the immune-modulatory properties, cimetidine was repurposed as adjuvant therapy for PFAPA (periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis) syndrome (Peridis et al. 2010) along with steroids and adenotonsillectomy, a syndrome initially described by Marshall et al. (Marshall et al. 1987). Levamisole, the levorotatory isomer of tetramisole (hydrochloride of 2,3,5,6-tetrahydro-6-phenylimidazo(2,1-b)thiazole), was used as an anthelminthic for ascariasis (Lionel et al. 1969). Later, immunomodulatory properties of Levamisole were identified with a possible proposed role in chronic infective or noninfective inflammatory disease and malignancies (Sampson and Lui 1976; Brunner and Muscoplat 1980) leading to the use of Levamisole in various dermatological conditions such as collagen vascular diseases, inflammatory skin diseases and leprosy (Scheinfeld et al. 2004), along with cimetidine in recalcitrant warts where the combination therapy was found to be more effective than cimetidine monotherapy (Parsad et al. 2001); and also in otolaryngology for the chronic mucosal manifestations (ulcers) of dermatological diseases such as lichen planus, erythema multiforme, and aphthous ulcers of the mouth where the addition of Levamisole to prednisolone had significantly better outcomes compared to prednisolone alone (Lu et al. 1998; Sharda et al. 2014). Similarly, other drugs primarily discovered as gastroprotective agents in peptic ulcer disease such as Irsoglandine (Nanke et al. 2008) and Rebamipide (Parvathi Devi et al. 2014) are also repurposed in chronic aphthous stomatitis with or without Behcet's disease. It has also been repurposed to prevent recurrent upper respiratory tract infections in children (Van Eygen et al. 1976) and for recurrent respiratory papillomatosis (Dubreuil et al. 1979; Schouten et al. 1981). Levamisole

has also been tried in various non ENT diseases like rheumatic diseases (Rosenthal 1978; Scheinberg et al. 1978; Rabson et al. 1980), colorectal carcinoma (Grem 1990), and most recently in COVID-19 infections for patients presenting with diarrhea (Uyaroglu et al. 2020) and as an immunomodulator to reduce cough and dyspnea (Roostaei Firozabad et al. 2021). However, Levamisole is not a harmless drug and can cause vasculitis and consequent necrosis of the skin of the ear and nose, too (George et al. 2019). Levamisole-adulterated cocaine has been shown to cause severe necrosis of the nose, ears and extremities (Lawrence et al. 2014). Hence, it is currently in practice only for veterinary practice as an anthelminthic (Maichomo et al. 2020).

Recent high-throughput analyses have identified possible drugs which can be repurposed for recurrent respiratory papillomatosis where drugs such as Panobinostat (histone deacetylase inhibitor(HDAC) used for multiple myeloma), Dinaciclib (CDK2, CDK5, CDK1, and CDK9 inhibitor), and Forskolin (ubiquitous activator of eukaryotic adenylyl cyclase approved for glaucoma) are found to be eligible for promising repurposing in RRP (Alkhilaiwi et al. 2019) to prevent and delay recurrences.

Mitomycin was discovered in 1956 as an antibiotic from *Streptomyces caespitosus* (Hata et al. 1956). However, due to its later discovered DNA crosslinking property (Iyer and Szybalski 1964; Tomasz and Palom 1997) and consequent double-strand breaks and apoptosis, it has been repurposed as an anti-neoplastic agent in various cancers such as esophageal cancers (Coia 1993), non–small-cell lung cancers (Luo et al. 2020), anal cancers (Glynne-Jones and Rao 2017), breast cancers (Navarro et al. 1989; Aarts et al. 2019; Varshosaz et al. 2019), cervical cancers (Al-Otaibi et al. 2018), and urothelial cancers (Dreicer 2020; Kleinmann et al. 2020). Furthermore, the antifibrotic properties of Mitomycin were discovered, which also resulted in the inhibition of proliferation of human T31 tracheal scar fibroblasts in vitro (Li et al. 2004). This antifibrotic property has significantly impacted otolaryngologic practice and led to the established use of Mitomycin c in laryngotracheal (airway) stenosis (Senders 2004; Ubell et al. 2006; Chen and Zhang 2013) and esophageal strictures (Machida et al. 2012; Bartel et al. 2016). However, there exists contrasting opinion regarding such antifibrotic use of Mitomycin for preventing airway stenosis (Roh et al. 2006; Gangar and Bent 2014). Vitamin D has been proven to have immunomodulatory action in preventing recurrent respiratory tract infection in children (Esposito and Lelii 2015).

12.2.1.2 Diseases of the EAR

Vitamin D is an essential micronutrient prescribed for various deficiency disorders and calcium and phosphate metabolism disorders. However, later discovered immune-modulatory activities (Di Rosa et al. 2012) have helped repurpose vitamin D for multiple indications. With the development of concepts of origin pf BPPV which cited various causes like decreased bone mineral density, estrogen deficiency, and lower serum vitamin D levels (Jeong et al. 2013; Jeong and Kim 2019), vitamin

D was repurposed (Buki et al. 2013) to prevent and alleviate symptoms of BPPV (Parham et al. 2013; Talaat et al. 2016) and has shown moderate results in reducing recurrences and symptoms (Jeong et al. 2020). On similar grounds and considering post viral etiology, vitamin D is being repurposed for Meniere's disease (Buki et al. 2018; Bakhshaee et al. 2022). For the combination of Meniere's disease and BPPV, gentamycin has also been repurposed with canal positioning man oeuvres but could produce moderate results only (Perez et al. 2002). Danhong, a Chinese traditional medicine, has also been repurposed for BPPV due to the properties of vasodilation and improvement of microcirculation, resulting in improvement in residual dizziness (Deng et al. 2014).

Similarly, progression of hearing loss, especially in cases of early onset age induced hearing loss, is a challenge in the field of otology. The key to the solution for the same is being observed with significantly slow progression of hearing loss seen in the elderly population among patients who are on hypolipidemic therapy (Choo et al. 2019) guiding future possibilities of role of hypolipidemic drugs in hearing preservation. Promising results have been seen with the use of systemic bisphosphonates in sensorineural hearing loss, where synaptic regeneration has been observed in mice with noise-induced loss of glutaminergic spiral ganglion synapses after 24 h of the administration of the drug (Seist et al. 2020), which can lead to repurposing of bisphosphonates for sensorineural hearing loss in future. For vestibular schwannomas where the surgery is challenging and always has the risk of facial nerve palsy associated with surgery. Currently, it is proposed that immunohistochemistry expression of COX-2 correlates with the growth of vestibular schwannomas, and hence nonsteroidal anti-inflammatory drugs can be repurposed as cytostatic for vestibular schwannomas (Dilwali et al. 2015; Kandathil et al. 2016; Behling et al. 2019). However, the meta-analysis by Ignacio et al. in 2021 implies that current clinical evidence is not sufficient (Ignacio et al. 2021) to form practice recommendations. Mifepristone, an antagonist of glucocorticoid and progesterone receptors, has also been shown to adversely affect the morphology, metabolic activity, and proliferation of primary human vestibular schwannoma cells and HEI-193 human schwannoma cells (Sagers et al. 2018). This fact can be used in the future for repurposing of mifepristone to manage vestibular schwannomas.

The most common ear disease being chronic suppurative otitis media (CSOM) and cholesteatoma formation being a prominent reason of CSOM, its management has always intrigued the otologists. The primary management of cholesteatoma is surgery; however, there are reports of complete spontaneous resolution of cholesteatoma with Infliximab (Chao et al. 2019), an antibody against TNF-alpha, primarily used for chronic inflammatory conditions. Hence, medical management of cholesteatoma can be a possibility too with the repurposing of such anticytokine drugs, though they come with associated side effects (Lichtenstein et al. 2015; Hemperly and Vande Casteele 2018).

12.2.1.3 Diseases of Head and Neck

Head and neck cancers and their management are significant for ENT physicians and surgeons as head and neck cancers constitute the most common cancer in males and the third most common in females in the southeast Asia region and India (Bray et al. 2018; Bakshi et al. 2020). Various drugs have been repurposed for the management of head and neck cancers to address the disease process. Currently, almost 335 molecules are being explored for repurposing in cancers of various types and sites (Pfab et al. 2021); however, those with a proposed role in head and neck cancers are significant in the current context.

Antimalarial drug artesunate is being explored in head and neck cancers due to the properties of induction of cell death and apoptosis via increased reactive oxygen species (ROS) and decreased Glutathione (GSH) (Roh et al. 2017). Artesunate in cell cultures and in vivo xenograft models have shown to effectively inhibit phosphorylation of proteins Akt, mTOR, and 4EBP1 in the Akt/mTOR pathway and to induce mitochondrial dysfunction by inhibiting mitochondrial resulting in oxidative stress via increasing levels of mitochondrial superoxide and cellular reactive oxygen species (ROS) leading to decreased ATP levels (Li et al. 2017). Other similar agents repurposed to induce ferroptosis include sulfasalazine and sorafenib (Xie et al. 2016). Hence repurposing is being explored in an oral cancer cell line (YD-10B) (Nam et al. 2007) and nasopharyngeal cancer (NPC) cell lines (C666-1, HONE-1, HK1, HNE1, CNE2 cultured in RPMI-1640 medium) (Li et al. 2017). Similar findings of Akt/mTOR inhibition in nasopharyngeal cell lines also produced by capsaicin (Lin et al. 2017), sodium butyrate (Huang et al. 2019), and chloroform extract from Sophora tonkinensis Gagnep (ShuoWang et al. 2021). In future, these molecules and strategies of Akt/mTOR inhibition might be repurposed for clinical use for effective management. Antiviral ribavirin, an FDA-approved antiviral for Hepatitis C, has shown to decrease cellular proliferation, migration, and invasion; and promote cell-cycle arrest and cell death in nasopharyngeal cancer cell lines by modulation of various proteins such as EZH2, Snail, eIF4E, IMPDH, mTOR, and cyclin D1 with replication of similar results in NPC xenograft models (Huq et al. 2020). Anthelminthic drug albendazole showed significant activity against 13 out of 14 HPV negative head and cancer lines, with an average IC50 of 152 nM by causing apoptosis, cell migration inhibition, G2/M phase arrest and altered tubulin distribution, though the responses were not that significant in HPV positive head and neck cancer cell lines (Ghasemi et al. 2017).

Antidiabetic drug metformin has shown better survival in hypopharyngeal cancers patients in a retrospective cohort study based on its role in the regulation of p38/JNK pathway to reduce Cyclin D1 and Bcl-2 and activation of AMPKα and MEK/ERK to phosphorylate p27 (Thr198) and inhibit mTOR phosphorylation leading to G1 cell cycle arrest, apoptosis, and autophagy in cancer cell lines of FaDu (ATCC HTB-43) (Tsou et al. 2021). The lipid-lowering agent fenofibrate suppressed tumorigenesis in oral cancer cells through downregulation of mTOR activity via TSC1/2-dependent signaling via either activation of AMPK and

inactivation of Akt or TSC1/2-independent pathway through direct suppression of raptor; and decreased incidence of tongue lesions, tumor size, multiplicity, and the immunoreactivities of VDAC and mTOR in oral cancer mouse models showing its future possible promising role in oral cancers (Jan et al. 2016). Other lipid-lowering agents such as statins have shown both individual and synergistic antitumor activity along with cisplatin in head and neck cancer cells and murine models through increased calreticulin exposure and endoplasmic reticulum stress signals, and activation of antigen-presenting cell (APC)s and tumor-specific CD8+ T cells (Kwon et al. 2021). Proton pump inhibitor esomeprazole has shown inhibition of tumor growth and dose-dependent enhancement of cellular killing by ionizing radiation in both wild-type and p53-mutant radioresistant cancer cells in mouse models of HNSCC and cell arrest in G1 phase in head and neck cancer cell lines by upregulation of p21 and inhibition of cyclin-dependent kinase(Cdk) types 1 (Cdk1) and 2 (Cdk2) (Hebert et al. 2021). There has been significant work in drug repurposing in ENT and head and neck diseases; however, most of the work has been done at the preclinical level in vitro in cells and in vivo in animal models. Hence, there are greater possibilities in the field for finding clinical solutions in ENT and head and neck diseases in the future.

Funding The research work discussed in this perspective was conducted in the Department of Otolaryngology and Head and Neck Surgery. The funding support was provided by Science and Engineering Research Board (SERB), Department of Science and Technology, Government of India under the following funding schemes SERB-CRG/2019/006745 and SERB-SPR/2019/001447.

References

Aarts BM, Klompenhouwer EG et al (2019) Intra-arterial mitomycin C infusion in a large cohort of advanced liver metastatic breast cancer patients: safety, efficacy and factors influencing survival. Breast Cancer Res Treat 176(3):597–605

Al-Hatmi AM, Al-Shuhoumi MA, Denning DW (2020) Estimated burden of fungal infections in Oman. J Fungi 7(1):5

Alkhilaiwi F, Paul S et al (2019) High-throughput screening identifies candidate drugs for the treatment of recurrent respiratory papillomatosis. Papillomavirus Res 8:100181

Al-Otaibi WA, Alkhatib MH et al (2018) Cytotoxicity and apoptosis enhancement in breast and cervical cancer cells upon coadministration of mitomycin C and essential oils in nanoemulsion formulations. Biomed Pharmacother 106:946–955

Antinori S, Galimberti L, Milazzo L, Ridolfo AL (2020) Bacterial and fungal infections among patients with SARS-CoV-2 pneumonia. Hospitals 85(77):90–96

Arai T, Umeyama T, Majima H, Inukai T, Watanabe A, Miyazaki Y, Kamei K (2021) Hmg1 mutations in Aspergillus fumigatus and their contribution to triazole susceptibility. Med Mycol 59(10):980–984

Arastehfar A, Carvalho A, van de Veerdonk FL, Jenks JD, Koehler P, Krause R, Cornely OA, S Perlin D, Lass-Flörl C, Hoenigl M. (2020a) COVID-19 associated pulmonary Aspergillosis (CAPA)—from immunology to treatment. J Fungi 6(2):91

Arastehfar A, Daneshnia F, Hafez A, Khodavaisy S, Najafzadeh MJ, Charsizadeh A, Zarrinfar H, Salehi M, Shahrabadi ZZ, Sasani E, Zomorodian K (2020b) Antifungal susceptibility,

genotyping, resistance mechanism, and clinical profile of Candida tropicalis blood isolates. Med Mycol 58(6):766–773
Arastehfar A, Lass-Flörl C, Garcia-Rubio R, Daneshnia F, Ilkit M, Boekhout T, Gabaldon T, Perlin DS (2020c) The quiet and underappreciated rise of drug-resistant invasive fungal pathogens. J Fungi 6(3):138
Aydın AF, Bingül İ, Küçükgergin C, Doğan-Ekici I, Doğru Abbasoğlu S, Uysal M (2017) Carnosine decreased oxidation and glycation products in serum and liver of high-fat diet and low-dose streptozotocin-induced diabetic rats. Int J Exp Pathol 98(5):278–288
Badjatia N, Rossi MJ, Bataille AR, Mittal C, Lai WK, Pugh BF (2021) Acute stress drives global repression through two independent RNA polymerase II stalling events in saccharomyces. Cell Rep 34(3):108640
Baggen J, Persoons L, Vanstreels E, Jansen S, Van Looveren D, Boeckx B, Geudens V, De Man J, Jochmans D, Wauters J, Wauters E (2021) Genome-wide CRISPR screening identifies TMEM106B as a proviral host factor for SARS-CoV-2. Nat Genet 53(4):435–444
Bakhshaee M, Moradi S et al (2022) Association between serum vitamin D level and Meniere's disease. Otolaryngol Head Neck Surg 166(1):146–150
Bakshi J, Kaur N et al (2020) Survival analysis of oral squamous cell carcinoma patients attending Tertiary Care Centre of North India. Indian J Surg Oncol:1–9
Barba-Aliaga M, Alepuz P, Pérez-Ortín JE (2021) Eukaryotic RNA polymerases
Bartel MJ, Seeger K et al (2016) Topical mitomycin C application in the treatment of refractory benign esophageal strictures in adults and comprehensive literature review. Dig Liver Dis 48(9): 1058–1065
Beattie SR, Krysan DJ (2020) Antifungal drug screening: thinking outside the box to identify novel antifungal scaffolds. Curr Opin Microbiol 57:1–6
Behling F, Ries V et al (2019) COX2 expression is associated with proliferation and tumor extension in vestibular schwannoma but is not influenced by acetylsalicylic acid intake. Acta Neuropathol Commun 7(1):105
Bingül İ, Yılmaz Z, Aydın AF, Çoban J, Doğru-Abbasoğlu S, Uysal M (2017) Antiglycation and anti-oxidant efficiency of carnosine in the plasma and liver of aged rats. Geriatr Gerontol Int 17(12):2610–2614
Borman AM, Fraser M, Patterson Z, Palmer MD, Johnson EM (2021) In vitro antifungal drug resistance profiles of clinically relevant members of the mucorales (mucoromycota) especially with the newer triazoles. J Fungi 7(4):271
Bowyer P, Bromley MJ, Denning DW (2020) Linking calcium signaling and mitochondrial function in fungal drug resistance. Proc Natl Acad Sci U S A 117(3):1254–1256
Bray F, Ferlay J et al (2018) Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68(6):394–424
Brogden RN, Heel RC et al (1978) Cimetidine: a review of its pharmacological properties and therapeutic efficacy in peptic ulcer disease. Drugs 15(2):93–131
Brown GD, Denning DW, Gow NA, Levitz SM, Netea MG, White TC (2012) Hidden killers: human fungal infections. Sci Transl Med 4(165):165rv13
Brown HE, Telzrow CL, Saelens JW, Fernandes L, Alspaugh JA (2020) Sterol-response pathways mediate alkaline survival in diverse fungi. MBio 11(3):e00719–e00720
Brunner CJ, Muscoplat CC (1980) Immunomodulatory effects of levamisole. J Am Vet Med Assoc 176:1159–1162
Bruno M, Kersten S, Bain JM, Jaeger M, Rosati D, Kruppa MD, Lowman DW, Rice PJ, Graves B, Ma Z, Jiao YN (2020) Transcriptional and functional insights into the host immune response against the emerging fungal pathogen Candida auris. Nat Microbiol 5(12):1516–1531
Buda De Cesare G, Cristy SA, Garsin DA, Lorenz MC (2020) Antimicrobial peptides: a new frontier in antifungal therapy. MBio 11(6):e02123–e02120
Buki B, Ecker M et al (2013) Vitamin D deficiency and benign paroxysmal positioning vertigo. Med Hypotheses 80(2):201–204

Buki B, Junger H et al (2018) Vitamin D supplementation may improve symptoms in Meniere's disease. Med Hypotheses 116:44–46

Caetano LA, Faria T, Springer J, Loeffler J, Viegas C (2019) Antifungal-resistant Mucorales in different indoor environments. Mycology 10(2):75–83

Cánovas-Márquez JT, Falk S, Nicolás FE, Padmanabhan S, Zapata-Pérez R, Sánchez-Ferrer Á, Navarro E, Garre V (2021) A ribonuclease III involved in virulence of Mucorales fungi has evolved to cut exclusively single-stranded RNA. Nucleic Acids Res 49(9):5294–5307

Carolus H, Pierson S, Muñoz JF, Subotić A, Cruz RB, Cuomo CA, Van Dijck P (2021) Genome-wide analysis of experimentally evolved Candida auris reveals multiple novel mechanisms of multidrug resistance. MBio 12(2):e03333–e03320

Caruso G, Caraci F, Jolivet RB (2019) Pivotal role of carnosine in the modulation of brain cells activity: multimodal mechanism of action and therapeutic potential in neurodegenerative disorders. Prog Neurobiol 175:35–53

Caruso G, Godos J, Castellano S, Micek A, Murabito P, Galvano F, Ferri R, Grosso G, Caraci F (2021) The therapeutic potential of carnosine/anserine supplementation against cognitive decline: a systematic review with meta-analysis. Biomedicine 9(3):253

Chao J, Dewyer N et al (2019) Spontaneous resolution of cholesteatoma in a patient on Long-term infliximab. Ann Otol Rhinol Laryngol 128(4):365–368

Chaturvedi V, de Hoog GS (2020) Onygenalean fungi as major human and animal pathogens. Mycopathologia 185(1):1–8

Chen N, Zhang J (2013) [The mechanism and progress of topical application of mitomycin C as an adjunctive therapy for benign airway stenosis]. Zhonghua Jie He He Hu Xi Za Zhi 36(8): 592–594

Choo OS, Yoon D et al (2019) Drugs for hyperlipidaemia may slow down the progression of hearing loss in the elderly: a drug repurposing study. Basic Clin Pharmacol Toxicol 124(4): 423–430

Chowdhary A, Tarai B, Singh A, Sharma A (2020) Multidrug-resistant Candida auris infections in critically ill coronavirus disease patients, India, April–July 2020. Emerg Infect Dis 26(11):2694

Coia LR (1993) The use of mitomycin in esophageal cancer. Oncology 50(Suppl 1):53–60

Cornely OA, Alastruey-Izquierdo A, Arenz D, Chen SC, Dannaoui E, Hochhegger B, Hoenigl M, Jensen HE, Lagrou K, Lewis RE, Mellinghoff SC (2019) Global guideline for the diagnosis and management of mucormycosis: an initiative of the European Confederation of Medical Mycology in cooperation with the Mycoses Study Group Education and Research Consortium. Lancet Infect Dis 19(12):e405–e421

d'Enfert C, Kaune AK, Alaban LR, Chakraborty S, Cole N, Delavy M, Kosmala D, Marsaux B, Fróis-Martins R, Morelli M, Rosati D (2021) The impact of the fungus-host-microbiota interplay upon Candida albicans infections: current knowledge and new perspectives. FEMS Microbiol Rev 45(3):fuaa060

Dagley MJ, Gentle IE, Beilharz TH, Pettolino FA, Djordjevic JT, Lo TL, Uwamahoro N, Rupasinghe T, Tull DL, McConville M, Beaurepaire C (2011) Cell wall integrity is linked to mitochondria and phospholipid homeostasis in Candida albicans through the activity of the post-transcriptional regulator Ccr4-Pop2. Mol Microbiol 79(4):968–989

Das BB, Anton K et al (2018) Cimetidine: a safe treatment option for cutaneous warts in pediatric heart transplant recipients. Med Sci (Basel) 6(2):30

Davey CL (1960) The significance of carnosine and anserine in striated skeletal muscle. Arch Biochem Biophys 89(2):303–308

Demuyser L, Swinnen E, Fiori A, Herrera-Malaver B, Verstrepen K, Van Dijck P (2017) Mitochondrial cochaperone Mge1 is involved in regulating susceptibility to fluconazole in Saccharomyces cerevisiae and Candida species. MBio 8(4):e00201–e00217

Deng W, Yang C et al (2014) Danhong enhances recovery from residual dizziness after successful repositioning treatment in patients with benign paroxysmal positional vertigo. Am J Otolaryngol 35(6):753–757

Denning DW, Perlin DS, Muldoon EG, Colombo AL, Chakrabarti A, Richardson MD, Sorrell TC (2017) Delivering on antimicrobial resistance agenda not possible without improving fungal diagnostic capabilities. Emerg Infect Dis 23(2):177

Di Rosa M, Malaguarnera G et al (2012) Immuno-modulatory effects of vitamin D3 in human monocyte and macrophages. Cell Immunol 280(1):36–43

Dilwali S, Kao SY et al (2015) Nonsteroidal anti-inflammatory medications are cytostatic against human vestibular schwannomas. Transl Res 166(1):1–11

Ding Q, Tanigawa K, Kaneko J, Totsuka M, Katakura Y, Imabayashi E, Matsuda H, Hisatsune T (2018) Anserine/carnosine supplementation preserves blood flow in the prefrontal brain of elderly people carrying APOE e4. Aging Dis 9(3):334

Dreicer R (2020) New mitomycin formulation for low-grade upper tract urothelial cancer. Lancet Oncol 21(6):735–736

Drug repurposing and repositioning: workshop summary. Washington, DC (2014)

Dubreuil C, Dumolard P, et al (1979) [Laryngeal papillomatosis in the child: a new treatment with levamisole]. J Fr Otorhinolaryngol Audiophonol Chir Maxillofac 28(7):465–470

Duval C, Macabiou C, Garcia C, Lesuisse E, Camadro JM, Auchère F (2020) The adaptive response to iron involves changes in energetic strategies in the pathogen Candida albicans. Microbiology Open 9(2):e970

Duvenage L, Munro CA, Gourlay CW (2019) The potential of respiration inhibition as a new approach to combat human fungal pathogens. Curr Genet 65(6):1347–1353

Ellsworth M, Ostrosky-Zeichner L (2020) Isavuconazole: mechanism of action, clinical efficacy, and resistance. J Fungi 6(4):324

Ershler WB, Hacker MP et al (1983) Cimetidine and the immune response. I. In vivo augmentation of nonspecific and specific immune response. Clin Immunol Immunopathol 26(1):10–17

Espinel-Ingroff A, Sasso M, Turnidge J, Arendrup M, Botterel F, Bourgeois N, Bouteille B, Canton E, Cassaing S, Dannaoui E, Dehais M (2021) Etest ECVs/ECOFFs for detection of resistance in prevalent and three nonprevalent Candida spp. to Triazoles and amphotericin B and Aspergillus spp. to Caspofungin: further assessment of modal variability. Antimicrob Agents Chemother 65(11):e01093–e01021

Esposito S, Lelii M (2015) Vitamin D and respiratory tract infections in childhood. BMC Infect Dis 15:487

Forgács L, Borman AM, Prépost E, Tóth Z, Kardos G, Kovács R, Szekely A, Nagy F, Kovacs I, Majoros L (2020) Comparison of in vivo pathogenicity of four Candida auris clades in a neutropenic bloodstream infection murine model. Emerg Microb Infect 9(1):1160–1169

Furukawa T, Van Rhijn N, Fraczek M, Gsaller F, Davies E, Carr P, Gago S, Fortune-Grant R, Rahman S, Gilsenan JM, Houlder E (2020) The negative cofactor 2 complex is a key regulator of drug resistance in Aspergillus fumigatus. Nat Commun 11(1):1–6

Gallego M, Aristoy MC, Toldrá F (2014) Dipeptidyl peptidase IV inhibitory peptides generated in Spanish dry-cured ham. Meat Sci 96(2):757–761

Gangar M, Bent JP (2014) The use of mitomycin C in pediatric airway surgery: does it work? Curr Opin Otolaryngol Head Neck Surg 22(6):521–524

Garbe E, Vylkova S (2019) Role of amino acid metabolism in the virulence of human pathogenic fungi. Curr Clin Microbiol Rep 6(3):108–119

García-Vidal G, Guzmán-Vilar L, Sánchez-Rodríguez A, Martínez-Vivar R, Pérez-Campdesuñer R, Uset-Ruiz F (2020) Facing post COVID-19 era, what is really important for Ecuadorian SMEs? Int J Eng Bus Manag 12:1847979020971944

Garg D, Muthu V, Sehgal IS, Ramachandran R, Kaur H, Bhalla A, Puri GD, Chakrabarti A, Agarwal R (2021) Coronavirus disease (Covid-19) associated mucormycosis (CAM): case report and systematic review of literature. Mycopathologia 186(2):289–298

Gebremariam T, Alkhazraji S, Soliman SS, Gu Y, Jeon HH, Zhang L, French SW, Stevens DA, Edwards JE Jr, Filler SG, Uppuluri P (2019) Anti-CotH3 antibodies protect mice from mucormycosis by prevention of invasion and augmenting opsonophagocytosis. Sci Adv 5(6): eaaw1327

George TC, Freet DJ et al (2019) Levamisole-induced vasculitis. JAAPA 32(1):23–27
Ghasemi F, Black M et al (2017) Repurposing Albendazole: new potential as a chemotherapeutic agent with preferential activity against HPV-negative head and neck squamous cell cancer. Oncotarget 8(42):71512–71519
Glass AT, Solomon BA (1996) Cimetidine therapy for recalcitrant warts in adults. Arch Dermatol 132(6):680–682
Glynne-Jones R, Rao S (2017) Treatment of the primary tumor in anal canal cancers. Surg Oncol Clin N Am 26(1):73–90
Gonzalez-Jimenez I, Lucio J, Amich J, Cuesta I, Sanchez Arroyo R, Alcazar-Fuoli L, Mellado E (2020) A Cyp51B mutation contributes to azole resistance in Aspergillus fumigatus. J Fungi 6(4):315
Grem JL (1990) Levamisole as a therapeutic agent for colorectal carcinoma. Cancer Cells 2(5): 131–137
Gupta M, Outten CE (2020) Iron–sulfur cluster signaling: the common thread in fungal iron regulation. Curr Opin Chem Biol 55:189–201
Gupta D, Garapati HS, Kakumanu AV, Shukla R, Mishra K (2020) SUMOylation in fungi: a potential target for intervention. Comput Struct Biotechnol J 18:3484–3493
Han G, Liu N, Li C, Tu J, Li Z, Sheng C (2020) Discovery of novel fungal lanosterol 14-α-demethylase (CYP51)/histone deacetylase dual inhibitors to treat azole-resistant candidiasis. J Med Chem 63(10):5341–5359
Harcourt JP, Worley G et al (1999) Cimetidine treatment for recurrent respiratory papillomatosis. Int J Pediatr Otorhinolaryngol 51(2):109–113
Hassan MI, Voigt K (2019) Pathogenicity patterns of mucormycosis: epidemiology, interaction with immune cells and virulence factors. Med Mycol 57(Supplement_2):S245–S256
Hata T, Hoshi T et al (1956) Mitomycin, a new antibiotic from Streptomyces. I. J Antibiot (Tokyo) 9(4):141–146
Heard KL, Hughes S, Mughal N, Moore LS (2020) COVID-19 and fungal superinfection. Lancet Microbe 1(3):e107
Hebert KA, Jaramillo S et al (2021) Esomeprazole enhances the effect of ionizing radiation to improve tumor control. Oncotarget 12(14):1339–1353
Hemperly A, Vande Casteele N (2018) Clinical pharmacokinetics and pharmacodynamics of infliximab in the treatment of inflammatory bowel disease. Clin Pharmacokinet 57(8):929–942
Hewlings S, Kalman D (2020) A review of zinc-L-carnosine and its positive effects on oral mucositis, taste disorders, and gastrointestinal disorders. Nutrients 12(3):665
Hipkiss AR (2020) COVID-19 and Senotherapeutics: any role for the naturally-occurring dipeptide carnosine? Aging Dis 11(4):737
Hisatsune T, Kaneko J, Kurashige H, Cao Y, Satsu H, Totsuka M, Katakura Y, Imabayashi E, Matsuda H (2016) Effect of anserine/carnosine supplementation on verbal episodic memory in elderly people. J Alzheimers Dis 50(1):149–159
Horianopoulos LC, Kronstad JW (2019) Connecting iron regulation and mitochondrial function in Cryptococcus neoformans. Curr Opin Microbiol 52:7–13
Huang W, Zeng C et al (2019) Sodium butyrate induces autophagic apoptosis of nasopharyngeal carcinoma cells by inhibiting AKT/mTOR signaling. Biochem Biophys Res Commun 514(1): 64–70
Huijghebaert S, Hoste L, Vanham G (2021) Essentials in saline pharmacology for nasal or respiratory hygiene in times of COVID-19. Eur J Clin Pharmacol 77(9):1275–1293
Humphries F, Shmuel-Galia L, Jiang Z, Wilson R, Landis P, Ng SL, Parsi KM, Maehr R, Cruz J, Morales A, Ramanjulu JM (2021) A diamidobenzimidazole STING agonist protects against SARS-CoV-2 infection. Sci Immunol 6(59):eabi9002
Huq S, Casaos J et al (2020) Repurposing the FDA-approved antiviral drug ribavirin as targeted therapy for nasopharyngeal carcinoma. Mol Cancer Ther 19(9):1797–1808
Idrees M, Mohammad AR, Karodia N, Rahman A (2020) Multimodal role of amino acids in microbial control and drug development. Antibiotics 9(6):330

Ignacio KHD, Espiritu AI et al (2021) Efficacy of aspirin for sporadic vestibular schwannoma: a meta-analysis. Neurol Sci 42(12):5101–5106
Iyer VN, Szybalski W (1964) Mitomycins and Porfiromycin: chemical mechanism of activation and cross-linking of DNA. Science 145(3627):55–58
Jafarzadeh A, Nemati M et al (2019) Immunomodulatory properties of cimetidine: its therapeutic potentials for treatment of immune-related diseases. Int Immunopharmacol 70:156–166
Jan CI, Tsai MH et al (2016) Fenofibrate suppresses oral tumorigenesis via reprogramming metabolic processes: potential drug repurposing for oral cancer. Int J Biol Sci 12(7):786–798
Jeong SH, Kim JS (2019) Impaired calcium metabolism in benign paroxysmal positional vertigo: a topical review. J Neurol Phys Ther 43(Suppl 2):S37–S41
Jeong SH, Kim JS et al (2013) Decreased serum vitamin D in idiopathic benign paroxysmal positional vertigo. J Neurol 260(3):832–838
Jeong W, Keighley C, Wolfe R, Lee WL, Slavin MA, Kong DC, Chen SA (2019) The epidemiology and clinical manifestations of mucormycosis: a systematic review and meta-analysis of case reports. Clin Microbiol Infect 25(1):26–34
Jeong SH, Kim JS et al (2020) Prevention of benign paroxysmal positional vertigo with vitamin D supplementation: a randomized trial. Neurology 95(9):e1117–e1125
Jiao L, Seow JY, Skinner WS, Wang ZU, Jiang HL (2019) Metal–organic frameworks: structures and functional applications. Mater Today 27:43–68
Johnston SR, Wright PL (1990) Control of metastatic Zollinger-Ellison syndrome with high-dose H2-receptor antagonists. Br J Clin Pract 44(12):792–793
Kandathil CK, Cunnane ME et al (2016) Correlation between aspirin intake and reduced growth of human vestibular schwannoma: volumetric analysis. Otol Neurotol 37(9):1428–1434
Katakura Y, Totsuka M, Imabayashi E, Matsuda H, Hisatsune T (2017) Anserine/carnosine supplementation suppresses the expression of the inflammatory chemokine CCL24 in peripheral blood mononuclear cells from elderly people. Nutrients 9(11):1199
Khunweeraphong N, Kuchler K (2021) Multidrug resistance in mammals and fungi—from MDR to PDR: a rocky road from atomic structures to transport mechanisms. Int J Mol Sci 22(9):4806
Kilis-Pstrusinska K (2020) Carnosine and kidney diseases: what we currently know? Curr Med Chem 27(11):1764–1781
Kim HR, Eom YB (2021) Antifungal and anti-biofilm effects of 6-shogaol against Candida auris. J Appl Microbiol 130(4):1142–1153
Kleinmann N, Matin SF et al (2020) Primary chemoablation of low-grade upper tract urothelial carcinoma using UGN-101, a mitomycin-containing reverse thermal gel (OLYMPUS): an open-label, single-arm, phase 3 trial. Lancet Oncol 21(6):776–785
Komlos L, Notmann J et al (1994) IN vitro cell-mediated immune reactions in herpes zoster patients treated with cimetidine. Asian Pac J Allergy Immunol 12(1):51–58
König A, Müller R, Mogavero S, Hube B (2021) Fungal factors involved in host immune evasion, modulation and exploitation during infection. Cell Microbiol 23(1):e13272
Kozłowska E, Brzezińska-Błaszczyk E, Rasmus P, Żelechowska P (2020) Fungal β-glucans and mannan stimulate peripheral blood mononuclear cells to cytokine production in Syk-dependent manner. Immunobiology 225(5):151985
Kumar A (1990) Cimetidine: an immunomodulator. DICP 24(3):289–295
Kurkcuoglu N, Tuglular T et al (1993) Cimetidine prevents erythema multiforme. Ann Allergy 70(2):180
Kwon M, Nam GH et al (2021) Statin in combination with cisplatin makes favorable tumor-immune microenvironment for immunotherapy of head and neck squamous cell carcinoma. Cancer Lett 522:198–210
Lawrence LA, Jiron JL et al (2014) Levamisole-adulterated cocaine induced skin necrosis of nose, ears, and extremities: case report. Allergy Rhinol (Providence) 5(3):132–136
Lax C, Pérez-Arques C, Navarro-Mendoza MI, Cánovas-Márquez JT, Tahiri G, Pérez-Ruiz JA, Osorio-Concepción M, Murcia-Flores L, Navarro E, Garre V, Nicolás FE (2020) Genes, pathways, and mechanisms involved in the virulence of mucorales. Genes 11(3):317

Li H, Liu Y et al (2004) Synthesis and biological evaluation of a cross-linked hyaluronan-mitomycin C hydrogel. Biomacromolecules 5(3):895–902
Li Q, Ni W et al (2017) Targeting nasopharyngeal carcinoma by artesunate through inhibiting Akt/mTOR and inducing oxidative stress. Fundam Clin Pharmacol 31(3):301–310
Li C, Zhu B, Son YM, Wang Z, Jiang L, Xiang M, Ye Z, Beckermann KE, Wu Y, Jenkins JW, Siska PJ (2019) The transcription factor Bhlhe40 programs mitochondrial regulation of resident CD8+ T cell fitness and functionality. Immunity 51(3):491–507
Lichtenstein L, Ron Y et al (2015) Infliximab-related infusion reactions: systematic review. J Crohns Colitis 9(9):806–815
Lin YT, Wang HC et al (2017) Capsaicin induces autophagy and apoptosis in human nasopharyngeal carcinoma cells by downregulating the PI3K/AKT/mTOR pathway. Int J Mol Sci 18(7): 1343
Lionel ND, Mirando EH et al (1969) Levamisole in the treatment of ascariasis in children. Br Med J 4(5679):340–341
Long JZ, Svensson KJ, Bateman LA, Lin H, Kamenecka T, Lokurkar IA, Lou J, Rao RR, Chang MR, Jedrychowski MP, Paulo JA (2016) The secreted enzyme PM20D1 regulates lipidated amino acid uncouplers of mitochondria. Cell 166(2):424–435
Lu SY, Chen WJ et al (1998) Response to levamisole and low-dose prednisolone in 41 patients with chronic oral ulcers: a 3-year open clinical trial and follow-up study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 86(4):438–445
Luo M, Wang F et al (2020) Mitomycin C enhanced the efficacy of PD-L1 blockade in non-small cell lung cancer. Signal Transduct Target Ther 5(1):141
Machida H, Tominaga K et al (2012) Locoregional mitomycin C injection for esophageal stricture after endoscopic submucosal dissection. Endoscopy 44(6):622–625
Maichomo MW, Nginyi JM et al (2020) Efficacy of VERYL(R) in the treatment of cattle naturally infected with gastro-intestinal nematodes in Kenya. Trop Anim Health Prod 52(4):1699–1705
Malagelada JR, Cortot A (1978) H2-receptor antagonists in perspective. Mayo Clin Proc 53(3): 184–190
Marshall GS, Edwards KM et al (1987) Syndrome of periodic fever, pharyngitis, and aphthous stomatitis. J Pediatr 110(1):43–46
Mastrangelo A, Germinario BN, Ferrante M, Frangi C, Li Voti R, Muccini C, Ripa M (2020) Candidemia in COVID-19 patients: incidence and characteristics in a prospective cohort compared to historical non-COVID-19 controls. Clin Infect Dis 30:ciaa1594
Masuoka N, Yoshimine C, Hori M, Tanaka M, Asada T, Abe K, Hisatsune T (2019) Effects of anserine/carnosine supplementation on mild cognitive impairment with APOE4. Nutrients 11(7):1626
Masuoka N, Lei C, Li H, Hisatsune T (2021) Influence of imidazole-dipeptides on cognitive status and preservation in elders: a narrative review. Nutrients 13(2):397
Maurer E, Binder U, Sparber M, Lackner M, Caramalho R, Lass-Flörl C (2015) Susceptibility profiles of amphotericin B and posaconazole against clinically relevant mucorales species under hypoxic conditions. Antimicrob Agents Chemother 59(2):1344–1346
Menini S, Iacobini C, Fantauzzi CB, Pugliese G (2020) L-carnosine and its derivatives as new therapeutic agents for the prevention and treatment of vascular complications of diabetes. Curr Med Chem 27(11):1744–1763
Miller A, Harel D et al (1989) Cimetidine as an immunomodulator in the treatment of herpes zoster. J Neuroimmunol 22(1):69–76
Mlcochova P, Kemp S, Dhar MS, Papa G, Meng B, Mishra S, Whittaker C, Mellan T, Ferreira I, Datir R, Collier DA (2021) SARS-CoV-2 B. 1.617. 2 Delta variant emergence and vaccine breakthrough
Montoir D, Guillon R, Gazzola S, Ourliac-Garnier I, Soklou KE, Tonnerre A, Picot C, Planchat A, Pagniez F, Le Pape P, Logé C (2020) New azole antifungals with a fused triazinone scaffold. Eur J Med Chem 189:112082

Morcoss MM, El Shimaa MN, Ibrahem RA, Abdel-Rahman HM, Abdel-Aziz M, Abou El-Ella DA (2020) Design, synthesis, mechanistic studies and in silico ADME predictions of benzimidazole derivatives as novel antifungal agents. Bioorg Chem 101:103956

Moser D, Biere K, Han B, Hoerl M, Schelling G, Choukér A, Woehrle T (2021) COVID-19 impairs immune response to Candida albicans. Front Immunol 12:640644

Mozdzan M, Szemraj J, Rysz J, Nowak D (2005) Antioxidant properties of carnosine re-evaluated with oxidizing systems involving iron and copper ions. Basic Clin Pharmacol Toxicol 96(5): 352–360

Muraleedharan M, Panda NK, Angrish P, Arora K, Patro SK, Bansal S, Chakrabarti A, Rudramurthy SM, Bakshi J, Mohindra S, Gupta R (2022) As the virus sowed, the fungus reaped! A comparative analysis of the clinico-epidemiological characteristics of rhino-orbital mucormycosis before and during COVID-19 pandemic. Mycoses 65(5):567–576

Naderpoor N, Mousa A, de Courten M, Scragg R, de Courten B (2018) The relationship between 25-hydroxyvitamin D concentration and liver enzymes in overweight or obese adults: cross-sectional and interventional outcomes. J Steroid Biochem Mol Biol 177:193–199

Nagy G, Kiss S, Varghese R, Bauer K, Szebenyi C, Kocsubé S, Homa M, Bodai L, Zsindely N, Nagy G, Vágvölgyi C (2021) Characterization of three pleiotropic drug resistance transporter genes and their participation in the azole resistance of mucor circinelloides. Front Cell Infect Microbiol 11:660347

Nam W, Tak J et al (2007) Effects of artemisinin and its derivatives on growth inhibition and apoptosis of oral cancer cells. Head Neck 29(4):335–340

Nanke Y, Kamatani N et al (2008) Irsogladine is effective for recurrent oral ulcers in patients with Behcet's disease : an open-label, single-centre study. Drugs R D 9(6):455–459

Navarro M, Bellmunt J et al (1989) Mitomycin-C and vinblastine in advanced breast cancer. Oncology 46(3):137–142

Neubauer M, Zhu Z, Penka M, Helmschrott C, Wagener N, Wagener J (2015) Mitochondrial dynamics in the pathogenic mold Aspergillus fumigatus: therapeutic and evolutionary implications. Mol Microbiol 98(5):930–945

Nivoix Y, Ledoux MP, Herbrecht R (2020) Antifungal therapy: new and evolving therapies. Semin Respir Crit Care Med 41(1):158–174. Thieme Medical Publishers

O'Brien B, Chaturvedi S, Chaturvedi V (2020) In vitro evaluation of antifungal drug combinations against multidrug-resistant Candida auris isolates from New York outbreak. Antimicrob Agents Chemother 64(4):e02195–e02119

Pagano L, Cattaneo C, Quattrone M, Oberti M, Mazzitelli M, Trecarichi EM (2020) Isavuconazole—animal data and clinical data. J Fungi 6(4):209

Parham K, Leonard G et al (2013) Prospective clinical investigation of the relationship between idiopathic benign paroxysmal positional vertigo and bone turnover: a pilot study. Laryngoscope 123(11):2834–2839

Parsad D, Pandhi R et al (2001) Cimetidine and levamisole versus cimetidine alone for recalcitrant warts in children. Pediatr Dermatol 18(4):349–352

Parvathi Devi MK, Ramesh DNSV et al (2014) Efficacy of rebamipide and levamisole in the treatment of patients with recurrent aphthous ulcer—a comparative study. J Clin Diagn Res 8(11):ZC119–ZC122

Paulovičová L, Paulovičová E, Farkaš P, Čížová A, Bystrický P, Jančinová V, Turánek J, Pericolini E, Gabrielli E, Vecchiarelli A, Hrubiško M (2019) Bioimmunological activities of Candida glabrata cellular mannan. FEMS Yeast Res 19(2):foz009

Perez N, Martin E et al (2002) Benign paroxysmal positional vertigo in patients with Meniere's disease treated with intratympanic gentamycin. Laryngoscope 112(6):1104–1109

Pérez-Arques C, Navarro-Mendoza MI, Murcia L, Lax C, Martínez-García P, Heitman J, Nicolás FE, Garre V (2019) Mucor circinelloides thrives inside the phagosome through an Atf-mediated germination pathway. MBio 10(1):e02765–e02718

Pérez-Arques C, Navarro-Mendoza MI, Murcia L, Navarro E, Garre V, Nicolás FE (2020) A non-canonical RNAi pathway controls virulence and genome stability in Mucorales. PLoS Genet 16(7):e1008611

Pérez-Arques C, Navarro-Mendoza MI, Murcia L, Navarro E, Garre V, Nicolás FE (2021) The RNAi mechanism regulates a new exonuclease gene involved in the virulence of Mucorales. Int J Mol Sci 22(5):2282

Peridis S, Pilgrim G et al (2010) PFAPA syndrome in children: a meta-analysis on surgical versus medical treatment. Int J Pediatr Otorhinolaryngol 74(11):1203–1208

Peters V, Yard B, Schmitt CP (2020) Carnosine and diabetic nephropathy. Curr Med Chem 27(11): 1801–1812

Peyclit L, Yousfi H, Rolain JM, Bittar F (2021) Drug repurposing in medical mycology: identification of compounds as potential antifungals to overcome the emergence of multidrug-resistant fungi. Pharmaceuticals 14(5):488

Pfab C, Schnobrich L et al (2021) Repurposing of antimicrobial agents for cancer therapy: what do we know? Cancers (Basel) 13(13):3193

Prakash H, Chakrabarti A (2019) Global epidemiology of mucormycosis. J Fungi 5(1):26

Prakash H, Rudramurthy SM, Gandham PS, Ghosh AK, Kumar MM, Badapanda C, Chakrabarti A (2017) Apophysomyces variabilis: draft genome sequence and comparison of predictive virulence determinants with other medically important Mucorales. BMC Genomics 18(1):1–3

Rabson A, Blank S et al (1980) Effects of levamisole on in vitro suppressor cell function in normal humans and patients with systemic lupus erythematosus. Immunopharmacology 2(2):103–108

Ramanjulu JM, Pesiridis GS, Yang J, Concha N, Singhaus R, Zhang SY, Tran JL, Moore P, Lehmann S, Eberl HC, Muelbaier M (2018) Design of amidobenzimidazole STING receptor agonists with systemic activity. Nature 564(7736):439–443

Ramos-Alonso L, Romero AM, Martínez-Pastor MT, Puig S (2020) Iron regulatory mechanisms in Saccharomyces cerevisiae. Front Microbiol 11:582830

Roh JL, Lee YW et al (2006) Can mitomycin C really prevent airway stenosis? Laryngoscope 116(3):440–445

Roh JL, Kim EH et al (2017) Nrf2 inhibition reverses the resistance of cisplatin-resistant head and neck cancer cells to artesunate-induced ferroptosis. Redox Biol 11:254–262

Rokicki J, Li L, Imabayashi E, Kaneko J, Hisatsune T, Matsuda H (2015) Daily carnosine and anserine supplementation alters verbal episodic memory and resting state network connectivity in healthy elderly adults. Front Aging Neurosci 7:219

Romero AM, Ramos-Alonso L, Alepuz P, Puig S, Martínez-Pastor MT (2020) Global translational repression induced by iron deficiency in yeast depends on the Gcn2/eIF2α pathway. Sci Rep 10(1):1

Roostaei Firozabad A, Meybodi ZA et al (2021) Efficacy and safety of levamisole treatment in clinical presentations of non-hospitalized patients with COVID-19: a double-blind, randomized, controlled trial. BMC Infect Dis 21(1):297

Rosam K, Monk BC, Lackner M (2020) Sterol 14α-demethylase ligand-binding pocket-mediated acquired and intrinsic azole resistance in fungal pathogens. J Fungi 7(1):1

Rosenthal M (1978) A critical review of the effect of levamisole in rheumatic diseases other than rheumatoid arthritis. J Rheumatol Suppl 4:97–100

Rothan HA, Zhong Y, Sanborn MA, Teoh TC, Ruan J, Yusof R, Hang J, Henderson MJ, Fang S (2019) Small molecule grp94 inhibitors block dengue and Zika virus replication. Antivir Res 171:104590

Saadah LM, Deiab GA, Al-Balas Q, Basheti IA (2020) Carnosine to combat novel coronavirus (nCoV): molecular docking and Modeling to cocrystallized host angiotensin-converting enzyme 2 (ACE2) and viral spike protein. Molecules 25(23):5605

Saenz-Santamaria MC, Gilaberte Y (1997) Cimetidine and warts. Arch Dermatol 133(4):530–531

Sagers JE, Brown AS et al (2018) Publisher correction: computational repositioning and preclinical validation of mifepristone for human vestibular schwannoma. Sci Rep 8(1):17449

Sahasrabudhe DM, McCune CS et al (1987) Inhibition of suppressor T lymphocytes (Ts) by cimetidine. J Immunol 138(9):2760–2763
Sampson D, Lui A (1976) The effect of levamisole on cell-mediated immunity and suppressor cell function. Cancer Res 36(3):952–955
Sari S, Barut B, Özel A, Saraç S (2021) Discovery of potent α-glucosidase inhibitors through structure-based virtual screening of an in-house azole collection. Chem Biol Drug Des 97(3): 701–710
Scheinberg MA, Santos L et al (1978) Decreased lymphocyte response to PHA, Con-A, and calcium ionophore (A23187) in patients with RA and SLE, and reversal with levamisole in rheumatoid arthritis. Arthritis Rheum 21(3):326–329
Scheinfeld N, Rosenberg JD et al (2004) Levamisole in dermatology : a review. Am J Clin Dermatol 5(2):97–104
Schouten TJ, Bos JH et al (1981) Levamisole in the treatment of juvenile laryngeal papillomatosis. Int J Pediatr Otorhinolaryngol 3(4):365–369
Schwarz C, Brandt C, Melichar V, Runge C, Heuer E, Sahly H, Schebek M, Köster H, Bouchara JP, Biedermann T, Meißner P (2019) Combined antifungal therapy is superior to monotherapy in pulmonary scedosporiosis in cystic fibrosis. J Cyst Fibros 18(2):227–232
Seist R, Tong M et al (2020) Regeneration of Cochlear synapses by systemic administration of a bisphosphonate. Front Mol Neurosci 13:87
Senders CW (2004) Use of mitomycin C in the pediatric airway. Curr Opin Otolaryngol Head Neck Surg 12(6):473–475
Shafiei M, Peyton L, Hashemzadeh M, Foroumadi A (2020) History of the development of antifungal azoles: a review on structures, SAR, and mechanism of action. Bioorg Chem 104: 104240
Shapira T, Monreal IA, Dion SP, Buchholz DW, Imbiakha B, Olmstead AD, Jager M, Désilets A, Gao G, Martins M, Vandal T (2022) A TMPRSS2 inhibitor acts as a pan-SARS-CoV-2 prophylactic and therapeutic. Nature 605:340–348
Sharda N, Shashikanth MC et al (2014) Levamisole and low-dose prednisolone in the treatment of recurrent aphthous stomatitis. J Oral Pathol Med 43(4):309–316
Sharma M, Vanam HP, Panda NK, Patro SK, Arora R, Bhadada SK, Rudramurthy SM, Singh MP, Koppula PR (2022) Deciphering the neurosensory olfactory pathway and associated neo-immunometabolic vulnerabilities implicated in COVID-associated mucormycosis (CAM) and COVID-19 in a diabetes backdrop—a novel perspective. Diabetology 3(1):193–235
Shen Q, Naqvi NI (2021) Ferroptosis and microbial pathogenesis. PLoS Pathog 17(3):e1009298
Shen Q, Liang M, Yang F, Deng YZ, Naqvi NI (2020) Ferroptosis contributes to developmental cell death in rice blast. New Phytol 227(6):1831–1846
ShuoWang, Song Z et al (2021) Chloroform extract from Sophora Tonkinensis Gagnep. inhibit proliferation, migration, invasion and promote apoptosis of nasopharyngeal carcinoma cells by silencing the PI3K/AKT/mTOR signaling pathway. J Ethnopharmacol 271:113879
Silva DL, Lima CM, Magalhães VC, Baltazar LM, Peres NT, Caligiorne RB, Moura AS, Fereguetti T, Martins JC, Rabelo LF, Abrahão JS (2021) Fungal and bacterial coinfections increase mortality of severely ill COVID-19 patients. J Hosp Infect 113:145–154
Singh K, Kumar S, Shastri S, Sudershan A, Mansotra V (2022) Black fungus immunosuppressive epidemic with Covid-19 associated mucormycosis (zygomycosis): a clinical and diagnostic perspective from India. Immunogenetics 74(2):197–206
Sinha A, Israeli R, Cirigliano A, Gihaz S, Trabelcy B, Braus GH, Gerchman Y, Fishman A, Negri R, Rinaldi T, Pick E (2020) The COP9 signalosome mediates the Spt23 regulated fatty acid desaturation and ergosterol biosynthesis. FASEB J 34(4):4870–4889
Smith EB (1938) The buffering of muscle in rigor; protein, phosphate and carnosine. J Physiol 92(3):336
Soliman MS, El-Deriny MM, Ibrahim DS, Zakaria H, Ahmed Y (2021) The nematode trapping fungus Arthrobotrys oligospora Fresenius, a potential bio-control agent suppressing the root-knot nematode Meloidogyne incognita on tomato plants. J Appl Microbiol 131(5):2402–2415

Song J, Liu X, Zhai P, Huang J, Lu L (2016) A putative mitochondrial calcium uniporter in A. fumigatus contributes to mitochondrial Ca2+ homeostasis and stress responses. Fungal Genet Biol 94:15–22

Song J, Zhou J, Zhang L, Li R (2020) Mitochondria-mediated azole drug resistance and fungal pathogenicity: opportunities for therapeutic development. Microorganisms 8(10):1574

Stashower ME, Yeager JK et al (1993) Cimetidine as therapy for treatment-resistant psoriasis in a patient with acquired immunodeficiency syndrome. Arch Dermatol 129(7):848–850

Sturm L, Geißel B, Martin R, Wagener J (2020) Differentially regulated transcription factors and ABC transporters in a mitochondrial dynamics mutant can alter azole susceptibility of Aspergillus fumigatus. Front Microbiol 11:1017

Sun B, Dong Y, Lei K, Wang J, Zhao L, Liu M (2019) Design, synthesis and biological evaluation of amide-pyridine derivatives as novel dual-target (SE, CYP51) antifungal inhibitors. Bioorg Med Chem 27(12):2427–2437

Szcześniak D, Budzeń S, Kopeć W, Rymaszewska J (2014) Anserine and carnosine supplementation in the elderly: effects on cognitive functioning and physical capacity. Arch Gerontol Geriatr 59(2):485–490

Talaat HS, Kabel AM et al (2016) Reduction of recurrence rate of benign paroxysmal positional vertigo by treatment of severe vitamin D deficiency. Auris Nasus Larynx 43(3):237–241

Tanaka KI, Kawahara M (2020) Carnosine and lung disease. Curr Med Chem 27(11):1714–1725

Thomas E, Roman E, Claypool S, Manzoor N, Pla J, Panwar SL (2013) Mitochondria influence CDR1 efflux pump activity, Hog1-mediated oxidative stress pathway, iron homeostasis, and ergosterol levels in Candida albicans. Antimicrob Agents Chemother 57(11):5580–5599

Tomasz M, Palom Y (1997) The mitomycin bioreductive antitumor agents: cross-linking and alkylation of DNA as the molecular basis of their activity. Pharmacol Ther 76(1–3):73–87

Treviño-Rangel RD, Villanueva-Lozano H, Bonifaz A, Castañón-Olivares LR, Andrade A, Becerril-García MA, Martínez-Reséndez MF, Ayala-Gaytán J, Montoya AM, González GM (2021) Species distribution and antifungal susceptibility patterns of Aspergillus isolates from clinical specimens and soil samples in Mexico. Med Mycol 59(10):1006–1014

Tripathi SK, Feng Q, Liu L, Levin DE, Roy KK, Doerksen RJ, Baerson SR, Shi X, Pan X, Xu WH, Li XC (2020) Puupehenone, a marine-sponge-derived sesquiterpene quinone, potentiates the antifungal drug caspofungin by disrupting hsp90 activity and the cell wall integrity pathway. Msphere 5(1):e00818–e00819

Truong L, Zheng YM, Wang YX (2019) Mitochondrial Rieske iron-sulfur protein in pulmonary artery smooth muscle: a key primary signaling molecule in pulmonary hypertension. Arch Biochem Biophys 664:68–75

Tsou YA, Chang WC et al (2021) Metformin increases survival in hypopharyngeal cancer patients with diabetes mellitus: retrospective cohort study and cell-based analysis. Pharmaceuticals (Basel) 14(3):191

Ubell ML, Ettema SL et al (2006) Mitomycin-c application in airway stenosis surgery: analysis of safety and costs. Otolaryngol Head Neck Surg 134(3):403–406

Urrea AA, Velez GM et al (1995) Psoriasis in an HIV-positive patient treated with cimetidine. Dermatology 191(1):77

Uyaroglu OA, Guven GS et al (2020) Can levamisole be used in the treatment of COVID-19 patients presenting with diarrhea? J Infect Dev Ctries 14(8):844–846

Vahdatpour T, Nokhodchi A, Zakeri-Milani P, Mesgari-Abbasi M, Ahmadi-Asl N, Valizadeh H (2019) Leucine–glycine and carnosine dipeptides prevent diabetes induced by multiple low-doses of streptozotocin in an experimental model of adult mice. J Diabetes Invest 10(5): 1177–1188

Van Eygen M, Znamensky PY et al (1976) Levamisole in prevention of recurrent upper-respiratory-tract infections in children. Lancet 1(7956):382–385

Varshosaz J, Sarrami N et al (2019) LHRH targeted chonderosomes of mitomycin C in breast cancer: an in vitro/in vivo study. Anticancer Agents Med Chem 19(11):1405–1417

Vianna MF, Pelizza S, Russo ML, Toledo A, Mourelos C, Scorsetti AC (2020) ISSR markers to explore entomopathogenic fungi genetic diversity: implications for biological control of tobacco pests. J Biosci 45(1):1

Vincent AE, Turnbull DM, Eisner V, Hajnóczky G, Picard M (2017) Mitochondrial nanotunnels. Trends Cell Biol 27(11):787–799

Wang-Eckhardt L, Bastian A, Bruegmann T, Sasse P, Eckhardt M (2020) Carnosine synthase deficiency is compatible with normal skeletal muscle and olfactory function but causes reduced olfactory sensitivity in aging mice. J Biol Chem 295(50):17100–17113

White WB, Ballow M (1985) Modulation of suppressor-cell activity by cimetidine in patients with common variable hypogammaglobulinemia. N Engl J Med 312(4):198–202

White PL, Dhillon R, Cordey A, Hughes H, Faggian F, Soni S, Pandey M, Whitaker H, May A, Morgan M, Wise MP (2021) A national strategy to diagnose coronavirus disease 2019–associated invasive fungal disease in the intensive care unit. Clin Infect Dis 73(7):e1634–e1644

Williams TJ, Gonzales-Huerta LE, Armstrong-James D (2021) Fungal-induced programmed cell death. J Fungi 7(3):231

Xie Y, Hou W et al (2016) Ferroptosis: process and function. Cell Death Differ 23(3):369–379

Xu CF, Wang J (2015) Delivery systems for siRNA drug development in cancer therapy. Asian J Pharm Sci 10(1):1–2

Xu H, Su X, Guo MB, An R, Mou YH, Hou Z, Guo C (2020a) Design, synthesis, and biological evaluation of novel miconazole analogues containing selenium as potent antifungal agents. Eur J Med Chem 198:112360

Xu H, Yu W, Sun S, Li C, Zhang Y, Ren J (2020b) Luteolin attenuates doxorubicin-induced cardiotoxicity through promoting mitochondrial autophagy. Front Physiol 11:113

Yakobov N, Fischer F, Mahmoudi N, Saga Y, Grube CD, Roy H, Senger B, Grob G, Tatematsu S, Yokokawa D, Mouyna I (2020) RNA-dependent sterol aspartylation in fungi. Proc Natl Acad Sci U S A 117(26):14948–14957

Yan L, Khan RA (2021) Biological control of bacterial wilt in tomato through the metabolites produced by the biocontrol fungus, Trichoderma harzianum. Egypt J Biol Pest Control 31(1): 1–9

Yang S, Yan D, Li M, Li D, Zhang S, Fan G, Peng L, Pan S (2022) Ergosterol depletion under bifonazole treatment induces cell membrane damage and triggers a ROS-mediated mitochondrial apoptosis in Penicillium expansum. Fungal Biol 126(1):1–10

Zhang S, Ntasis E, Kabtni S, van den Born J, Navis G, Bakker SJ, Krämer BK, Yard BA, Hauske SJ (2016) Hyperglycemia does not affect iron mediated toxicity of cultured endothelial and renal tubular epithelial cells: influence of L-carnosine. J Diabetes Res 2016:8710432

Chapter 13
Repurposing of Immunomodulators for the Treatment of Cancer with QSAR Approaches

Rajiv K. Tonk, Vivek Yadav, and Ramesh K. Goyal

Abstract Natural products with immunomodulatory activity are widely used to treat many diseases, including autoimmune diseases, inflammatory disorders and other cancer treatments. Over the last decades, the immunomodulators agent increased their interest as the therapeutic agents against various conditions, including cancer diseases. Furthermore, they also provide inexpensive and less toxic extracts from the plants compared to other chemical poisonous chemotherapy and radiotherapy types of treatment. The advances in the computational field technology, methods such as QSAR studies, docking and receptor–drug interaction, provide better-promising drug repurposed options for cancer treatment with lesser side effects. The CADD studies enhance the repurposed drug uses with the detailed insight into biological mechanism understanding through the binding and interaction role with the natural target, which results in improved outcomes therapeutically. We have described the immunomodulatory mechanism associated with the cancer treatment involving the cytokines, adjuvants, checkpoints, and agonists and hypothesized restricting tumour development by interrupting the tumour cell cycle and promoting apoptosis. Among several therapeutic classes, immunomodulators have become a hot new area of research in oncology that is erupting with the activity, which is the main focus of this chapter.

Keywords QSAR · Chemotherapy · Repurposing · Immunomodulator

13.1 Introduction

The multiplication and development of abnormal or undesired cells causes a dangerous disease, i.e. cancer. Chemotherapy, radiotherapy, photodynamic therapy (PDT) and active immunotherapy comes among the most well-known therapies used for the treatment of various types of cancer. However, they had limited success

R. K. Tonk (✉) · V. Yadav · R. K. Goyal
Delhi Pharmaceutical Sciences and Research University, New Delhi, India
e-mail: rajiv.tonk@dpsru.edu.in

R. C. Sobti et al. (eds.), *Drug Repurposing for Emerging Infectious Diseases and Cancer*, https://doi.org/10.1007/978-981-19-5399-6_13

due to their destructive effect not only on cancer cells but also on surrounding normal cells, as well as severe side effects and immunosuppressive activity. During the last decades, there is an increasing interest towards the recent advancement techniques like computational methods involving QSAR studies, docking, receptor–drug interaction to provide better promising drug for the cancer treatment with lesser side effects. The main problem associated with the cancer treatment is the adverse side effects towards neighbouring cells and associated organs. The CADD study might be useful in the future for building more effective and safe anticancer medications by reducing unwanted effects and increasing therapeutic efficacy. This chapter deals with the anticancer properties of immunomodulator drugs, which are suppressive or promote immune system utilizing QSAR techniques.

13.2 Immunotherapy as Anticancer

Immunomodulator drugs are a form of immunotherapy that boosts the immune system's ability to fight cancer. The mechanism of immunotherapy as an anticancer treatment is vague; however, natural immunomodulators are hypothesized to be able to restrict tumour development by interrupting the tumour cell cycle and promoting apoptosis (shown in Fig. 13.1). Moreover, the biological immune modulator drugs, cytokines and bacterial-induced derivatives like BCG and other natural products are involved in the treatment approaches. Interferons (INFs) and interleukins (ILs), produced by white blood cells, play a crucial part in our body's regular immune responses as well as the immune system's capacity to respond to cancer cells.

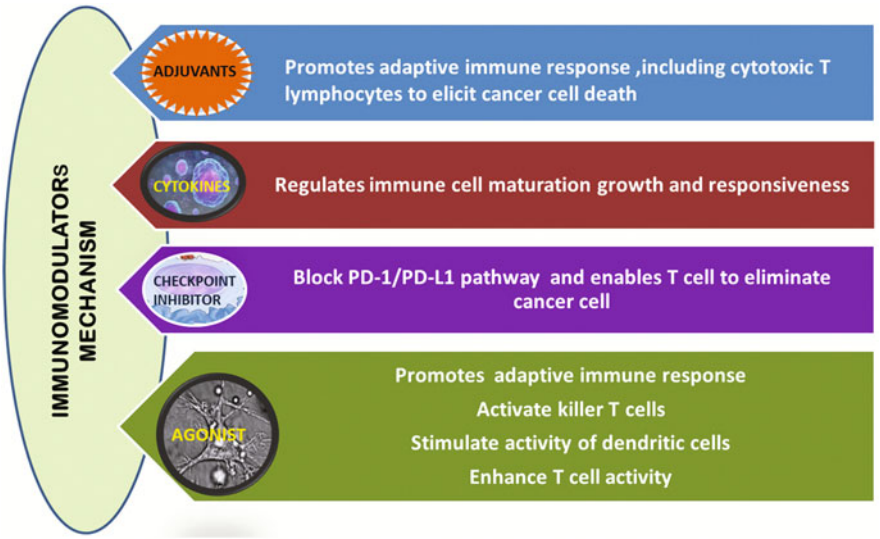


Fig. 13.1 Immunotherapy mechanism

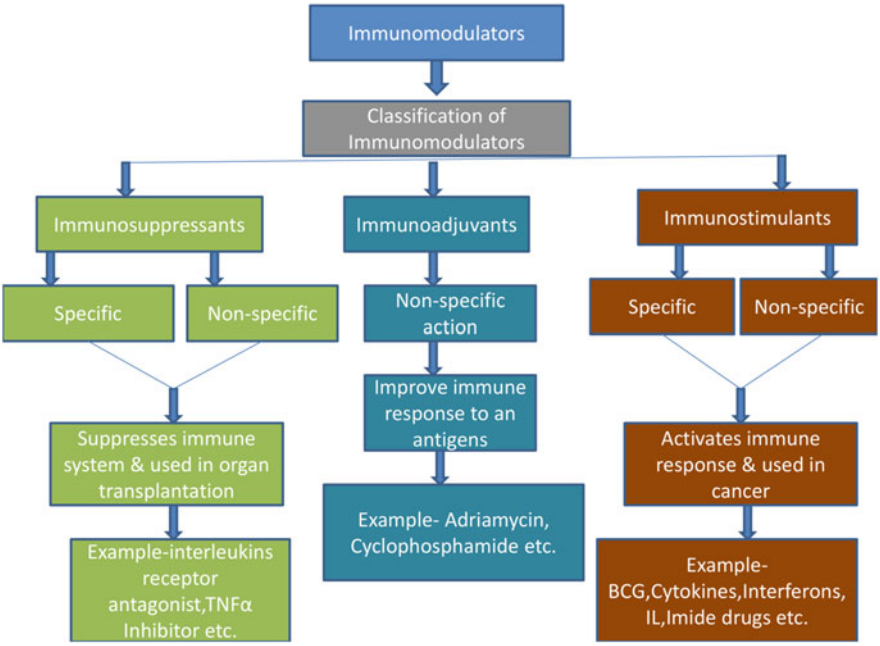


Fig. 13.2 A typical classification of immunomodulators

Interferon, also known as INF-alfa, improves the immune response to cancer cells by enabling particular white blood cells, such as natural killer cells and dendritic cells.

Hematopoietic growth factors (HGFs) are cytokines that encourage the formation of blood cells that have been damaged by chemotherapy, therefore minimizing the side effects of cancer treatment. Erythropoietin, which improves red blood cell synthesis, IL-2, which increases platelet formation, and Granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF), both of which enhance white blood cell production and hence reduce infections. G-CSF and GM-CSF can boost the immune system's anticancer response by boosting the number of cancer-fighting T cells. The Bacillus–Calmette–Guerin (BCG) is used to treat bladder cancer through stimulating an immune response against cancer cells when injected into the bladder with a catheter (Fig. 13.2).

13.3 Prospects for Repurposing Drugs for Cancer Treatment

In light of the numerous linked elements that contribute to the failure of cancer treatment, drug repurposing is a viable method. Repurposed drugs for cancer can expedite the first phases of drug development as tolerability studies and

pharmacokinetic/pharmacodynamic (PK/PD) screening have been undertaken during assessment for other medical disorders (Lee and Bhakta 2021). Repurposing is extremely desirable in view of the narrowing new therapeutic development pipeline due to the potential for lower financial expenditures and speedier implementations of novel treatment regimens.

The earliest successful examples of drug repurposing were mostly the product of chance discoveries, but systematic methodologies for identifying non-oncology pharmaceuticals with the possibility for repositioning in cancer therapy evolved (De Lellis et al. 2021; Pushpakom et al. 2018; Zhang et al. 2020). Such methodologies can be classified as: computational and experimental techniques (Shim and Liu 2014). The former technique makes use of high-throughput studies in conjunction with bioinformatics methods (such as pathway/network mapping, QSAR, signature matching, molecular docking, pharmacophore) or it makes use of patients' electronic health records (EHRs) through systematic evaluation, for example retrospective clinical analyses. The latter is more activity-based and utilizes binding assays (such as proteomics and chemical genetic approaches) to look for relevant interactions between new targets and known drugs, or cell-based phenotypic testing based on the choice of prevalent phenotypic criteria (such as proliferation, exosome biogenesis modulation, cell cycle profiling) without previous understanding of the target influence.

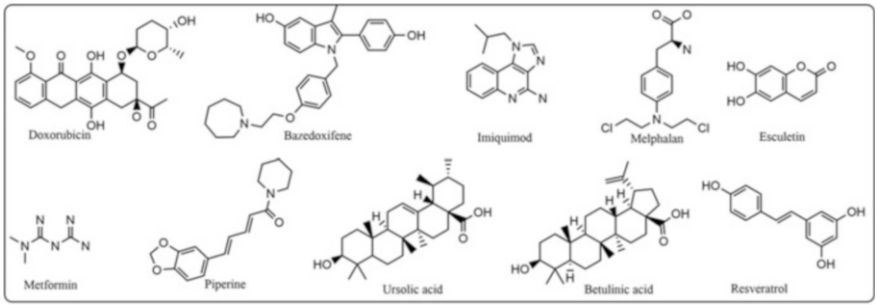
A number of drugs used in non-oncology treatments have been effectively repurposed for use in cancer treatment at the present time. Doctors used to prescribe pregnant women thalidomide, which was supposed to help them get over their morning sickness, but it was taken off the market because it had bad effects on babies. Then, it was discovered that erythema nodosum leprosum (ENL) could be used for the treatment. In 2006, it was approved as a first-line treatment for elderly patients with advanced multiple myeloma who was also taking melphalan and prednisone (Palumbo et al. 2006). Similarly, the beta-blocker propranolol was widely used off-label to treat infantile haemangiomas (IHs) until being approved by the FDA in 2014 as a novel oral paediatric composition for IH therapy. Another example is raloxifene, a selective oestrogen receptor modulator (SERM) that was initially recommended to treat postmenopausal women's osteoporosis. This medicine was effectively repurposed and approved by the FDA in 2007 to treat postmenopausal women with ER+/PR+ advanced breast cancer after retrospective analyses of clinical trial data (Aggarwal et al. 2021). Compounds of the artemisinin (ARS) class have superior antimalarial activity with few adverse effects and drug resistance (Efferth and Oesch 2021). Additionally, it has been shown that ARS and its derivatives function in vitro and in vivo against a variety of tumour types, including acute leukaemia. As a result, ARS-type chemicals may be ideal for leukaemia therapeutic repurposing.

These instances show that developing, commercializing, and clinically accepting low-cost non-oncology drugs for cancer treatment represents a considerable possibility, particularly for difficult-to-treat, uncommon cancers. Among several classes of therapeutics, immunomodulators have now become a hot new area of research in

the field of oncology that are erupting with activity which is the main focus of this chapter.

13.4 Natural Derivatives as a Source of Immunomodulator

Natural products such as plants include bioactive chemicals that have anticancer and immunomodulatory properties while posing little danger of cytotoxicity and adverse effects. The natural plants showed potential role as anticancer through immunomodulation such as boswilic acid, phyllanthus, mushroom and other natural products.



13.4.1 Maslinic Acid

A field-based 3D-QSAR model which is a virtual screening tool utilized for the identification of anticancer agents, with the goal of reducing the number of clinical trials required for the drug development process. This 3D-QSAR model defined molecular information and structure-activity areas like average shape, hydrophobic regions and electrostatic patterns to virtually screen potential analogues for the triterpene maslinic acid and its analogues. P-902 was shown to be a good maslinic acid analogue, and it has cytotoxic/anticancer action against the MCF7 breast cancer cell line (Alam and Khan 2017) (Fig. 13.3).

The red colour circle denotes electrostatic field spots that are responsible for the expected activity drop. The purple colour triangles represent steric field points that regulate the projected activity rise, whereas the blue colour squares represent electrostatic field points that control the predicted activity increase.

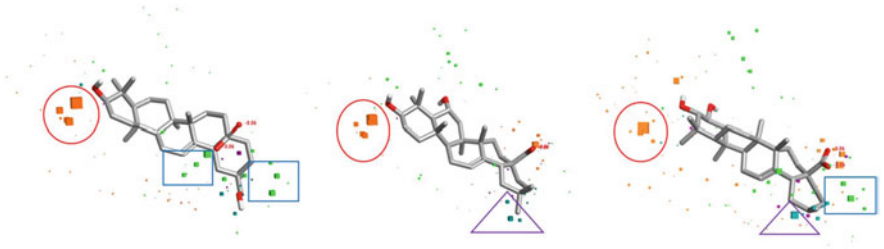


Fig. 13.3 Molecular SAR mechanisms of maslinic acid derivatives, which show how different shapes of fields contribute to their predicted activity (Alam and Khan 2017)

13.4.2 Mushroom Species

Numerous mushroom species, edible and toxic, have bioactive chemicals that are beneficial to human health are used as immunomodulator. Two critical chemicals are found in mushroom cell walls: chitin and β-glucans. Among them, β-glucans $(1 \rightarrow 3)$, $(1 \rightarrow 4)$, and $(1 \rightarrow 6)$ contribute to the mushroom's importance in terms of health and therapy of various ailments. It has been discovered that more than 50 mushroom species contain potential immunotherapeutics that exhibit immunomodulatory and anticancer effects in vitro and animal models, as well as in human tumours. They activate lymphocytes, natural killer cells and macrophages; increase cytokine production; reduce cancer cell growth; boost apoptosis and limit angiogenesis, in addition to being cytotoxic to cancer. The mushrooms have been linked to the therapy of a variety of cancers, including breast, colon, cervical, skin, liver, ovarian, bladder, prostate, gastric, skin, lung, leukaemia and stomach cancers, among others. The activities of mushroom constituents result in the death of cancer cells, the arrest of the cell cycle, and the prevention of angiogenesis and metastasis (Ayeka 2018) (Table 13.1).

13.4.3 Curcumin

Curcumin is a polyphenol derived from the rhizomes of the turmeric plant, Curcuma longa, that possesses anti-inflammatory and anticancer activities. Curcumin is found in high concentrations in turmeric. Curcumin exerts anticancer activity through regulating a variety of immune modulators, including cytokines, cyclooxygenase-2 (COX-2) and reactive oxygen species (ROS). Additionally, it is involved in the downregulation of growth factors, protein kinases, oncogenic molecules and a variety of signalling pathways, including nuclear factor kappa-light-chain-enhancer of activated B cells (NF-B), c-Jun N-terminal kinase (JNK) and signal transducer and activator of transcription 3 (STAT3) signalling.

Curcumin binds directly to ROS scavengers, perhaps slowing cancer growth and metastasis. NF-B regulates and controls the expression of cytokines and interferons.

Table 13.1 List of drugs repurposed on different targets for anticancer activity

Repurpose drugs	Mechanism	Target	Type of cancer	PDB id
Adriamycin	Stimulation of macrophages leading to stimulation of T cells and inhibition of T suppressor cell	Alpha-1-antichymotrypsin	Breast cancer	6FTP
Phenylalamine mustard (Melphalan)	Can induce the release of pro-inflammatory cytokines and growth factors, deplete regulatory T cells, and create space to facilitate the expansion of infused tutor-reactive T cells	Trypanothione reductase (oxidized form) (protein)	Multiple myeloma Ovarian cancer Neuroblastoma Rhabdomyosarcoma Breast cancer	3GRT
Vinca alkaloids	VLB preferentially affected T cells whereas VCR preferentially affected B cells functions	Stathmin-4 (protein) Tubulin alpha chain (protein) Tubulin beta chain (protein)	Non-small cell lung cancer, acute leukaemia, neuroblastoma, Wilms' tumour	7A69
Bazedoxifene	Inhibited the production of a tumour vaccine-induced macrophage suppressor cell	Estrogen receptor (protein)	Colon cancer	6PSJ
Imide drugs	Inhibit production of tumour necrosis factor, interleukin 6 and VEGF co stimulates T cells and NK cells and interleukin 2 production	Protein cereblon	Multiple myeloma (MM)	4CL1,4CL2
Imiquimod	Increased mRNA in cell lysates and/or cytokine levels in supernatants of IFN-, interleukin (IL)-1, IL-6 and tumour necrosis factor (TNF)	Protein cereblon	Multiple myeloma (MM)	5LBT
Curcumin	Acts on cytokines, cyclooxygenase-2 (COX-2), and reactive oxygen species (ROS)	NADP-dependent oxidoreductase and curcumin synthase	Prostate cancer	5ZTN
Metformin	Enhancement of CD8+ T lymphocytes expands and transform into effector cytotoxic T lymphocytes (CTL) which targets cancer	ATP-binding cassette sub-family C member 8 isoform X2 (protein) ATP-sensitive inward rectifier potassium channel 11	Ovarian cancer	5G5J
Piperine		Acetylcholinesterase	Breast, lung, prostate, cervical	6LZL

(continued)

Table 13.1 (continued)

Repurpose drugs	Mechanism	Target	Type of cancer	PDB id
	Combination with gamma-aminobutyric acid (GABA) mediated p38 and JNK MAPK activation			
6-Gingerol	Act as an antioxidant, anti-inflammatory, anti-angiogenesis and antiproliferative	COX-2, NF-κB, AP-1, iNOS, VEGF, Bcl-2, Bax, MMP-9	Cervical cancer cell (HeLa, CaSki and SiHa), colon cancer cell line (HCT 116)	

Both inflammation and cancer progression share these proteins. Curcumin inhibits the NF-B-dependent pathway, causing tumour suppression and death. Cytokines are immune system regulators that affect leucocyte proliferation, survival, differentiation, and death. Curcumin inhibits the interaction of nuclear proteins to interleukins or interferons, reducing the expression of pro-inflammatory cytokines (Giordano and Tommonaro 2019).

13.4.4 Piperine

Piperine's immunomodulatory potential has been demonstrated and it is reported that incubation of tumour cell lines with 5-fluorouracil (5-FU) inhibited the growth as measured by decreased IC_{50} values for 5-FU. Combined usage of piperine and 5-FU reduced leucopenia, indicating better immunocompetence limited by 5-FU. According to Bernardo and co-workers, piperine inhibits the proliferative response induced by lipopolysaccharide (LPS) and immunoglobulin—IgM antibody in vitro (Sunila and Kuttan 2004).

13.4.5 Cardamom (Elettaria Cardamomum)

Cardamom is a spice manufactured from the seeds of plants in the Zingiberaceae family, Elettaria and Amomum. Cardamom aqueous extracts greatly increase splenocyte proliferation in a dose-dependent, synergistic manner. Cardamom increases and decreases splenocyte T helper 1 (Th1) cytokine output in enzyme-linked immunosorbent assays. Cardamom, on the other hand, significantly reduces and increases splenocyte Th2 cytokine secretion. Cardamom extracts dramatically increase the cytotoxic activity of natural killer cells, indicating possible anticancer properties (Majdalawieh and Carr 2010).

13.4.6 Gingerol

It is derived from Zingiber officinalis (ginger) and has antioxidant, anti-inflammatory, antiangiogenesis and antiproliferative effects (Mohamed et al. 2017).

13.4.7 Adriamycin

There are many malignancies that can be treated with Adriamycin. This drug is also used to treat non-Hodgkin lymphoma and certain leukaemias. The

immunomodulatory effects that could be used therapeutically include stimulating macrophages, which may stimulate T cells, and inhibiting T suppressor cells. It's worth noting that when the treatment generates macrophages, perhaps M-1 macrophages, this appears to result in tumour suppression, in contrast to the macrophage- and inflammation-induced tumour-promoting effects frequently established with M2 macrophages under various settings (Mihich 2007).

13.4.8 Imide Drugs

They are chemotherapeutic agents that inhibit molecular pathways involved in tumour formation and secondary spread. The most well-known member is thalidomide. Thalidomide is a sedative and antinausea medication. The FDA has repurposed thalidomide twice. It was first approved in 1998 for leprosy treatment and then in 2006 for MM therapy. The amino group on the phthaloyl ring of lenalidomide and pomalidomide was added to thalidomide to boost anticancer effectiveness and efficacy while reducing negative effects. Their anticancer activity has been linked to several pathways (Dinić et al. 2020). Thalidomide and its derivatives compete with CD147 and MCT1 for CRBN binding, allowing them to exert a wide range of anticancer properties. Thorazine appears to have mechanisms underpinning immunomodulatory, antiproliferative and antiangiogenic effects, and CRBN appears to play an important role in all of these activities. Thalidomide was initially investigated as a potential anticancer drug because of its putative antiangiogenic properties.

QSAR of Thalidomide: Thalidomide and its derivatives have showed therapeutics ability to inhibit angiogenesis for suppression of the tumour cell growth. Leeper E.R. et al. have used a series of 39 compounds based on its metabolites to generate a 3D QSAR study utilizing the CoMFA and CoMSIA methods. Both models have provided useful insights into the structural requirements for activity of thalidomide analogues as angiogenesis inhibitors. The compound 6 displays the best inhibition activity due to (a) presence of glutarimide ring, (b) presence of hydrophobic amino acids in the receptor around the bulky group, (c) presence of positive and negative charged groups on the ligand and (d) presence of hydrogen bond acceptor on receptor near the positively charged group (Fig. 13.4). Thus, based on such features further molecules can be optimized with improved inhibition activity.

Thalidomide is reported to be a multi-target drug where its activities are reported as inhibition of peptidases, glucosidases, androgen receptors, COX, TNF-alpha, NF-kB, etc. (PMID: 25942060, 18389516). Additionally, SAR investigations demonstrated that phthalimide is a critical pharmacophore fragment, with N-phenylphthalimides of particular interest due to their inhibitory activity against TNF-alpha, COX, and tubulins. Typically, they have been used in the development of anti-inflammatory, immunomodulatory, antiangiogenic and anticancer therapeutic candidates. Cardoso et al. worked on novel phthalimide derivatives with anticancer and immunomodulatory activities in order to investigate molecular hybridization

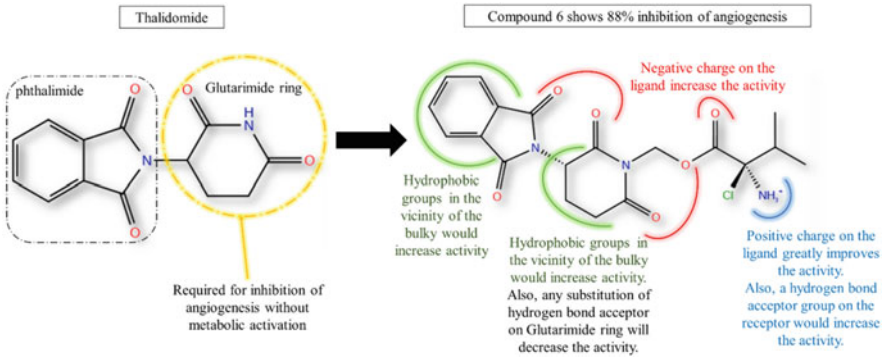


Fig. 13.4 A pictorial representation of Thalidomide derivative, compound 6 with predicted structure activity correlations from CoMFA and CoMSIA methods

and bioisoterism techniques between the thalidomide, thiosemicarbazone, thiazolidinone and thiazole derivatives. They evaluated that the immunosuppressive and antiproliferative efficacy results against TNFa, IFNg, IL-2 and IL-6 production (Fig. 13.5). Their compounds show potential inhibition activity in both in-vitro and in-silico studies as they show high cytotoxicity levels in human cancer cell lines, with more affinity towards IKKbeta protein.

Imiquimod—(TLR-7 agonist, immune response modifier) Imiquimod (Aldara, R-837, S-26308) is a low-molecular-weight molecule that belongs to a novel class of chemicals known as imidazoquinolinamines. It has been demonstrated to have immune response modulating capabilities both in vitro and in vivo, as well as antiviral and antitumour action through the synthesis of endogenous cytokines in the body. IFN-, interleukin (IL)-1, interleukin (IL)-6 and tumour necrosis factor-alpha (TNF-alpha) mRNA levels in cell lysates and/or cytokine levels in supernatants of nonhuman or human monocytes treated with imiquimod have been reported in in vitro studies using nonhuman or human monocytes treated with imiquimod (TNF). It was shown that the IFN-fold induction was larger than that of other cytokines when compared to LPS or viral stimulation (Chi et al. 2017).

13.4.9 Metformin

Metformin is a medication that is used to treat type 2 diabetes. Since 2005, when it was initially reported to reduce the incidence of cancer in diabetics, a vast number of preclinical and clinical studies have highlighted its potential function as a preventative and adjuvant therapy for a wide range of cancers. Currently, there is no FDA-approved treatment for this condition.

As previously stated, the immune system plays a significant role in the prevention and control of cancer, as well as in the interaction with metabolic and inflammatory pathways. Metformin, like other cancer immunostimulants, acts in a multifaceted

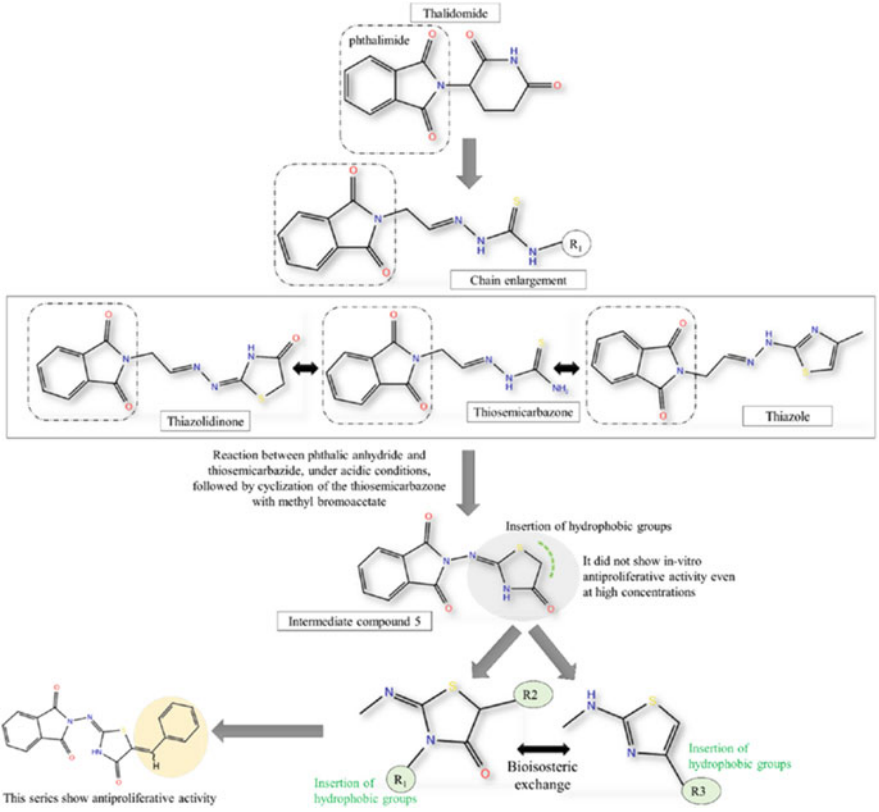


Fig. 13.5 A pictorial representation of phthalamides with dual immunomodulatory and antiproliferative activities

manner to bolster immunity against cancer, affecting almost every aspect of the immune system, particularly in relation to cancer immunity. Metformin enhances CD8+ T lymphocytes and recovers them from depletion. These cells can grow and change into effector cytotoxic T lymphocytes (CTL) that target malignancy. Rescue of tired CD8+ T cells has been demonstrated in leukaemia, melanoma, renal cell, NSCLC, gastrointestinal and breast cancers in vitro. Also, metformin-induced AMPK activation enhances immunological checkpoint programmed death ligand 1 (PD-L1) degradation, allowing CTL-mediated tumour cell killing. Metformin can also boost local and systemic cytokine responses to malignancies (Chang 2021).

13.4.9.1 QSAR Approaches

Bioactivity and three-dimensional (3D) structure of molecules are linked in SAR research. Once a hit's structure is known, its biological effects are predicted using

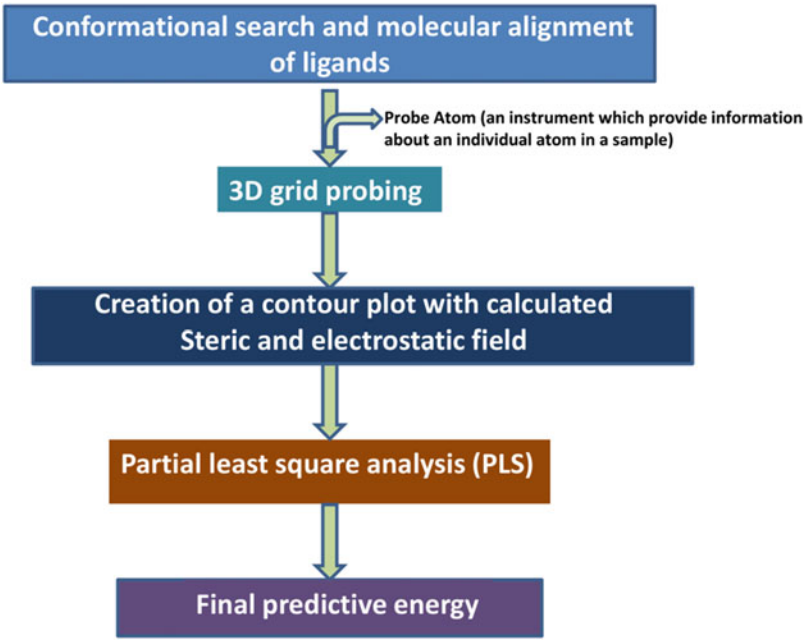


Fig. 13.6 A general workflow of 3D-QSAR

data from other similar drugs. Molecular structure can influence physical and biological qualities. Binding site interactions are identified using computational chemistry and molecular modelling technologies. SAR is vital in drug discovery and development. It is used to discover new compounds and assess the health risks of existing ones. For example, SAR analysis can identify which chemical groups are crucial in evoking an organism's desired activity. This allows for logical modification of a bioactive compound's action or potency by adding or removing chemical groups (SAR n.d.). The most sensitive toxicological endpoints like carcinogenicity or cardiotoxicity may be used in risk assessment.

A subset of SAR, the quantitative SAR (QSAR) model, is used to estimate the activity of molecules by linking a collection of 'predictor' factors to the response variable's potency. Rather than simply labelling structures as 'active' or 'inactive', this novel approach enables researchers to foresee their biological activity or potency. An approach for predicting the physical and biological characteristics of tiny molecules is known as QSAR (quantitative structure–activity relationship). The three pillars that QSAR is built on are biological data, chemical understanding and modelling techniques. QSAR is the end product of computer processes that begin with a sufficient description of molecular structure and conclude with inferences, hypotheses and predictions about the behaviour of molecules in the environmental, physicochemical and biological systems under investigation. QSAR computations result in a collection of mathematical equations that link chemical structure to biological activity. The general workflow of this approach is depicted in Fig. 13.6.

3D QSAR is defined as the method in which the three-dimensional properties of a molecule are considered as a whole rather than by considering individual substituents or moieties. The 3D QSAR philosophy is based on the assumption that the most important features about a molecular if these features can be defined, then it is possible to study how they affect biological properties. There are several approaches to 3D QSAR but the most important ascendancy gained by CoMFA (comparative molecules field analysis), due to its overall size and shape and its electronic properties (Patrick 2011). The various 3D-QSAR methods used for the chemometric techniques are comparative molecular field analysis (CoMFA), comparative molecular similarity indices analysis (COMSIA), comparative molecular movement analysis (COMMA), adaptation of field for molecular comparison (AFMoC), genetically evolved receptor models GERM) and self-organizing molecular field analysis (SoMFA).

References

Aggarwal S, Verma SS, Aggarwal S, Gupta SC (2021) Drug repurposing for breast cancer therapy: old weapon for new battle. Semin Cancer Biol 68:8–20. https://doi.org/10.1016/j.semcancer.2019.09.012

Alam S, Khan F (2017) 3D-QSAR studies on maslinic acid analogs for anticancer activity against breast cancer cell line MCF-7. Sci Rep 7:1–13. https://doi.org/10.1038/s41598-017-06131-0

Ayeka PA (2018) Potential of mushroom compounds as immunomodulators in cancer immunotherapy: a review. Evid Based Complement Altern Med 2018:7271509. https://doi.org/10.1155/2018/7271509

Chang R (2021) Repurposing of metformin as a multifaceted and multitasking preventative and treatment for cancer, repurposed drugs for cancer [working title]. https://doi.org/10.5772/intechopen.96101

Chi H, Li C, Zhao FS, Zhang L, Ng TB, Jin G, Sha O (2017) Anti-tumor activity of toll-like receptor 7 agonists. Front Pharmacol 8:1–10. https://doi.org/10.3389/fphar.2017.00304

De Lellis L, Veschi S, Tinari N, Mokini Z, Carradori S, Brocco D, Florio R, Grassadonia A, Cama A (2021) Drug repurposing, an attractive strategy in pancreatic cancer treatment: preclinical and clinical updates. Cancers (Basel) 13:1–39. https://doi.org/10.3390/cancers13163946

Dinić J, Efferth T, Garcia-Sosa AT, Grahovac J, José M (2020) This is the peer reviewed version of the following article: Dinić J, Efferth T, García-Sosa AT, Grahovac J, Padrón JM, Pajeva I, Rizzolio F, Saponara S, Spengler G, Tsakovska I. Repurposing old drugs to fight multidrug resistant cancers. Drug Resist Updat. © 2020 Elsevier Ltd

Efferth T, Oesch F (2021) The immunosuppressive activity of artemisinin-type drugs towards inflammatory and autoimmune diseases. Med Res Rev 41:3023–3061. https://doi.org/10.1002/med.21842

Giordano A, Tommonaro G (2019) Curcumin and cancer. Nutrients 11:2376. https://doi.org/10.3390/nu11102376

Lee C, Bhakta S (2021) The prospect of repurposing immunomodulatory drugs for adjunctive chemotherapy against tuberculosis: a critical review. Antibiotics 10:91. https://doi.org/10.3390/antibiotics10010091

Majdalawieh AF, Carr RI (2010) In vitro investigation of the potential immunomodulatory and anticancer activities of black pepper (Piper nigrum) and cardamom (Elettaria cardamomum). J Med Food 13:371–381. https://doi.org/10.1089/jmf.2009.1131

Mihich E (2007) Anticancer drug-induced immunomodulation and cancer therapeutics. Curr Cancer Ther Rev 3:174–193. https://doi.org/10.2174/157339407781368350

Mohamed SIA, Jantan I, Haque MA (2017) Naturally occurring immunomodulators with antitumor activity: an insight on their mechanisms of action. Int Immunopharmacol 50:291–304. https://doi.org/10.1016/j.intimp.2017.07.010

Palumbo A, Bringhen S, Caravita T, Merla E, Capparella V, Callea V, Cangialosi C, Grasso M, Rossini F, Galli M, Catalano L, Zamagni E, Petrucci MT, De Stefano V, Ceccarelli M, Ambrosini MT, Avonto I, Falco P, Ciccone G, Liberati AM, Musto P, Boccadoro M (2006) Oral melphalan and prednisone chemotherapy plus thalidomide compared with melphalan and prednisone alone in elderly patients with multiple myeloma: randomised controlled trial. Lancet 367:825–831. https://doi.org/10.1016/S0140-6736(06)68338-4

Patrick GL (2011) An introduction to medicinal chemistry, 4th edn (2011)

Pushpakom S, Iorio F, Eyers PA, Escott KJ, Hopper S, Wells A, Doig A, Guilliams T, Latimer J, McNamee C, Norris A, Sanseau P, Cavalla D, Pirmohamed M (2018) Drug repurposing: progress, challenges and recommendations. Nat Rev Drug Discov 18:41–58. https://doi.org/10.1038/nrd.2018.168

SAR and QSAR models—creative biolabs. n.d.. https://www.creative-biolabs.com/drug-discovery/therapeutics/sar-and-qsar-models.htm

Shim JS, Liu JO (2014) Recent advances in drug repositioning for the discovery of new anticancer drugs. Int J Biol Sci 10:654–663. https://doi.org/10.7150/ijbs.9224

Sunila ES, Kuttan G (2004) Immunomodulatory and antitumor activity of Piper longum Linn. and piperine. J Ethnopharmacol 90:339–346. https://doi.org/10.1016/j.jep.2003.10.016

Zhang Z, Zhou L, Xie N, Nice EC, Zhang T, Cui Y, Huang C (2020) Overcoming cancer therapeutic bottleneck by drug repurposing. Signal Transduct Target Ther 5:113. https://doi.org/10.1038/s41392-020-00213-8

Chapter 14
Reverse Translational Approach in Repurposing of Drugs for Anticancer Therapy

Geeta Aggarwal, Pankaj Musyuni, Bharti Mangla, and Ramesh K. Goyal

Abstract Cancer treatment costs are rising due to high expense of clinical trials and a low success rate of around 12%. This necessitates cost-effective and safe anticancer medications. Repurposing of existing drugs may be beneficial in alleviating current medicine scarcity issues in anticancer therapy. It is conceivable to expedite the drug repurposing process by utilising non-oncology therapies, which account for the vast majority of treatments. Drug repurposing is the notable example of reverse translation, wherein patients' 'real-life' experiences in a clinical trial are reverse translated in order to determine the molecular roots of these experiences and other examination manifestations. The Food & Drug Administration, the World Health Organization's list of essential medications and the National Institutes of Health have endorsed this concept. Repurposing, according to on-going research in this field, could lead to the discovery of new anticancer drugs. Throughout this chapter, an overview of drug repositioning for anticancer applications, with a specific emphasis on activity-based drug repositioning of non-cancer medicines is provided. Several effective case studies of drug repurposing based on reverse translational research and their strategies have also been reviewed.

Keywords Reverse translational research · Drug repurposing · Anticancer drugs · Drug discovery · Precision medicine · Innovation

14.1 Introduction

Cancer has long been cited as a significant global health concern. It is a deadly disease that is the second largest cause of mortality in the world. Cancer is defined by the National Cancer Institute (NCI) as a spectrum of illnesses in which abnormal cells divide and spread to surrounding tissue (National Cancer Institute (NCI) 2021). As per the World Health Organization (WHO), cancer is expected to kill one out of every six individuals in the world each year. The survey found that over 9.6 million

G. Aggarwal (✉) · P. Musyuni · B. Mangla · R. K. Goyal
Delhi Pharmaceutical Sciences and Research University, New Delhi, India

R. C. Sobti et al. (eds.), *Drug Repurposing for Emerging Infectious Diseases and Cancer*, https://doi.org/10.1007/978-981-19-5399-6_14

individuals died from cancer every year from 2018. The American Cancer Society predicted more than 18 lakh new cancer cases and 6 lakh cancer in 2020. Every day in the United States, more than 5000 new cases and 1600 deaths are reported (World Health Organization (WHO) 2021).

Cancer is treated with surgery and other therapies such as targeted radiation, hormone therapy, stem cell transplant, immunotherapy, cytotoxic chemotherapy and precision medicine. Treatment options are usually determined by the stage of cancer and progression rate. Resistance to medications, whether traditional chemotherapeutic agents or innovative targeted drugs, is a hurdle and a key challenge in cancer therapies, despite multiple attempts. The mode of action of numerous anticancer chemotherapeutic agents is geared toward direct DNA damage, resulting in non-specificity and increased toxicity (Arruebo et al. 2011; Falzone et al. 2018).

The technological advances and a better understanding of the disease itself, the development of new treatments for human neoplastic illness is now possible. Bioinformaticians, data scientists and computational biologists are able to analyse large-scale studies, mine databases, review clinical data, validate findings and enhance treatment options for cancer patients (Falzone et al. 2018). On the other hand, these novel drugs have taken significantly longer time than predicted to enter clinical testing. At least 13–15 years are required to develop a new medicine. Preclinical animal studies are also required to assess medication efficacy, toxicity, pharmacokinetics and pharmacodynamics in human subjects. The cost of transporting a single innovative medicine from the laboratory to the patient's bedside is close to $3 billion (Andrade et al. 2016). Novel compounds rarely make it to the general market, despite massive investments. Newer pharmaceutical companies have given up hope of producing new medications because of this situation.

The repurposing or repositioning of drugs for cancer therapy is a contentious issue in the scientific community presently. Repurposing is driven by the high expense of developing a new medication, as well as the time it takes to determine its safety and specificity. New cancer drugs could take years or decades to complete clinical trials and get Food and Drug Authority (FDA) approval before they are available on the market. Since just 0.2% of people are diagnosed with chronic myeloid leukaemia (CML), it took over a decade for the first therapeutic tyrosine kinase inhibitor (TKI) to become common practice. Processing times can be significantly longer for medications with smaller patient pools or less clear therapeutic effects. Omacetaxine (a plant alkaloid) was approved for the treatment of TKI-resistant CML after a 30-year hiatus. It operates by interfering with the translation of proteins. CML and acute lymphoblastic leukaemia (ALL) with the Philadelphia chromosome have been treated with Asciminib (ABL kinase, allosteric inhibitor), which was licenced in the United States in 2014 and scheduled to be approved in Europe after 2020 (Baker et al. 2018; Gonzalez-Fierro and Dueñas-González 2021). Surmounting these obstacles repurposing has emerged as one of the most intriguing areas of pharmacology in the last decade. It is possible to reduce the time and expense of developing new pharmaceuticals by having thorough data on their pharmacology, formulation, safety and adverse effects.

To find new uses for existing medications, repurposing through reverse translational approach focuses on diseases that are not related to their primary use. It's impossible to deny that the process of reverse translational approach is both fascinating and challenging in evaluating the new use of existing marketed drug or a drug failed in clinical trials. Drug repurposing through reverse translational approach has become a hot topic in cancer research because of the growing need for new anticancer treatments. Although resistance to cancer therapies and pharmaceuticals is a hallmark of cancer, researchers are constantly looking for new ways to combat the disease. There are many examples wherein non-cancer medications are well understood and widely used as anticancer treatments by oncologists (Olgen and Kotra 2019).

Translational approach or traditional development of drug entails target selection, high-throughput screening lead identification, lead optimisation, efficacy testing, pharmacokinetic study, pharmacodynamics, toxicity, specificity study and drug interaction in animal models throughout preclinical research. Due to the high expense of developing cancer medications, the pharmaceutical industry is forced to rely on marketing laws to ensure that these medications are successfully marketed to cancer patients. So, most modern cancer products are not only prohibitively expensive for most the world's cancer patients based on current norms but may also be clinically ineffective. Furthermore, no link has been established between medicine prices and societal or patient benefits. This essential issue has prompted non-profit academic organisations to focus on cancer treatment development alternatives (Siddiqui and Rajkumar 2012). Consequently, drug repurposing through reverse translational approach is identified as an alternate technique for therapeutic development. Drugs that are approved for the treatment of non-malignant illnesses and whose cancer targets have been identified are referred to as anticancer medications. Despite the fact that sildenafil was originally intended to treat angina, the drug's side effect of prolonged erections in human volunteers has gained attention. This is the most well-known example of a repurposing for non-oncological condition in the medical literature (Nowak-Sliwinska et al. 2019).

Thalidomide, a repurposed cancer drug, is the standard treatment for patients. People diagnosed with multiple myeloma in 1998 were given this medication by the FDA to be used in conjunction with dexamethasone. In conjunction with bortezomib and dexamethasone, the *National Comprehensive Cancer Network* (NCCN) now recommends it as a primary therapy choice, 'useful in some conditions'. Arsenic trioxide and all-trans retinoic acid (ATRA), which have been used to treat skin diseases since 1962 and are utilised in traditional Chinese medicine to treat acute promyelocytic leukaemia (APML). Based on published pharmacokinetic studies, it appears that the proposed drug's antitumour efficacy in vitro can be pharmacologically obtained for repositioning cancer targets. Due to the documented safety and toxicity, including rare side effects, clinical trials can proceed more quickly. When it comes to the first indication, it's typically because they have a lot of experience. Once it is on the WHO Essential Medicines list, a medicine candidate can be available worldwide (Cavallo et al. 2007; Burnett et al. 2015; Zhu et al. 2016).

14.2 Prospective of Reverse Translational Research Approach in Drug Development for Cancer Therapy

Many opportunities for drug repurposing can arise during exploratory drug screening and based on analysis, a few pharmaceuticals have been examined as an alternative for testing for various diseases, a process known as 'shifting from bench to bedside' or 'reverse translational research' (Cook et al. 2014). There are several intriguing preclinical candidates in the drug development pipeline that have failed in clinical trials. One of the most prominent causes of these failures is preclinical models' incapacity to accurately imitate human physiology. Despite progress in both in vitro and in vivo studies, a more accurate portrayal of human physiological systems remains a problem (Scannell et al. 2012).

Due to a major sudden uptick in translational medicine research, new medications are entering clinical trials and, in many cases, get FDA approval or get failed. Some firms are creating new technologies that can mine a wide range of sources for relevant information by merging data from published academic research and clinical trials in the scientific literature for reverse translational research. Predicting which treatment will be the most effective for treating a certain illness is done using an increasingly complicated algorithm. Even in oncology, the advent of immunotherapy has changed the treatment picture (Williams 2018).

An example of reverse translational approach for repurposing includes a medicine developed for pancreatic cancer that has failed in clinical trials may help treat glioblastoma multiforme (GBM). To perform reverse translation, it is first essential to identify the underlying causes of human pharmacological reactions. Researchers conclude that the electronic health record can be an effective and cost-effective tool for gathering real-world, pharmacokinetic and pharmacodynamic data in reverse translational research (Robinson et al. 2018). There is a huge amount of pre-existing data on pharmaceuticals that biotechnology and pharmaceutical companies can access for new treatment therapies in cancer patients, which saves them millions of dollars in drug research (Terranova et al. 2021).

14.3 Opportunities in Drug Repurposing Approach

In May 2012, the NIH started a project entitled 'Discovery of New Therapeutic Uses for Existing Molecules'. This project aimed to find new ways to use molecules that are already in use to help people. In addition, this programme looked in the field of new therapeutic indications and new treatment options in the process of making new drugs (Strovel et al. 2016).

Drug re-concept is very important to polypharmacology, which studies how drugs interact with both their target and non-target proteins. There are different ways that the same protein can work on different cells. One of the network's cascades may have a protein that doesn't work the way it should, which makes

people sick. If the same protein makes a mistake in another physiological process, it can have a completely different effect. If the drug works for both diseases where the same protein is important for another set of responses, it is usually acceptable (Boran and Iyengar 2010; Reddy and Zhang 2013).

Reverse translational research backwards from clinical or clinical trial data to determine what occurs at the molecular level. Each patient observation results in the development of new testable hypotheses that aid in the improvement and direction of the subsequent cycle of benchtop therapeutic research, which then advances to the next stage. This model of research has three stages: benchtop therapies, clinical trials and human experience. The reverse translation approach investigates real-world patient experiences in clinical trials. A molecular basis for each of these experiences is discovered by starting with the experiences themselves and working backwards from there. To better understand the human condition, doctors are constantly coming up with new theories to test, which leads to new clinical trials and new human experiences. Clinical pharmacology and patient-centred translational therapies, as well as the question of reverse translation in translational medicine, are major topics in the field of reverse translation (Fernandez-Moure 2016).

Deep learning and big data mining, according to the researchers, are two more examples of reverse translation that can be utilised to repurpose pharmaceuticals across a wide range of therapeutic domains. Some firms are creating software that can gather significant information from various sources by mining the immense reservoir of knowledge accessible in scientific literature—both published academic research and data from clinical trials—in order to make big discoveries. This data is compiled using complex algorithms to forecast, with increasing precision, which drug will be most beneficial in treating a certain ailment (Qian et al. 2019).

In most cases, an approved drug with a specific side effect is discovered by chance to have greater therapeutic potential in a different application. Failure of exploratory drugs has ramifications for new therapeutic fields. Saracatinib, an anticancer failure drug developed by AstraZeneca, showed significant symptom reversal in an Alzheimer's disease (AD) mouse model. This drug also passed phase I Alzheimer's disease trials (NCT01864655) (Cummings et al. 2019).

14.4 Strategies for Drug Repurposing

Repurposing medicine is a well-known example of reverse translation. In this instance, clinical evidence of a medication's efficacy for a therapeutic benefit unrelated to its original therapeutic application resulted in the medication's repurposing. This phenomenon can occur because of a drug acting off target or because of an unanticipated downstream effect of a drug acting on target, as has been postulated in the case of cholesterol lowering with 3hydroxy3methylglutaryl coenzyme. Recently, it was discovered that rosuvastatin, a reductase inhibitor, may protect the kidneys in children with cystic fibrosis. The process of repurposing statins to protect the kidneys demonstrates the critical role of reverse translation in

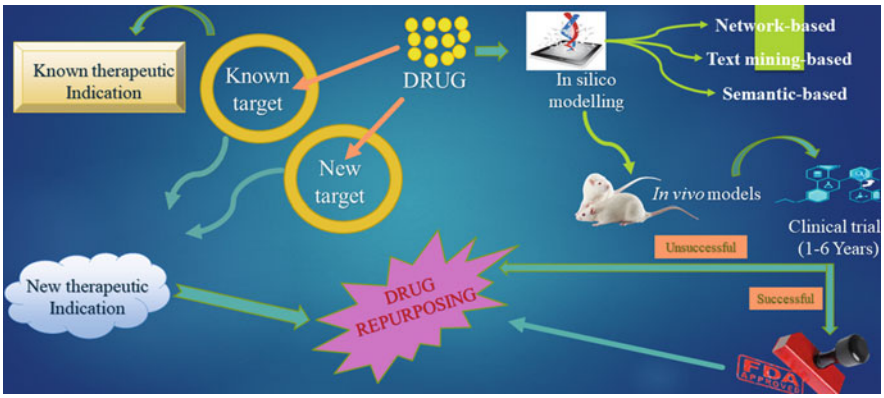


Fig. 14.1 Strategies involved in the drug repurposing

comprehending pharmacological mechanisms of action. Additionally, it demonstrates how real-world clinical data influences benchtop research and future therapeutic iterations.

Repurposing is accomplished in two stages. After conducting in vitro and in vivo research into specific pathophysiological pathways associated with the disease of interest, the first phase consists of screening licenced or commercially available medicines against a specific therapeutic target in a computer simulation. It is the second phase of the drug repurposing process to enrol in clinical studies for the new indication that has been identified. Repurposing drugs is possible at any stage of development, beginning with the discovery phase (Menduti et al. 2020). Figure 14.1 illustrates the off target, on target and various strategies involved in drug repurposing.

Off-label use of FDA-approved drugs has been shown to provide antiangiogenesis effects. For pharmacological repurposing to be successful, a thorough examination of the drug profile as well as the targeted illness ultra-mechanisms in the literature must be performed. To advance their research, scientists want the most up-to-date and credible information on previously undiscovered or innovative pathways related to the genesis and progression of the disease, as well as biomarkers associated with distinct phases of the disease. Any study focusing on genetic disorders must include literature demonstrating the drug's possible modifying influence at the genomic level, such as gene expression profiles, transcriptional studies, and other similar studies, among other things. The selection of the most appropriate technique for repurposing the medicine is an important step in this approach. Itraconazole, which is generally used as an antifungal, has been shown to have antiangiogenesis properties. The phase II clinical trials to determine the efficacy of the chemical as a second-line therapeutic agent in the treatment of lung cancer (NCT02357836) and prostate cancer (NCT02357837) were completed in late 2017 (NCT00887458) (Ioannidou et al. 2021; Zhong et al. 2021).

14.5 Necessity of Drug Repurposing for Managing Cancer

Most people had less than a 50% response rate to first-line treatment for different types of cancers after they spread. Most cancer patients become resistant to anticancer drugs because their genes and epigenetics have been changed in ways that are not normal, as well as because cancer stem cells keep growing even after other tumour cells die. In people who have a lot of different types of genes, repurposing drugs that work on a lot of different parts of tumours is important. In the last few years, new genomic and proteomic techniques for studying cancer-specific biochemical pathways that lead to new treatment targets have become more popular (Wang et al. 2017). Because this is the case, a whole new world of drug repurposing possibilities opens. People can be affected by almost any treatment that they get. As a result, if the drugs' targets are very similar to those found in cancer, there is a good chance they will help other people with cancer (Housman et al. 2014; Zhang et al. 2020).

On the other hand, drug repurposing has a long history of being random and fortuitous. In none of the successful drug repurposing situations, precision treatments have yet to be used. As a result, metformin, an antidiabetic drug, was unintentionally discovered to be effective in the treatment of a range of malignancies, even though the mechanisms underlying its anticancer activity are unknown. Because of its precision and versatility, the technique based on oncogene targets is sure to boost pharmaceutical repurposing (Sahoo et al. 2021).

Recent years have seen advancements in cancer prevention and therapy, as well as a breakthrough in our understanding of cancer's molecular underpinning. Over the last two decades, the concept of tailored treatment, also known as targeted therapy, has gained popularity. This resulted in the development of powerful targeted medications that have significantly improved the lives of cancer survivors. However, only a small number of the thousands of targeted drugs that have been made can help people. Resistance to anticancer medicines, both targeted and non-targeted, is very common. For pharmaceutical companies, it is extremely difficult to discover and invest in new lead medications, which shortens the therapeutic response and puts a lot of pressure on them (Ciliberto et al. 2020).

14.6 Reverse Translation for Drug Repurposing in Anticancer Therapies

Numerous effective medications have been developed for a variety of purposes, including oncology. Four repurposed drugs are already included in the European Society for Medical Oncology (ESMO) and *National Comprehensive Cancer Network* (NCCN) guidelines. Thalidomide is used to treat multiple myeloma, retinoic acid is used to treat acute promyelocytic leukaemia, zoledronic acid is used to treat solid tumours with bone metastases, and non-steroidal anti-inflammatory drugs are used to treat desmoid tumours (NCCN). There is considerable demand in both

pre-clinical and clinical settings for the discovery of novel applications for medications already approved for use in other contexts. The following examples demonstrate how novel cancer therapies can be combined with currently used drugs in treatment and in the real world. The following is a brief overview of some relevant literature.

In an early study, Medina Jiménez and Monroy-Torres (Medina-Jiménez and Monroy-Torres 2020) used a customised food intervention to help cervical cancer patients who were getting radiotherapy. They found that a customised nutrition plan increased muscle mass, weight, haemoglobin levels and gastrointestinal side effects, all of which led to better radiation treatment outcomes. In addition, the authors suggested that cervical cancer patients who were taking repurposed medications should be given a specialised diet to improve their effectiveness and, thus, their quality of life.

In their review Martinez-Escobar et al. (2021) concluded that drug repurposing in conjunction with CRISPR-dCas9-based artificial transcription factors (ATFs) could be an effective cancer treatment strategy. ATFs based on CRISPR-dCas9 technology can alter DNA and target genes, activate tumour suppressor genes, suppress oncogenes and diminish tumour resistance mechanisms in a variety of cancers. In cancer research, it is critical to develop new therapeutic combinations that function synergistically to increase the effectiveness of treatment.

One of the ways to treat cancer is through nanomedicine, which may use nanoparticles (NPs) that have been coated with sugar. NPs can transport and distribute cancer-targeting chemicals and drugs in a way that avoids the severe side effects that come with non-targeted drug delivery in traditional chemotherapy regimens. They work with lectin receptors, glucose transporters (GLUT) and immunological receptors for cell death, all of which have been overexpressed in tumour cells (Torres-Pérez et al. 2020).

It is reported in a book review by Hu and Carraway (2020), cationic amphiphilic medications such as antidepressants, antiarrhythmics and diuretics have been shown to cause lysosomal membrane permeabilisation (LMP) and cell death (LCD) in treatment-resistant cancer cells (Montaño-Samaniego et al. 2020). The recombinant DNA technologies and gene therapies are being employed to fulfil the increased demand for new cancer treatments. But additional research is needed to see if it can be used in conjunction with other drugs.

Montalvo-Casimiro et al. 2020 summarised the use of epidrugs, which are epigenetic regulators that offer cancer treatment options. There are drugs like hydralazine, decitabine, valproic acid and vorinostat (SAHA) being worked on to improve precision medicine by improving epigenetic therapy in cancer. These multidisciplinary approaches are a good way to get around problems with repurposing anticancer drugs. The Table 14.1 and data below enlist non-oncological drugs that have been used to treat cancer in clinical trials and are still going on or are about to start. They are grouped by differentiating mechanism for treating cancer and specific hallmarks.

Table 14.1 Some repurposed drugs used in cancer therapy

Drug	Original indication	Pathways	New indication as anticancer	Anticancer mechanism
Rapamycin	Immunosuppressant, antirestenosis agent	mTOR and associated signalling networks	Rectum, breast, prostate cancer etc.	Sustaining proliferative signalling
Prazosin	Hypertension	PKCδ-dependent AKT signalling pathway	Adrenal incidentalomas	Sustaining proliferative signalling
Indomethacin	Rheumatic disease	Shc-ERK axis, PKCζ-p38-DRP1 axis, Wnt/β-catenin	Colorectal, oesophageal, ovarian cancer etc.	Sustaining proliferative signalling
Quinacrine	Malaria, giardiasis, rheumatoid arthritis	p53, FACT-CK2-p53 axis	Prostatic, non–small-cell lung cancer, etc.	Evading growth suppressors
Ritonavir	Human immunodeficiency virus	p53, CDKs-RB axis, AKT-E2F-1-RB axis	Breast cancer, Kaposi's sarcoma, etc.	Evading growth suppressors
Artemisinin and related derivatives	Malaria	Ferroptosis, autophagy, oncosis, anoikis	Breast, colorectal, lung cancer, etc.	Resisting cell death
Chloroquine and related derivatives	Malaria, rheumatoid arthritis	Autophagy, PPT1	Pancreatic, breast cancer, chondrosarcoma, etc.	Resisting cell death
Curcumin	Dermatological diseases	hTERT, Wnt/β-catenin, Hippo/YAP	Breast, prostate cancer, multiple myeloma, etc.	Enabling replicative immortality
Genistein	Menopause, osteoporosis, obesity	Colorectal, bladder, breast cancer etc.	hTERT, Wnt/β-catenin	Enabling replicative immortality
Thalidomide	Sedative, antiemetic	Prostate, ovarian, colorectal cancer etc.	Various proangiogenic factors, VEGF receptor, NF-κB	Inducing angiogenesis
Itraconazole	Antifungal agent	Prostate, lung cancer etc.	mTOR-cholesterol trafficking, VDAC1, PDGF-Akt–mTOR axis	Inducing angiogenesis
Berberine	Bacterial diarrhoea	Gastric, colorectal, lung cancer etc.	Ephrin-B2, MMP-2/MMP-9, EMT, miR-101, VEGF	Activating invasion and metastasis
Niclosamide	Antihelminthic drug	Colorectal, prostate cancer etc.	Wnt/β-catenin, STAT3, NF-κB	Activating invasion and metastasis
Triamterene	Diuretic	Acute myelocytic leukaemia, etc.		

(continued)

Table 14.1 (continued)

Drug	Original indication	Pathways	New indication as anticancer	Anticancer mechanism
			Nucleotide excision repair, thymidylate synthase	Genome instability and mutation
Mebendazole and related-derivatives	Intestinal helminthiasis	Medulloblastoma, glioma, astrocytoma etc.	Chk2, Nbs1, PARP-1, DHODH	Genome instability and mutation
Aspirin	Pain, fever	Gastrointestinal, oesophageal cancer etc.	COX-1/2, ANXA1-NF–κB axis, CDX2, COMMD1–RelA axis	Tumour-promoting inflammation
Metformin	Obese type 2 diabetes	Prostate, breast, colorectal cancer etc.	AMPK, PI3K-mTOR pathways, BACH1	Reprogramming energy metabolism
Disulfiram	Alcohol-aversion drug	Prostate, breast cancer, melanoma etc.	ALDH, NAD^+-dependent proteins	Reprogramming energy metabolism

14.7 Antibiotics

Antibiotics are produced by bacteria, fungi and actinomycetes, as well as higher animals and plants, in the course of their lives. Other living cells can be stopped by these antipathogens or other actions. Antibiotics have been used to treat infectious diseases for over a century and have contributed significantly to people living longer lives. Antibiotics have undeniably made it easier for doctors to treat patients, particularly those with cancer who are more likely to get bacterial infections as a result of their disease or treatment side effects. A better strategy to employ antibiotics in cancer treatment is required because they can be a double-edged sword. As seen in the paragraph below, antibiotics are employed as an anticancer treatment.

14.7.1 Clarithromycin

Clarithromycin is a medication that has been approved by the FDA. Additionally, it is available as a generic. It is a broad-spectrum antibiotic that is effective against both Gram-positive and Gram-negative bacteria. It inhibits cell growth by covalently binding to the bacteria's 50S ribosomal subunit. This antibiotic may be beneficial if you have pneumonia or bronchitis (Fair and Tor 2014). Additionally, it can be used to treat oral infections, such as foul breath. There is a wealth of evidence suggesting clarithromycin is effective in the treatment of cancer, including in vitro, in vivo, and clinical trials. It was discovered in 1997 that it could benefit persons with lung cancer and was therefore put into usage. Since Hodgkin lymphoma was discovered in the 1970s, extensive study has been conducted. Clarithromycin is typically used alone to reduce lung cancer cell survival, but it is significantly more effective when coupled with other medications (Bayat et al. 2017). Bortezomib and clarithromycin in combination killed more melanoma cells than either treatment alone. When combined with bortezomib and vorinostat, clarithromycin in combination with carboplatin or cyclophosphamide has been shown to be beneficial for adenocarcinoma, myeloma and breast cancer. Clarithromycin increases natural killer T-cell receptor and CD8+ (cytotoxic) T-cell cytotoxicity after vindesin sulphate and cisplatin treatment. This leads to an increase in Interferon gamma (IFN)- and interleukin (IL-4) producing toxicity (T) cells and a better prognosis. Clarithromycin kills by preventing angiogenesis, or the formation of endothelial cell tubes (Van Nuffel et al. 2015).

14.7.2 Doxycycline

The FDA has approved doxycycline, a tetracycline antibiotic with a broad spectrum of activity. Doxycycline inhibits the synthesis of proteins in bacteria by binding to

the 16S ribosomal RNA. It is used to treat infections of the skin, respiratory system, gastro-intestinal tract and urinary tract. Additionally, it is used to prevent malaria (Fuoco 2012). Doxycycline can chelate divalent cations like Mg^{2+} and Ca^{2+}, reducing their bioavailability in patients' blood. Doxycycline, repurposed as an anticancer drug, inhibits inducible nitric oxide synthase (iNOS), an enzyme involved in tumour growth, development and angiogenesis. Doxycycline inhibits colon cancer cell growth by causing G0/G1 arrest and decreasing matrix metalloproteinase. The antiproliferative and antiapoptotic properties of doxycycline influence the proliferation and apoptosis of cervical cancer cells. When used in vitro, doxycycline inhibits the expression of stem cell factors (Oct4, Sox2, Nanog and CD44) as well as autophagy markers in breast cancer cells (LC-3BI and LC-3BII) (Nanda et al. 2016). Doxycycline has been shown to prevent tumour-sphere formation in a variety of cancer stem cells in a dose-dependent manner. It has been demonstrated that doxycycline inhibits mitochondrial biogenesis. The effect is not cancer-specific, and it has been shown to prevent tumour formation in its early stages. Through signal transducer and activator of transcription (STAT1/3), Sonic Hedgehog (Shh), Notch, and WNT/TGF-beta signalling, this medication inhibits glycolysis and mitochondrial activity in cancer cells. Lamb et al. (2015) in subsequent analyses describe the effect of doxycycline on DNA-PK, an enzyme that repairs irradiation DNA damage.

14.7.3 Minocycline

Minocycline is a tetracycline antibiotic with a broad spectrum that has been approved by the FDA. Minocycline is an antibiotic prescribed for the treatment of Lyme disease, urinary tract infections, and skin infections (i.e. acne). It inhibits protein synthesis by attaching to the 30S subunit of the bacterial ribosome. It, for example, stops the cell cycle and downregulates cyclins A, B and E, thereby inhibiting the formation and proliferation of ovarian cancer cells. Minocycline reduces interleukin (IL-6) production, modifies the IL-6 receptor system and inhibits the transforming growth factor beta-activated kinase (TGF-1-TAK1-IB) signalling pathway in ovarian cancer cells. In breast cancer, however, the combination of minocycline and celecoxib inhibits tumour cell proliferation, decreases micro-vessel density and decreases vascular endothelial growth factor (VEGF) and activated matrix metalloproteinase (MMP) expression (Garrido-Mesa et al. 2013).

14.7.4 Tigecycline

The FDA has approved tigecycline, a tetracycline antibiotic with a wide spectrum of activity. For multidrug-resistant Gram-positive and Gram-negative infections, tigecycline should only be administered as a last resort antibiotic. Antiproliferative properties of the ribosomal subunit can be inhibited by binding to it. Myeloid

leukaemia, glioma, non–small-cell lung cancer and RB1 negative breast cancer are all examples of malignancies that can be treated with tigecycline. Phase I research is now being conducted using this treatment due to its effectiveness. Tigecycline inhibits cell proliferation, migration/invasion, angiogenesis and oxidative stress, as well as cell cycle arrest, autophagy and death. When it comes to treating triple-negative breast cancer, tigecycline is an excellent option. Mitochondrial protein production and proliferation are accelerated in these cells. Tigecycline inhibits mitochondrial protein production, making it an effective inhibitor of RB-deficient cells, according to Xiong et al. (2018).

14.7.5 Nitroxoline

Nitroxoline, a hydroxyquinoline derivative, is used to treat bacterial infections, including Gram-positive and Gram-negative, as well as sprouts. For pathogens to thrive, they must be able to chelate divalent cations like Zn^2, Mg^2, Ca^2 and Fe^{2+}. Researchers have found numerous ways to employ nitroxoline to treat a wide range of malignancies, both in vitro and in vivo. Cyclin D1, Cdc25A and phosphorylated Rb are all decreased in nitroxoline-treated prostate cancer cells. To decrease cell development, nitroxoline activates AMPK, which suppresses the mTOR-p70S6K signalling pathway. Nitroxoline, on the other hand, inhibits MetAP2 activity and increases senescence in bladder and breast cancer cells. This lowers the density of micro-vessels and hinders the growth of endothelial tubes. Many cell types, including lymphoma, leukaemia and pancreatic cancer, benefit from the addition of copper to the nitroxoline treatment regimen (Cherdtrakulkiat et al. 2019).

14.7.6 Cephalosporins

Cephalosporins, or ß-lactam antibiotics, are commonly used to treat both Gram-positive and Gram-negative bacteria. The ß-lactam ring, which is unique to this class of antibiotics, is responsible for preventing the formation of bacterial cell walls and thus the antibiotics' effects. In vitro and in vivo studies have shown that the cephalosporins (cefaclor, cephradine and cefixime) are effective radio-sensitisers for breast, head and neck cancer. Among the cephalosporins previously described, only cefepime, a manganese complex, can suppress breast tumour cell proliferation and colon cancer cell proliferation without irradiation (Leone et al. 2019).

14.7.7 Fluoroquinolones

Bacterial gyrase activity is inhibited by fluoroquinolones, a broad-spectrum antibiotic drug, which results in a defect in DNA replication. Fleroxacin and ciprofloxacin are among the fluoroquinolones that have been shown to have powerful anticancer properties. As an example, ciprofloxacin, for example, is an anticancer agent that can treat a wide range of cancers, including colon, bladder, prostate and bone cancers such as osteoblastoma and osteosarcoma. Damage to mitochondria, DNA breakage and cell cycle arrest are all caused by ciprofloxacin, as well as an increase in TGF-ß. Prostate, breast and cervical cancer cells are all inhibited by enoxacin, which also causes cell cycle arrest and apoptosis (Pham et al. 2019). Enoxacin, a cancer-targeted regulator of miRNA production, is unusual. Repurposing fluoroquinolones as anticancer medications in combination with 5-fluorouracil and as a metal-ion complex is supported by a wealth of evidence, according to Yadav and Talwar (2019).

14.8 Antivirals

Not only genetic abnormalities and an unhealthy lifestyle contribute to the development of cancer; viral infections such as human papillomavirus (HPV) and human immunodeficiency virus (HIV), as well as the Epstein–Barr virus (EBV), can also cause cancer to thrive. According to the WHO, 32 million people worldwide are HIV positive in 2019. There are numerous diseases to which HIV patients are predisposed, including lymphomas and Kaposi's sarcoma, due to their weaker immune systems. Antivirals have been found to have a beneficial effect on cancer therapy when administered alone or in combination with other therapies in patients infected with viruses. The following are a few of the most exciting new medications.

14.8.1 Ganciclovir

The FDA has established ganciclovir's efficacy in treating patients infected with Herpes virus and Epstein–Barr virus. When ganciclovir's triphosphate analogue is introduced into infected host cells, viral kinases convert it to its triphosphate. This compound inhibits viral DNA polymerases, thereby reducing viral DNA elongation. Prostate cancer cells were transduced with an adenovirus (ADV) containing the HSV-tk gene derived from a replication-defective recombinant adenovirus. After a single dose of ganciclovir, cancer cells were killed and apoptotic. Additionally, 23 men with prostate cancer received neoadjuvant HSV-tk gene therapy to make the cancer cells more susceptible to the chemotherapy drug ganciclovir. In this case, intraprostatic viral infection and ganciclovir therapy were used. In surgical specimens taken 2–4 weeks after viral injection, apoptosis induction and decreased

micro-vessel density were observed. Experiments in vitro and in vivo with herpes simplex virus thymidine kinase (HSV-TK) transduced tumour cells, such as cervical cancer cells or lung cancer cells, demonstrate promising results (Cheon et al. 2000; Yanagisawa et al. 2021).

14.8.2 Lopinavir

Lopinavir, an FDA-approved antiviral medication, is used in conjunction with ritonavir to treat HIV-infected patients. Premature, non-infectious viruses are formed when HIV-1 protease and HIV-2 proteases are inhibited by lopinavir. Lopinavir and ritonavir combo therapy has received attention for its potential cancer-fighting properties. AMP-activated protein kinase and tumour necrosis factor-related apoptosis-inducing receptor (TRAIL) receptor expression levels are increased in urological cancer cells due to this combination. Using the same mixture in lung cancer cells results in apoptosis and cell cycle arrest. As an additional treatment, lopinavir and its produced lopinavir nitric oxide derivative decrease cell growth, cause morphological alterations, and cause reactive oxygen species generation and apoptosis in melanoma (Meini et al. 2020).

14.8.3 Indinavir

Indinavir is an FDA-approved antiviral medication that blocks HIV protease activity. Patients are usually provided indinavir as part of a retroviral combination therapy (e.g. with ritonavir) to prevent the development of resistance. This pro-carcinogenic protein (alpha-7 nicotinic acetylcholine receptor) and the malignancy promoter matrix metalloproteinase-2 (MMP-2) have been reported to be modulated by indinavir in an artificial computer model. Indinavir reduces cell viability, inhibits MMP-2 and MMP-9 production, and promotes apoptosis in HPV-induced cervical cancer cells. It also influences tumour size in the animal model. Indinavir also suppresses MMP-2 proteolytic activation, reduces angiogenesis, promotes apoptosis and slows tumour growth in vivo in patients with hepatocarcinoma. In Kaposi's sarcoma, the same results were seen (Barillari et al. 2018).

14.8.4 Cidofovir

AIDS patients with cytomegalovirus (CMV) infections can be treated with the FDA-approved antiviral medication cidofovir, which inhibits viral DNA polymerase. Efforts to repurpose cidofovir are taking place both in vitro and in vivo. Cidofovir, for example, has been proven to cause apoptosis in glioblastoma, boost

p53 and p-pRb levels in HPV-infected cervical cancer, and is a powerful radiosensitiser (Deutsch et al. 2016).

14.8.5 Efavirenz

Efavirenz, an FDA-approved antiviral medication, is given to HIV-1-infected individuals as part of an antiretroviral combination treatment. Efavirenz has antiviral properties because it inhibits viral DNA synthesis by binding to reverse transcriptase. The repurposing of efavirenz for cancer therapy is supported by a large body of data. In vitro, efavirenz inhibits colorectal, pancreatic and lung cancers, as well as glioblastoma and leukaemia. While this medication can be used alone, it has been shown to enhance the effects of radiation therapy. Although the effect of efavirenz 600 mg on prostate cancer was not statistically significant in a phase II clinical trial, some patients experienced a favourable outcome in terms of tumour suppression (Everson et al. 2018).

14.8.6 Maraviroc

Maraviroc, an antiretroviral medication, is the most frequently prescribed CCR5 co-receptor antagonist for HIV treatment. When CCL5 binds to CCR5, it prevents the virus from entering the cell by preventing CCL5 from binding to CCR5. Numerous in vivo and in vitro studies, as well as one clinical trial (NCT01276236), demonstrate that maraviroc may be used to cure cancers such as Kaposi's sarcoma and lymphomas, which are frequently associated with HIV infection. On the other hand, maraviroc appears to be effective against a variety of cancers, including breast, colon and gastric cancers, as well as a variety of leukaemia types. Maraviroc induces apoptosis in colorectal cancer, arrests cell cycle progression in colorectal and pancreatic cancer, decreases monocyte accumulation in the lymphoma tumour microenvironment and enhances the efficacy of a wide variety of medications (Kim et al. 2016).

14.8.7 Nelfinavir

Nelfinavir, a protease inhibitor and antiretroviral medication, is used to treat HIV-1. Numerous studies support nelfinavir's anticancer properties. By inhibiting the proteasome in cancers such as glioma, myeloma, laryngeal/laryngeal/ovarian/lung, it induces ER stress and upregulation of the UPR response, which results in apoptosis. Additionally, it inhibits the production and phosphorylation of the HER2 protein, as well as AKT and ERK1/2 signalling in breast cancer cells.

Nelfinavir also inhibits MnSOD protein expression in cervical cancer, resulting in apoptosis and G1 cell cycle arrest. On the other hand, nelfinavir synergises with several chemotherapeutic agents, indicating its significant potential for cancer treatment (Koltai 2015).

14.8.8 Ritonavir

Ritonavir is an antiretroviral drug that inhibits the HIV-1 protease. The role of ritonavir in cancer treatment is well-known. Ritonavir inhibits NF-B activity and subsequent expression of NF-B-regulated gene products and cytokines in T-cell leukaemia and Kaposi's sarcoma. It induces G1 cell cycle arrest in breast, lung and ovarian cancers. Ritonavir's chymotrypsin-like proteasome activity has been shown to stimulate and inhibit T-cell leukaemia and glioma cells, respectively. Additionally, ritonavir interacts synergistically with a variety of other medications. In clinical trials, DS-8201a, doxorubicin, lopinavir, metformin and ritonavir are frequently used in combination with ritonavir (Cao et al. 2020).

14.8.9 Ribavirin

Ribavirin can be used to treat hepatitis C in combination with interferon, but the mechanism by which it does so has not been fully elucidated. Ribavirin has been shown to have promising anticancer effects in the treatment of cancers such as oral cavity cancer, breast cancer, acute lymphoblastic leukaemia (ALL), osteosarcoma and glioblastoma. Ribavirin modulation of eIF4E, EZH2, Snail and EZH2 results in decreased migration and adhesion in nasopharyngeal cancer (NPC), osteosarcoma and glioma. Ribavirin is currently being studied in phase I and II clinical trials for AML, breast, head and neck cancer, and hepatocellular carcinoma (HCC) (Casaos et al. 2019).

14.8.10 Zidovudine

AZT, also known as zidovudine, is an antiretroviral drug. It is a thymidine analogue that stops chain elongation, which is bad for people who have HIV. Zidovudine reduces the activity of telomerase, in various types of cancers like colorectal, breast, parathyroid, oesophageal or ovarian. Several clinical trials are looking into zidovudine for Kaposi's sarcoma and lymphoma treatment, as well as interferon and zidovudine in combination for T-cell leukaemia treatment in a phase II clinical study.

14.8.11 Amantadine

Influenza is protected by amantadine, an FDA-approved medication used to treat the symptoms of Parkinson's disease. A virus infection is prevented by blocking the viral M2 ion channel, which prevents the virus from entering the host cell. Amantadine, in contrast to the medications indicated above, can be used for cancer detection but not for cancer treatment, as opposed to the other drugs. SSAT (spermidine/spermine N-acetyltransferase) is a unique enzyme that is only found in high concentrations in cancer cells and is responsible for the metabolisation of amantadine to N-acetylamantadine. Urine can be tested for the presence of N-acetylamantadine, which is believed to be a non-invasive, inexpensive and quick technique of cancer screening. An amantadine clinical phase II experiment is in underway to see whether it may be used as a potential diagnostic biomarker for cancer detection (Maksymiuk et al. 2018).

14.9 Antifungals

14.9.1 Itraconazole

Itraconazole is a triazole antifungal medicine that is licenced by the FDA and is used by mouth to treat infections inside the body. It stops the production of ergosterol, which stops the production of membranes. Itraconazole has been demonstrated in numerous trials to not only be effective at treating fungal infections, but it also has potential anticancer properties. For example, itraconazole, which is used to treat melanoma, stops tumour cell growth, cell proliferation and colony formation. It was discovered that the expression of Gli-1 and Gli-2, Wnt3A, -catenin and cyclin D1 had decreased, resulting in a blockage of the Hedgehog and WNT signalling pathways. It also inhibits the phosphorylation of p70S6K, 4E-BP1 and AKT, which in turn inhibits the PI3K/mTOR signalling pathway. When combined with chemotherapy, itraconazole considerably increases the overall survival of patients with pancreatic and ovarian malignancies. This is because itraconazole changes the Hedgehog signalling pathway and stops angiogenesis by its triazole unit. Itraconazole slows down the growth of endothelial cells in both animals and in the lab. It also stops the cell cycle and stops the growth of the endothelial growth factor, which stops angiogenesis. Regarding various cancers, it is currently under clinical phase I and II trials, with the results expected soon (Tsubamoto et al. 2014, 2015).

14.9.2 Ketoconazole

People who get infections on their skin, like foot fungus, can take a drug called ketoconazole. This drug is used to fight off fungus. It is used in the formulation of various lotions and shampoos. Ketoconazole prevents the production of the fungal sterol ergosterol and causes harm to the cell membrane. Another use for ketoconazole is to treat Cushing's syndrome, where it stops the adrenal gland from making steroids and cholesterol by blocking the cytochrome P450 system. Because ketoconazole is an antiandrogen, it is also good at fighting prostate cancer. Even though ketoconazole had promising outcomes in clinical trials at high doses, its usage is restricted due to side effects in the intestines. However, more clinical trials are being done to see if it helps fight prostate and breast cancer (Chen et al. 2019).

14.9.3 Clioquinol

Clioquinol is a fungicide or a bactericide that is used to treat infections that occur on the outside of the body that are caused by fungi or bacteria. Because it is most usually applied to the skin, it can be found in a variety of ointments for wound healing and neurodermatitis. Clioquinol's ability to bind to sulphydryl groups is what makes it an antimicrobial drug. This stops the pathogens from making important enzymes. Moreover, because of its propensity to chelate divalent cations such as Zn^{2+} and Cu^{2+}, it has been found to be an effective treatment for Alzheimer's disease, which has a high concentration of these cations in the bloodstream. Clioquinol also has promising effects on a lot of different types of cancer cells, and it has already been used in a clinical trial for leukaemia. Its ability to act as a Zn^{2+} ionophore is a big part of how it fights cancer cells. Shortly, when cancer cells are treated with clioquinol and zinc, the zinc level inside them rises. This causes the lysosomes to break down, which causes bid to be broken down and apoptosis to be caused. In addition to targeting lysosomes, it also stops the NF-B activity on its own (Bareggi and Cornelli 2012; Lu et al. 2018).

14.9.4 Clotrimazole

Clotrimazole is a broad-spectrum antifungal medication that has been licenced by the FDA for treating fungal infections on the skin and in the vaginal canal. Clotrimazole has been shown to be effective against a variety of cancers, including breast, melanoma, colon and lung tissues, both in the real world and in the laboratory. Clotrimazole functions by removing glycolytic enzymes from the cytoskeleton, such as hexokinase, aldolase and fructokinase. This causes the enzymes to break free from

the cytoskeleton. Clotrimazole's anticancer effects may potentially be mediated via Ca^{2+}-activated potassium channels, according to Kadavakollu et al. (2014).

14.9.5 Terbinafine

An antifungal drug called terbinafine is approved by the FDA to treat fungal infections on the nails and on the skin. There are many ways to apply it to your skin. It can be used as a cream, gel, solution, spray or even sprayed. This drug doesn't compete with the squalene oxidase, which stops the fungus from making ergosterol. Ergosterol is used to make cell walls in the fungus. Using an in vivo model, Labay and his team found that terbinafine can make radio waves more sensitive in mice with melanoma (Labay et al. 2016).

14.10 Antimalarial Drugs

There are also three antimalarial drugs (chloroquine, artesunate and mefloquine) that could one day be used to fight cancer, too. It can also help with lupus, rheumatoid arthritis and discoid. Chloroquine, which is approved by the FDA, can also help with malaria. When chloroquine is used with standard-of-care chemotherapy drugs, they work better together. On the other hand, chloroquine boosts tumour protein-p53 and makes breast cancer tumours more sensitive to phosphoinositide-3-kinase–protein kinase (PI3K/AKT) inhibitors in breast cancer cells, which makes them more likely to die from the drug. Artesunate, which is used to treat malaria, causes the parasite to make reactive oxygen species (ROS) and break DNA double-strand breaks because the drug cuts endoperoxide bridges. Artesunate has been shown to fight cancer in many studies, both in the real world and in the lab. Artesunate, for example, causes the production of ROS and apoptosis. This is linked to the release of cytochrome C and the cleavage of caspase-9, which happens when these things happen. Artesunate also stops the growth of Kaposi sarcoma tumours. This drug, which stops angiogenesis, also stops the growth of the tumours. Reduced phosphorylation of the vascular endothelial growth factor receptor-2 (VEGFR2) in renal cell carcinoma has been shown to cut cell growth, migration, metastasis and angiogenesis, among other things. Studies by Hamacher-Brady et al. (2011) have shown that artesunate influences endolysosomal traffic and slows down the flow of autophagy in breast cancer cells, which makes them more likely to die. Artesunate is also a radiosensitiser for lung cancer cells, and it works well with a lot of other medicines, like sorafenib, doxorubicin, paclitaxel and cisplatin. Malaria medicine is used to treat it. People who looked at this study say that mefloquine appears to influence a parasite's 80S-ribosome subunit, which could influence protein synthesis.

14.11 Anthelmintic Agents

14.11.1 Mebendazole, Niclosamide, Albendazole and Ivermectin

Mebendazole, niclosamide, albendazole and ivermectin are all common anthelmintic medications, with the FDA having approved the latter two of them. Each of them has a distinct method of treating worm infestations. Mebendazole and albendazole bind to tubulin and reduce worm tubulin production, whereas ivermectin operates via modulating aminobutyric acid receptors and glutamate-gated signalling pathways. Niclosamide prevents the worm from digesting glucose, which results in the worm becoming dehydrated and starving. By activating caspase-3, mebendazole inhibits colony formation, reduces angiogenesis and increases cytotoxicity in meningioma cells, all of which are detrimental to their survival. Mebendazole's antitumour properties are boosted when it is exposed to radiation. In the medulla blastoma cells, mebendazole inhibits the production of primary cilia, which is a tubulin-based organelle that serves as a signalling hub for the Hedgehog pathway, resulting in a reduction in cell proliferation. Mebendazole also has an immunostimulatory effect, enhancing T-cell activation as well as the ability of CD14+ myeloid cells to eliminate tumour cells in the body. Mebendazole also has the additional effect of increasing the body's tumour necrosis factor (TNF) and interferon (IFN) levels. Specifically, albendazole inhibits the formation and proliferation of ovarian cancer cells by interfering with the production of tubulin, which results in a reduction in malignant cells development and angiogenesis. Because of its limited solubility in water, it has been investigated in vivo as a BSA-albendazole nanoparticle and showed greater inhibitory effect on vascular endothelial growth factor. Albendazole's anticancer efficacy against advanced tumours was confirmed in a phase I clinical trial. It is now being evaluated in a phase II clinical trial for the treatment of neoplasms. Niclosamide also has synergistic effects with sorafenib, a protein kinase inhibitor (Son et al. 2020).

14.11.2 Ivermectin

Ectoparasites and threadworms are effectively treated with ivermectin, an anthelmintic medication. It induces DNA damage in ovarian cancer by producing double-strand DNA breaks, which results in the disease spreading. It also causes intrinsic apoptosis by rupturing the mitochondrial membrane, which results in increased BAX/BCL-2 production as well as the release of cytochrome C from the cell. Patients with liver cancer who are treated with ivermectin have the same effects. It's not the same way that ivermectin causes leukaemia cells to die, though. It raises intracellular chloride ions and reactive oxygen species (ROS) levels via hyperpolarising plasma membranes. Ivermectin works by suppressing the WNT

pathway, which is dependent on T-cell factor (TCF), and hence prevents colon cancer cells from growing and destroying. Moreover, it also stops the growth of new blood vessels and stops the phosphatidylinositol-3-kinase (Akt/mTOR) signalling pathway in glioblastoma when there is a lot of stress on the mitochondria and a lot of ROS. Ivermectin, like glioblastoma, stops the growth of stem-like breast cancer cells by lowering the number of self-renewal transcription factors. Studies show that the anthelmintic medicines can fight cancer in both animals and humans (Chai et al. 2021; Zaidi and Dehgani-Mobaraki 2021).

14.11.3 Nitazoxanide

Nitazoxanide was initially developed as an anthelmintic drug due to its ability to inhibit pyruvate-ferredoxin-oxidoreductase, which is required for parasites' anaerobic metabolism. The primary target of nitazoxanide treatment in colon cancer cells has been identified as glutathione-S-transferase P1 (GSTP1). Nitazoxanide has been shown to inhibit both cell proliferation and chromatin condensation, as well as genomic instability and cell cycle arrest. In colon cancer, the AMPK pathway is activated, and the c-Myc, WNT and MTOR signalling pathways are downregulated (Lokhande and Devarajan 2021). Nitazoxanide restricts autophagy modulation in glioblastoma cells by upregulating the expression of inhibitor growth protein 1 (ING1), which inhibits late-stage lysosome acidification, reduces cell growth and causes cell cycle arrest (ING1). It inhibits the c-Myc expression in breast tumour cells, resulting in the suppression of tumour development and the induction of apoptosis. An in-depth examination of nitazoxanide's further anticancer capabilities is provided by Di Santo and Ehrisman (2014) in their review.

14.11.4 Praziquantel

Praziquantel is an antiparasite medicine licenced by the FDA, although its mechanism of action is yet unknown. There are still several possible pharmacological synergistic targets that Thomas and Timson believe could be identified. Praziquantel has been shown to cause worm muscle to spasm due to a rapid Ca^{2+} influx inside the worm (Thomas and Timson 2020). Praziquantel's anticancer properties have been demonstrated in vitro and in vivo, and it is now being utilised in a clinical trial to treat cancer. In colon (DLD-1) and lung (H1299) cancer cells, downregulation of the apoptosis protein XIAP by praziquantel enhances paclitaxel's ability to limit cell growth.

14.11.5 Levamisole

Levamisole inhibits male worm reproduction by binding to nicotinic acetylcholine receptors. Depending on the concentration and time of application, levamisole can either activate or suppress the human immune system. Additionally, it can either activate or depress human immune systems. Levamisole is an effective treatment for lung cancer, melanoma and myeloma, both in vivo and in vitro. CD138 is a heparin-sulphate glycoprotein that is overexpressed in malignant cells, and levamisole inhibits this transmembrane protein while increasing IL-6 production, which inhibits myeloma cells. Inhibiting cJNK phosphorylation in lung cancer cells results in TNF-induced cell death and cell cycle arrest (Martin et al. 2012; Nageshwari and Merugu 2017).

14.12 Expanding Opportunities of Drug Repurposing

14.12.1 Treatment of COVID-19 Along with Cancer

The coronavirus disease 2019 (COVID-19) does not has any treatment option accessible at this point. A significant amount of effort is being put forward to identify effective medications and produce innovative vaccinations, which is a continuing endeavour. According to the findings of the studies against COVID-19, several FDA-approved pharmaceuticals that are currently in use for the treatment of other disorders specially cancer could be repurposed for the treatment of COVID-19. When it comes to the COVID-19 outbreak, repurposing has some merits. It is considered to be a very effective strategy for drug discovery because it requires less time and effort to discover a therapeutic agent when compared to the de novo drug discovery process, which takes longer and makes it more expensive. Broadly many FDA-authorised pharmaceuticals including antivirals, antimalarials, monoclonal antibodies, statins, angiotensin-converting enzyme inhibitors and receptor blockers are used in the treatment of various disorders, but these treatments are now a days employed in the treatment of coronavirus (Fig. 14.2). These medications are also being repurposed for the treatment of a variety of malignancies, including breast, prostate, colon, colorectal, skin and other forms of leukaemia. In several clinical investigations on COVID-19 patients, it has been observed that remdesivir, arbidol, ribavirin, favipiravir, tocilizumab or combination of lopinavir and ritonavir has demonstrated benefits; hence, these medications may be considered as prospective drug therapies in the future. Additionally, both Angiotensin-converting enzyme and receptor blockers have the capability to be used as supportive therapy in said treatment. A number of medications, including ivermectin and auranofin, are now in the development phase against the COVID-19 virus. It is anticipated that molecular docking would be the primary technique used to find potential therapeutic drugs for COVID-19 patients as in anticancer therapy. The screened medicines will then be evaluated for their efficacy in both in vitro and in vivo trials.

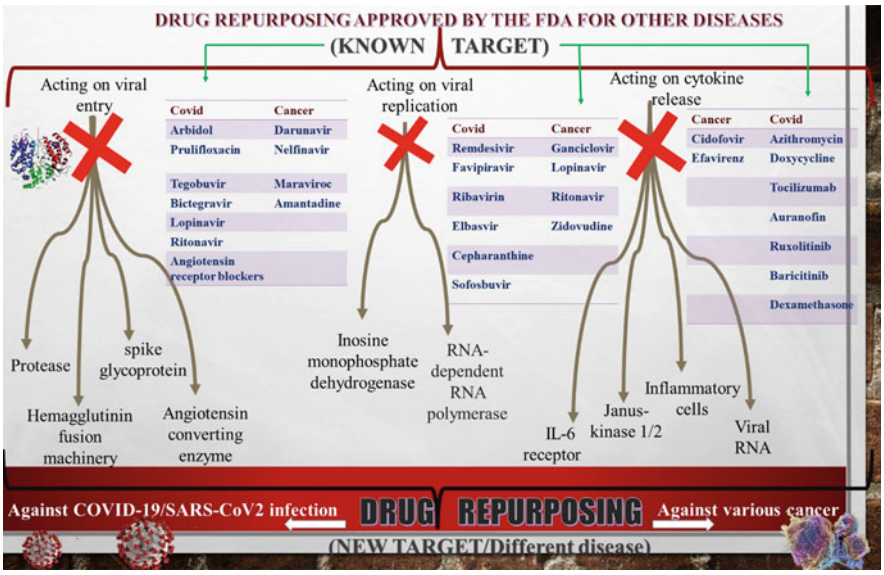


Fig. 14.2 Depiction of well-known disease medications used in the COVID-19 and cancer therapy

14.12.2 Precision Medicines Development

Precision medicine considers a patient's unique genetic, environmental and behavioural variability when deciding on or continuing a treatment. Drugs that are more effective or safer may be personalised to an individual's genome, transcriptome, proteome and metabolic activities or to specific disorders. For precision medicine, the repositioning technique has been extremely beneficial in identifying previously unknown mechanisms of action for medications through the investigation of novel metabolic pathways, off-targets and target-specific mechanisms/genetic expression for even inherited disorders. Genomic and transcriptome data have flooded our hands as a result of genomic advancements such as next-generation sequencing, microarray data and transcriptomics. Network biology and systems biology techniques may provide molecular or genetic insights into drug–target interaction patterns, hence revealing novel mechanisms of action. Repositioning pharmaceuticals demands a higher degree of understanding, which can be attained only through the use of a combination of computational and experimental methods. By contrast, drug repurposing can be used to find and develop innovative medications with very precise and successful therapeutic indications for human diseases. As illustrated in Fig. 14.3, translational precision medicine enables the integration of translational medicine, reverse translational medicine and precision medicine.

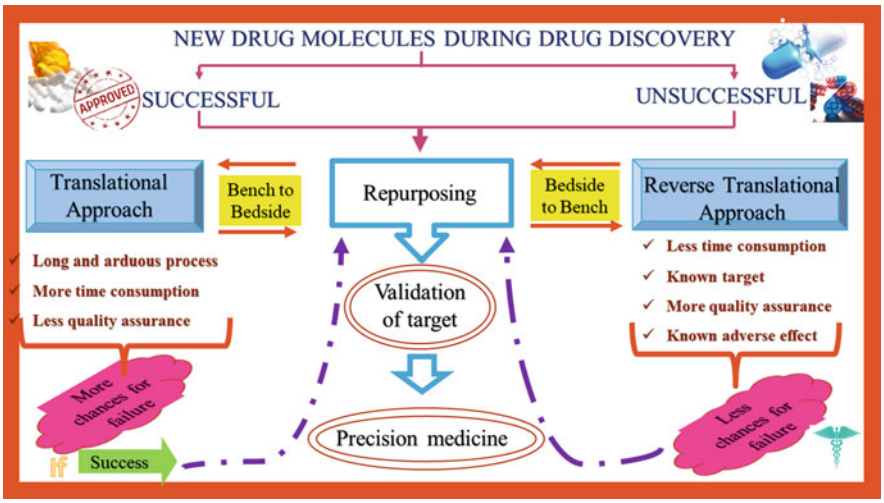


Fig. 14.3 Illustration the connection between the forward translation, reverse translation and precision medicine

14.13 Conclusion and Future Prospects

The high expense of developing a new treatment in cancer drives the practice of repurposing of existing drugs that have been approved for use in other disease or the drug molecules which are failed in clinical trials during drug discovery or withdrawn during post-marketing surveillance. As the application of repurposing, various non-oncology drugs can target multiple cancer hallmarks and related cancer biology, rather than merely killing cancer cells. This approach will be crucial in the establishment of a new paradigm of individualised and precision treatment. This review identified significant prospects for pharmacological repurposing using publicly available biological data.

It was concluded that establishment of new targets of existing drugs is feasible through repurposing based on reverse translational approach. Further reverse translational research in repurposing, being an innovative approach, can be helpful to investigate pharmaceutical combination therapy to improve clinical outcomes. Targeting cancer-related behaviours like downstream off-target effects, parallel pathways and compensatory signals is typical when utilising pharmaceutical combinations. A new route for pharmaceutical research has opened up due to repurposing. Aside from cancer, drug repurposing has been used to treat a variety of ailments, including tuberculosis, hepatitis, asthma, high blood pressure, etc. Finally, despite several setbacks, repurposing has opened a new path for pharmaceutical development, including but not restricted to cancer therapy.

References

Andrade EL, Bento AF, Cavalli J, Oliveira SK, Schwanke RC, Siqueira JM, Freitas CS, Marcon R, Calixto JB (2016) Non-clinical studies in the process of new drug development—part II: good laboratory practice, metabolism, pharmacokinetics, safety and dose translation to clinical studies. Braz J Med Biol Res 49(12):e5646. https://doi.org/10.1590/1414-431X20165646

Arruebo M, Vilaboa N, Sáez-Gutierrez B, Lambea J, Tres A, Valladares M, González-Fernández Á (2011) Assessment of the evolution of cancer treatment therapies. Cancers 3(3):3279–3330. https://doi.org/10.3390/cancers3033279

Baker NC, Ekins S, Williams AJ, Tropsha A (2018) A bibliometric review of drug repurposing. Drug Discov Today 23(3):661–672. https://doi.org/10.1016/j.drudis.2018.01.018

Bareggi SR, Cornelli U (2012) Clioquinol: review of its mechanisms of action and clinical uses in neurodegenerative disorders. CNS Neurosci Ther 18(1):41–46. https://doi.org/10.1111/j.1755-5949.2010.00231.x

Barillari G, Monini P, Sgadari C, Ensoli B (2018) The impact of human papilloma viruses, matrix metallo-proteinases and HIV protease inhibitors on the onset and progression of uterine cervix epithelial tumors: a review of preclinical and clinical studies. Int J Mol Sci 19(5):1418. https://doi.org/10.3390/ijms19051418

Bayat MR, Homayouni TS, Baluch N, Morgatskaya E, Kumar S, Das B, Yeger H (2017) Combination therapy in combating cancer. Oncotarget 8(23):38022–38043. https://doi.org/10.18632/oncotarget.16723

Boran AD, Iyengar R (2010) Systems approaches to polypharmacology and drug discovery. Curr Opin Drug Discov Devel 13(3):297–309

Burnett AK, Russell NH, Hills RK, Bowen D, Kell J, Knapper S, Morgan YG, Lok J, Grech A, Jones G, Khwaja A, Friis L, McMullin MF, Hunter A, Clark RE, Grimwade D, UK National Cancer Research Institute Acute Myeloid Leukaemia Working Group (2015) Arsenic trioxide and all-trans retinoic acid treatment for acute promyelocytic leukaemia in all risk groups (AML17): results of a randomised, controlled, phase 3 trial. Lancet Oncol 16(13):1295–1305. https://doi.org/10.1016/S1470-2045(15)00193-X

Cao B, Wang Y, Wen D, Liu W, Wang J, Fan G, Ruan L, Song B, Cai Y, Wei M, Li X, Xia J, Chen N, Xiang J, Yu T, Bai T, Xie X, Zhang L, Li C, Yuan Y, Wang C (2020) A trial of Lopinavir-ritonavir in adults hospitalized with severe Covid-19. N Engl J Med 382(19):1787–1799. https://doi.org/10.1056/NEJMoa2001282

Casaos J, Gorelick NL, Huq S, Choi J, Xia Y, Serra R, Felder R, Lott T, Kast RE, Suk I, Brem H, Tyler B, Skuli N (2019) The use of ribavirin as an anticancer therapeutic: will it go viral? Mol Cancer Ther 18(7):1185–1194. https://doi.org/10.1158/1535-7163.MCT-18-0666

Cavallo F, Boccadoro M, Palumbo A (2007) Review of thalidomide in the treatment of newly diagnosed multiple myeloma. Ther Clin Risk Manag 3(4):543–552

Chai JY, Jung BK, Hong SJ (2021) Albendazole and Mebendazole as anti-parasitic and anti-cancer agents: an update. Korean J Parasitol 59(3):189–225. https://doi.org/10.3347/kjp.2021.59.3.189

Chen Y, Chen HN, Wang K, Zhang L, Huang Z, Liu J, Zhang Z, Luo M, Lei Y, Peng Y, Zhou ZG, Wei Y, Huang C (2019) Ketoconazole exacerbates mitophagy to induce apoptosis by downregulating cyclooxygenase-2 in hepatocellular carcinoma. J Hepatol 70(1):66–77. https://doi.org/10.1016/j.jhep.2018.09.022

Cheon J, Kim HK, Moon DG, Yoon DK, Cho JH, Koh SK (2000) Adenovirus-mediated suicide-gene therapy using the herpes simplex virus thymidine kinase gene in cell and animal models of human prostate cancer: changes in tumour cell proliferative activity. BJU Int 85(6):759–766. https://doi.org/10.1046/j.1464-410x.2000.00516.x

Cherdtrakulkiat R, Lawung R, Nabu S, Tantimavanich S, Sinthupoom N, Prachayasittikul S, Prachayasittikul V (2019) Nitroxoline: a potent antimicrobial agent against multidrug resistant Enterobacteriaceae. EXCLI J 18:445–453. https://doi.org/10.17179/excli2019-1378

Ciliberto G, Mancini R, Paggi MG (2020) Drug repurposing against COVID-19: focus on anticancer agents. J Exp Clin Cancer Res 39(1):86. https://doi.org/10.1186/s13046-020-01590-2

Cook D, Brown D, Alexander R, March R, Morgan P, Satterthwaite G, Pangalos MN (2014) Lessons learned from the fate of AstraZeneca's drug pipeline: a five-dimensional framework. Nat Rev Drug Discov 13(6):419–431. https://doi.org/10.1038/nrd4309. Epub 2014 May 16

Cummings JL, Tong G, Ballard C (2019) Treatment combinations for Alzheimer's disease: current and future pharmacotherapy options. J Alzheimers Dis 67(3):779–794. https://doi.org/10.3233/JAD-180766

Deutsch E, Haie-Meder C, Bayar MA, Mondini M, Laporte M, Mazeron R, Adam J, Varga A, Vassal G, Magné N, Chargari C, Lanoy E, Pautier P, Levy A, Soria JC (2016) Phase I trial evaluating the antiviral agent Cidofovir in combination with chemoradiation in cervical cancer patients. Oncotarget 7(18):25549–25557. https://doi.org/10.18632/oncotarget.8224

Di Santo N, Ehrisman J (2014) A functional perspective of nitazoxanide as a potential anticancer drug. Mutat Res 768:16–21. https://doi.org/10.1016/j.mrfmmm.2014.05.005

Everson F, Genis A, Ogundipe T, De Boever P, Goswami N, Lochner A, Blackhurst D, Strijdom H (2018) Treatment with a fixed dose combination antiretroviral therapy drug containing tenofovir, emtricitabine and efavirenz is associated with cardioprotection in high calorie diet-induced obese rats. PLoS One 13(12):e0208537. https://doi.org/10.1371/journal.pone.0208537

Fair RJ, Tor Y (2014) Antibiotics and bacterial resistance in the 21st century. Persp Med Chem 6: 25–64. https://doi.org/10.4137/PMC.S14459

Falzone L, Salomone S, Libra M (2018) Evolution of cancer pharmacological treatments at the turn of the third millennium. Front Pharmacol 9:1300. https://doi.org/10.3389/fphar.2018.01300

Fernandez-Moure JS (2016) Lost in translation: the gap in scientific advancements and clinical application. Front Bioeng Biotechnol 4:43. https://doi.org/10.3389/fbioe.2016.00043

Fuoco D (2012) Classification framework and chemical biology of tetracycline-structure-based drugs. Antibiotics (Basel) 1(1):1–13. https://doi.org/10.3390/antibiotics1010001

Garrido-Mesa N, Zarzuelo A, Gálvez J (2013) Minocycline: far beyond an antibiotic. Br J Pharmacol 169(2):337–352. https://doi.org/10.1111/bph.12139

Gonzalez-Fierro A, Dueñas-González A (2021) Drug repurposing for cancer therapy, easier said than done. Semin Cancer Biol 68:123–131. https://doi.org/10.1016/j.semcancer.2019.12.012

Hamacher-Brady A, Stein HA, Turschner S, Toegel I, Mora R, Jennewein N, Efferth T, Eils R, Brady NR (2011) Artesunate activates mitochondrial apoptosis in breast cancer cells via iron-catalyzed lysosomal reactive oxygen species production. J Biol Chem 286(8):6587–6601. https://doi.org/10.1074/jbc.M110.210047

Housman G, Byler S, Heerboth S, Lapinska K, Longacre M, Snyder N, Sarkar S (2014) Drug resistance in cancer: an overview. Cancers 6(3):1769–1792. https://doi.org/10.3390/cancers6031769

Hu M, Carraway KL (2020) Repurposing cationic amphiphilic drugs and derivatives to engage lysosomal cell death in cancer treatment. Front Oncol 10:605361. https://doi.org/10.3389/fonc.2020.605361

Ioannidou E, Moschetta M, Shah S, Parker JS, Ozturk MA, Pappas-Gogos G, Sheriff M, Rassy E, Boussios S (2021) Angiogenesis and anti-angiogenic treatment in prostate cancer: mechanisms of action and molecular targets. Int J Mol Sci 22(18):9926. https://doi.org/10.3390/ijms22189926

Kadavakollu S, Stailey C, Kunapareddy CS, White S (2014) Clotrimazole as a cancer drug: a short review. Med Chem 4(11):722–724. https://doi.org/10.4172/2161-0444.1000219

Kim MB, Giesler KE, Tahirovic YA, Truax VM, Liotta DC, Wilson LJ (2016) CCR5 receptor antagonists in preclinical to phase II clinical development for treatment of HIV. Expert Opin Investig Drugs 25(12):1377–1392. https://doi.org/10.1080/13543784.2016.1254615

Koltai T (2015) Nelfinavir and other protease inhibitors in cancer: mechanisms involved in anticancer activity. F1000Res 4:9. https://doi.org/10.12688/f1000research.5827.2

Labay E, Mauceri HJ, Efimova EV, Flor AC, Sutton HG, Kron SJ, Weichselbaum R (2016) Repurposing cephalosporin antibiotics as pro-senescent radiosensitizers. Oncotarget 7(23): 33919–33933. https://doi.org/10.18632/oncotarget.8984

Lamb R, Fiorillo M, Chadwick A, Ozsvari B, Reeves KJ, Smith DL, Clarke RB, Howell SJ, Cappello AR, Martinez-Outschoorn UE, Peiris-Pagès M, Sotgia F, Lisanti MP (2015) Doxycycline down-regulates DNA-PK and radiosensitizes tumor initiating cells: implications for more effective radiation therapy. Oncotarget 6(16):14005–14025. https://doi.org/10.18632/oncotarget.4159

Leone S, Cascella M, Pezone I, Fiore M (2019) New antibiotics for the treatment of serious infections in intensive care unit patients. Curr Med Res Opin 35(8):1331–1334. https://doi.org/10.1080/03007995.2019.1583025

Lokhande AS, Devarajan PV (2021) A review on possible mechanistic insights of Nitazoxanide for repurposing in COVID-19. Eur J Pharmacol 891:173748. https://doi.org/10.1016/j.ejphar.2020.173748

Lu S, Ke Y, Wu C, Zhong Y, Xie C, Zhou Y, Zhou F, Yu H (2018) Radiosensitization of clioquinol and zinc in human cancer cell lines. BMC Cancer 18(1):448. https://doi.org/10.1186/s12885-018-4264-2

Maksymiuk AW, Sitar DS, Ahmed R, Cheng B, Bach H, Bagchi RA, Aroutiounova N, Tappia PS, Ramjiawan B (2018) Spermidine/spermine *N1*-acetyltransferase-1 as a diagnostic biomarker in human cancer. Future Sci OA 4(10):FSO345. https://doi.org/10.4155/fsoa-2018-0077

Martin RJ, Robertson AP, Buxton SK, Beech RN, Charvet CL, Neveu C (2012) Levamisole receptors: a second awakening. Trends Parasitol 28(7):289–296. https://doi.org/10.1016/j.pt.2012.04.003

Martinez-Escobar A, Luna-Callejas B, Ramón-Gallegos E (2021) CRISPR-dCas9-based artificial transcription factors to improve efficacy of cancer treatment with drug repurposing: proposal for future research. Front Oncol 10:604948. https://doi.org/10.3389/fonc.2020.604948

Medina-Jiménez AK, Monroy-Torres R (2020) Repurposing individualized nutritional intervention as a therapeutic component to prevent the adverse effects of radiotherapy in patients with cervical cancer. Front Oncol 10:595351. https://doi.org/10.3389/fonc.2020.595351

Meini S, Pagotto A, Longo B, Vendramin I, Pecori D, Tascini C (2020) Role of Lopinavir/ritonavir in the treatment of Covid-19: a review of current evidence, guideline recommendations, and perspectives. J Clin Med 9(7):2050. https://doi.org/10.3390/jcm9072050

Menduti G, Ras DM, Stanga S, Boido M (2020) Drug screening and drug repositioning as promising therapeutic approaches for spinal muscular atrophy treatment. Front Pharmacol 11:592234. https://doi.org/10.3389/fphar.2020.592234

Montalvo-Casimiro M, González-Barrios R, Meraz-Rodriguez MA, Juárez-González VT, Arriaga-Canon C, Herrera LA (2020) Epidrug repurposing: discovering new faces of old acquaintances in cancer therapy. Front Oncol 10:605386. https://doi.org/10.3389/fonc.2020.605386

Montaño-Samaniego M, Bravo-Estupiñan DM, Méndez-Guerrero O, Alarcón-Hernández E, Ibáñez-Hernández M (2020) Strategies for targeting gene therapy in cancer cells with tumor-specific promoters. Front Oncol 10:605380. https://doi.org/10.3389/fonc.2020.605380

Nageshwari B, Merugu R (2017) Effect of levamisole on expression of CD138 and interleukin-6 in human multiple myeloma cell lines. Indian J Cancer 54(3):566–571. https://doi.org/10.4103/ijc.IJC_349_17

Nanda N, Dhawan DK, Bhatia A, Mahmood A, Mahmood S (2016) Doxycycline promotes carcinogenesis & metastasis via chronic inflammatory pathway: an *in vivo* approach. PLoS One 11(3):e0151539. https://doi.org/10.1371/journal.pone.0151539

National Cancer Institute (NCI) (2021) What is cancer. www.cancer.gov. https://www.cancer.gov/about-cancer/understanding/what-is-cancer. Accessed 24 Dec 2021

Nowak-Sliwinska P, Scapozza L, Altaba AR (2019) Drug repurposing in oncology: compounds, pathways, phenotypes and computational approaches for colorectal cancer. Biochim Biophys Acta Rev Cancer 1871(2):434–454. https://doi.org/10.1016/j.bbcan.2019.04.005

Olgen S, Kotra LP (2019) Drug repurposing in the development of anticancer agents. Curr Med Chem 26(28):5410–5427. https://doi.org/10.2174/0929867325666180713155702

Pham T, Ziora ZM, Blaskovich M (2019) Quinolone antibiotics. MedChemComm 10(10):1719–1739. https://doi.org/10.1039/c9md00120d

Qian T, Zhu S, Hoshida Y (2019) Use of big data in drug development for precision medicine: an update. Expert Rev Precis Med Drug Dev 4(3):189–200. https://doi.org/10.1080/23808993.2019.1617632

Reddy AS, Zhang S (2013) Polypharmacology: drug discovery for the future. Expert Rev Clin Pharmacol 6(1):41–47. https://doi.org/10.1586/ecp.12.74

Robinson JR, Denny JC, Roden DM, Van Driest SL (2018) Genome-wide and phenome-wide approaches to understand variable drug actions in electronic health records. Clin Transl Sci 11(2):112. https://doi.org/10.1111/cts.12522

Sahoo BM, Ravi Kumar B, Sruti J, Mahapatra MK, Banik BK, Borah P (2021) Drug repurposing strategy (DRS): emerging approach to identify potential therapeutics for treatment of novel coronavirus infection. Front Mol Biosci 8:628144. https://doi.org/10.3389/fmolb.2021.628144

Scannell JW, Blanckley A, Boldon H, Warrington B (2012) Diagnosing the decline in pharmaceutical R&D efficiency. Nat Rev Drug Discov 11:191–200. https://doi.org/10.1038/nrd3681

Siddiqui M, Rajkumar SV (2012) The high cost of cancer drugs and what we can do about it. Mayo Clin Proc 87(10):935–943. https://doi.org/10.1016/j.mayocp.2012.07.007

Son DS, Lee ES, Adunyah SE (2020) The antitumor potentials of Benzimidazole anthelmintics as repurposing drugs. Immune Netw 20(4):e29. https://doi.org/10.4110/in.2020.20.e29

Strovel J, Sittampalam S, Coussens NP, Hughes M, Inglese J, Kurtz A, Andalibi A, Patton L, Austin C, Baltezor M, Beckloff M (2016) Early drug discovery and development guidelines: for academic researchers, collaborators, and start-up companies. Assay guidance manual [Internet]

Terranova N, Venkatakrishnan K, Benincosa LJ (2021) Application of machine learning in translational medicine: current status and future opportunities. AAPS J 23(4):74. https://doi.org/10.1208/s12248-021-00593-x

Thomas CM, Timson DJ (2020) The mechanism of action of Praziquantel: can new drugs exploit similar mechanisms? Curr Med Chem 27(5):676–696. https://doi.org/10.2174/0929867325666180926145537

Torres-Pérez SA, Torres-Pérez CE, Pedraza-Escalona M, Pérez-Tapia SM, Ramón-Gallegos E (2020) Glycosylated nanoparticles for cancer-targeted drug delivery. Front Oncol 10:605037. https://doi.org/10.3389/fonc.2020.605037

Tsubamoto H, Sonoda T, Yamasaki M, Inoue K (2014) Impact of combination chemotherapy with itraconazole on survival for patients with recurrent or persistent ovarian clear cell carcinoma. Anticancer Res 34(4):2007–2014

Tsubamoto H, Sonoda T, Ikuta S, Tani S, Inoue K, Yamanaka N (2015) Combination chemotherapy with Itraconazole for treating metastatic pancreatic cancer in the second-line or additional setting. Anticancer Res 35(7):4191–4196

Van Nuffel AM, Sukhatme V, Pantziarka P, Meheus L, Sukhatme VP, Bouche G (2015) Repurposing drugs in oncology (ReDO)-clarithromycin as an anti-cancer agent. Ecancermedicalscience 9:513. https://doi.org/10.3332/ecancer.2015.513

Wang M, Zhao J, Zhang L, Wei F, Lian Y, Wu Y, Gong Z, Zhang S, Zhou J, Cao K, Li X, Xiong W, Li G, Zeng Z, Guo C (2017) Role of tumor microenvironment in tumorigenesis. J Cancer 8(5):761–773. https://doi.org/10.7150/jca.17648

Williams JA (2018) Using PDX for preclinical cancer drug discovery: the evolving field. J Clin Med 7(3):41. https://doi.org/10.3390/jcm7030041

World Health Organization [WHO] (2021) Cancer. www.who.int. https://www.who.int/news-room/fact-sheets/detail/cancer. Accessed 24 Dec 2021

Xiong Y, Liu W, Huang Q, Wang J, Wang Y, Li H, Fu X (2018) Tigecycline as a dual inhibitor of retinoblastoma and angiogenesis via inducing mitochondrial dysfunctions and oxidative damage. Sci Rep 8(1):11747. https://doi.org/10.1038/s41598-018-29938-x

Yadav V, Talwar P (2019) Repositioning of fluoroquinolones from antibiotic to anti-cancer agents: an underestimated truth. Biomed Pharmacother 111:934–946. https://doi.org/10.1016/j.biopha.2018.12.119

Yanagisawa N, Satoh T, Tabata KI, Tsumura H, Nasu Y, Watanabe M, Thompson TC, Okayasu I, Murakumo Y, Baba S, Iwamura M (2021) Cytopathic effects and local immune responses in

repeated neoadjuvant HSV-*tk* + ganciclovir gene therapy for prostate cancer. Asian J Urol 8(3): 280–288. https://doi.org/10.1016/j.ajur.2020.06.004

Zaidi AK, Dehgani-Mobaraki P (2021) The mechanisms of action of Ivermectin against SARS-CoV-2: an evidence-based clinical review article. J Antibiot 75:122. https://doi.org/10.1038/s41429-021-00430-5

Zhang Z, Zhou L, Xie N, Nice EC, Zhang T, Cui Y, Huang C (2020) Overcoming cancer therapeutic bottleneck by drug repurposing. Signal Transduct Target Ther 5(1):113. https://doi.org/10.1038/s41392-020-00213-8

Zhong L, Li Y, Xiong L, Wang W, Wu M, Yuan T, Yang W, Tian C, Miao Z, Wang T, Yang S (2021) Small molecules in targeted cancer therapy: advances, challenges, and future perspectives. Signal Transduct Target Ther 6(1):201. https://doi.org/10.1038/s41392-021-00572-w

Zhu HH, Hu J, Gu XF (2016) Arsenic as traditional Chinese medicine provides new hope for overcoming high treatment costs of acute promyelocytic leukemia. J Global Oncol 2(6): 442–443. https://doi.org/10.1200/JGO.2016.005405

Chapter 15
Therapeutic Targeting of Antineoplastic Drugs in Alzheimer's Disease: Discovered in Repurposed Agents

Mini Dahiya, Anil Kumar, Monu Yadav, Pratibha Dhakla, and Shiva Tushir

Abstract The heterogeneity and complexity of various pathological marker and few therapeutics options make Alzheimer's disease (AD) a prime perpetrator to universal health that rationalize the requirement for new and effective drug discovery approaches. Clinical trials of new drugs for AD have consumed more time and resources with large failure of chances. Repurposing of drugs is developing approaches that give a boost to the conventional drug discovery approaches by exploring new therapeutic effects of available drugs. Drug repurposing already verified by FDA for other disorders is a more frequent and exclusive method. The mutual biological process and inverse tuning between cancer and AD give weightage to the concept of repurposing antineoplastic agents as novel therapeutics. Various studies revealed the successful use of antineoplastic drugs as a repurposed approach. The purpose of this review, to study the possible repurposing role of already approved antineoplastic drugs for AD treatment and shed light on the role of various multiple protein kinases in AD. In this, limitation of anticancer drugs such as

M. Dahiya
University Institute of Pharmaceutical Sciences (UIPS), UGC Centre of Advanced Studies (UGC-CAS), Panjab University, Chandigarh, Punjab, India

University Institute of Pharmaceutical Sciences (UIPS), UGC Centre of Advanced Studies (UGC-CAS), Panjab University, Chandigarh, Haryana, India

A. Kumar (✉)
University Institute of Pharmaceutical Sciences (UIPS), UGC Centre of Advanced Studies (UGC-CAS), Panjab University, Chandigarh, Punjab, India

University Institute of Pharmaceutical Sciences, UGC Centre of Advanced Studies (UGC-CAS), Panjab University, Chandigarh, Haryana, India

M. Yadav · P. Dhakla
Department of Pharmacy, School of Medical & Allied Sciences, GD Goenka University, Gurgaon, Haryana, India

S. Tushir
Department of Pharmacy, Panipat Institute of Engineering and Technology (PIET), Samalkha, Panipat, Haryana, India

R. C. Sobti et al. (eds.), *Drug Repurposing for Emerging Infectious Diseases and Cancer*, https://doi.org/10.1007/978-981-19-5399-6_15

their neurotoxic effect are also highlighted. This chapter boosts further research to decode the unseen potential of antineoplastic drugs to develop repurposed agents with negligible toxicity for AD.

Keywords Alzheimer's disease · Antineoplastic drugs · Repurposed agents · MAPK pathway · Wnt pathway

15.1 Introduction

Alzheimer's disease (AD) is one of the major alarming disorders which adversely affect the nervous system and brain. Moreover, the present gap in the understanding of the pathophysiology of AD and the slow pace of new drug development process makes the limited choice of therapeutics draws the attention of research groups and pharmaceutical companies toward exploring new alternatives. Moreover, cancer and AD represent two of the leading causes of mortality and disability worldwide. Both diseases have traditionally been associated with different pathological mechanisms and phenotypic appearances, but researchers have recently been explored their probable common relationship. Moreover, AD is defined as a CNS disorder characterized by abnormal Aβ (amyloid-β) accumulation, tau hyperphosphorylation, oxidative stress, and hyperactivation of microglial cells. Approximately, 35.6 million people are persisting with dementia, and the number is assumed to be three times by the next 30 years (Savva et al. 2019). The World Health Organization (WHO) reports show that in the next 20 years, a neurodegenerative disease affecting cognitive dysfunction will be the second most prevalent reason for death in humans (Rodriguez et al. 2021). The latest data by the Alzheimer's association demonstrated that only five drugs are FDA-approved which are currently used for AD. The failure rate of AD drugs is >99%, and for the disease-modifying therapies, it is 100%. Approximately, more than 20 years has been passed away, there are no novel therapeutics is approved for AD. The development of new drugs is a lengthy and expensive process with a low success rate, approx. 70% of projects probably failed between phase II and phase III of clinical trials (Mottini et al. 2019). Furthermore, various lead compounds are not fully developed to explore their potential due to lack of funds (Kumar et al. 2019). The researchers are doing a continuous effort for developing new therapeutics; one of the examples of this effort is drug repurposing.

Drug repositioning and repurposing is productive way to utilize already approved drugs for different disease sharing common mechanisms of action. This method has been successful in various conditions such as CVS diseases, Parkinson's disease, obesity, cancer, psychosis, irritable bowel syndrome, and AD (Kumar et al. 2017). To promote the use of repurposed drugs, the National Institute of Aging provides grants of $2.8 million to Case Western Reserve University School of Medicine to recognize potential FDA-approved medicines as repurposed agents for AD. The major benefit of drug repurposing is that the toxicology and pharmacokinetic properties of the approved drugs have already been tested. It reduces the time of drug development and cost factors. There are two main approaches adopted for repurposing in which the first approach is to take the drugs for new therapeutic

purposes within the same mechanism for which they are marketed, whereas the second more futuristic approach is to identify new therapeutic targets of the existing drugs. Some repurposed drugs entered clinical trials for neurodegenerative disease specifically for AD. Anticancer, antidiabetic, antimicrobial, antihypertensive, antipsychotic, and antiasthmatic drugs have shown promising results as AD therapeutics (Appleby et al. 2013). Therefore, the chapter aims to indicate the repurposing of approved antineoplastic drugs in clinical trials for AD.

15.2 The Common Shared Link Between Cancer and AD

The major signaling mechanism explored in cancer pathology has considerable links with neurodegenerative diseases. Cancer cells have the potential to grow uncontrollably, whereas, in AD, neurons face premature cell death (Advani et al. 2020). Extensive literature demonstrated that the commonly mutated genes in different neurodegeneration diseases have crosstalk with other genes which are responsible to cause cancer, for example, p53 is the most frequently altered gene in various types of cancers, has also possessed neuroprotective functions (Lanni et al. 2012). Moreover, p53 expression is found to be downstream-regulated in different cancers, whereas it is upstream regulated in AD, Parkinson's disease (PD), and Huntington's disease (HD) (Checler and Alves Da Costa 2014). In the case of AD, amyloid precursor protein (APP) expression is regulated by p53. The C-terminal of an intracellular fragment of APP is found to activate the promoter region of the p53 gene which is responsible for tau phosphorylation. In the case of the cellular stress situation, mouse double minute 2 homolog (MDM2) levels are low followed by elevated levels of p53 and glycogen synthase kinase 3β (GSK3β), which phosphorylates tau protein. Exciting crosstalk exists between p53 and Presenilin (PS) isoforms. P53 expression was reduced by PS1 and increased by overexpressed PS2 (Checler and Dunys 2014; Proctor and Gray 2010). The crosstalk between cancer and AD unlocks new areas for the repurposing of anticancer drugs for neuroprotective effect with some limitations. Various therapeutic are in clinical trials, and some are on the experimental level (Table 15.1). Furthermore, kinase inhibitors have shown the significant effects among other approved antineoplastic agents indicate their potential therapeutic action in neurodegeneration also. Furthermore, the frequently mutated genes associated with AD are described as significant relationships with oncogenes as mentioned in Table 15.2.

15.3 Pathophysiological Pathways Shared Between Cancer and AD Cell Cycle

The cell cycle is a basic cellular process typically classified into four stages:

- G1 (Gap 1) phase.
- S (DNA replication) phase,
- G2 (gap 2) phase.
- M (cell division) phase.

The cell cycle is completely regulated by a series of proteins, i.e., cyclins and cdks (cyclin-dependent kinases). Cancer is developed due to abnormal cell cycle events, characterized by genetic mutation encoding cell cycle proteins (Otto and Sicinski 2017), whereas the neuron cells once differentiated remain in a dormant state for the rest of their lives. In a case stress situation, the mature neurons can re-enter into the

Table 15.1 The list of antineoplastic drugs which are under clinical trial repurposing for neurodegenerative disease

S. no.	Study	Year	Clinical phase	Results	Status
1	Quercetin+ Dasatinib NCT04063124	2020	Phase I/II	Ongoing trial	Recruiting
2	Trametinib (NCT04326283)	2020	Phase I/II	Ongoing trial	Recruiting
3	Daratumumab NCT04070378	2019	Phase II	Ongoing trial	Recruiting
4	Saracatinib NCT03661125	2019	Phase I	Trial is well tolerated at Phase I and further moved toward Phase II. Final outcome is still under experimentations	Recruiting
5	Bosutinib NCT03888222	2019	Phase II	Ongoing trial	Recruiting
6	Rituximab NCT03979456	2018	Phase III	Ongoing trial	Recruiting
7	Imatinib NCT03674099	2018	Phase II	Ongoing trial	Recruiting
8	Nilotinib NCT02947893	2017	Phase II	Ongoing trail	Recruiting
9	Bexarotene NCTO1782742	2013	Phase II	Completed	No amyloid reduction in ApoE4 carriers, the reduction was found in ApoE4 noncarriers. Increased levels of blood lipid levels were found
10	Tamibarotene NCT01120002	2010	Phase II	Unknown	A trial is still under the experimental approach. Phase III still under consideration.
11	Thalidomide NCT01094340	2010	Phase I/II	Unknown	Result unavailable

Table 15.2 Interlinked proteins which are commonly mutated in AD and cancer

Name of protein	Role in AD	Role in cancer	References
P53	Downregulation of PS1, upregulation of GSK3β and tau phosphorylation	Tumor suppressor	Proctor and Gray (2010)
Tau	The major component of neurofibrillary tangles in AD	Down expression in certain tumors	Rossi et al. (2018)
PTEN	Regulation of tau phosphorylation	Tumor suppressor	Domanskyi et al. (2011), Ogino et al. (2016)
APP	Increased amyloid genic processing of APP in AD	Increased nonamyloid genic processing of APP	Kucheryavykh et al. (2019)
ATM	ATM inactivation causes cerebellar neuronal loss, reduced activity in AD brains	Tumor suppressor. Mutated in many cancer types	Choi et al. (2016)
Presenilin	Presenilin constitutes the catalytic core of the γ-secretase complex. Aids in APP processing	PS1 leads to tumor invasion, and metastasis in cancer, loss of function of PS2 promotes lung cancer development, regulation of PTEN	Li et al. (2016), Zhang et al. (2018)
Pin1	Downregulated in AD. Aids in tau dephosphorylation. Regulates APP processing	Overexpressed, Induction of multiple oncogenic pathways	Chen et al. (2018), Xu et al. (2017), Zhou and Lu (2016)
CDK4	Increased expression in AD brains	Expression in various human cancers	Baker and Reddy (2012)
CDK5	Causes AD-related pathophysiology hyperphosphorylation of tau and APP	Associated with tumor proliferation, angiogenesis, chemotherapy resistance, and antitumor immunity	Kimura et al. (2014), Liu et al. (2016), Pozo and Bibb (2016)

cell cycle and it leads to cell death and neurodegeneration (Bonda et al. 2010). Various evidence has demonstrated that the primary role of cell cycle adverse events in various neurodegeneration disorders. The key genes which are indulged in the pathology of AD, such as Presenilin 1 and Presenilin 2 (PS1/2), AβPP, are also marked as the role players in cell cycle regulation. It has been proposed that cell cycle alteration would lead to neurodegeneration and cancer (Seo and Park 2019).

15.3.1 MAPK Pathway

Mitogen-activated protein kinases (MAPKs) regulate various cellular functions like cell differentiation, growth, and cell death. MAPK signaling has three types of major kinases: MAPK, MAPK2K, and MAPK3K. The role of MAPK kinases is widely studied in cancer pathology. Furthermore, ERK kinases-B-Raf and K-Ras mutation

are commonly observed in various human tumors (Advani et al. 2020). Additionally, MAPK has exciting roles in neurodegenerative disorders including AD in which MAPK is responsible for tau phosphorylation which leads to the formation of tau tangle. Mitochondrial dysfunction has a link with AD which is primarily caused by extracellular signal-regulated kinase (ERK), and downregulation of this can restore the mitochondrial alterations in AD (Kim and Choi 2015). Moreover, oxidative stress stimulates JNK (c-Jun N-terminal kinase) and p38 activates the APP expression processing BACE1 enzyme.

15.3.2 Wnt Pathway

Wnt is a key pathway for various cellular processes mostly studied in tumors such as embryonic development, cellular differentiation, and tissue development. Wnt pathway stimulation supports tumor growth and subsequently protects against neurodegeneration (Behrens et al. 2009). Moreover, the Wnt pathway is abnormally expressed in various cancers and is downregulated in AD. It has a protective role in AD pathology by inhibiting Aβ induces neurotoxic effects. Wnt ligands and frizzled receptors expression are found to be downstream-regulated inpatient brain having AD (Folke et al. 2019; Palomer et al. 2019).

15.3.3 Redox Signaling Pathway

Redox homeostasis plays an important role in the cellular process, and any aberrant change in the signaling processes leads to neurodegeneration, aging, and cancer. Oxidative stress promotes cancer induction via DNA damage, DNA alteration, and cancer metastasis. AD is positively linked with oxidative stress and altered redox mechanisms, for example, oxidative stress stimulates BACE1 (β-secretase 1) release and Aβ synthesis, which further causes oxidative stress. Various metals such as Fe (iron), Cu (copper), and Zn (zinc) contents are found to increase in amyloid plaques as compared to the surrounding tissues (Advani et al. 2020).

15.3.4 PI3K/AKT/mTOR Pathway

The PI3K/AKT/mTOR signaling pathway is crucial for an array of cellular functions such as cell survival, growth, and metabolism. PI3K family is consisted of catalytic subunits (p110α, p110β, p110γ, and p110δ) and non-catalytic or regulatory subunits (p87, p85, and p101) (Kobayashi et al. 2020). PI3K signaling is marked as the major regulator of cancer and this pathway is altered in various human tumors via different ways like PTEN inactivation, PI3K mutation, and activation of upstream regulator of

PI3K (Yang et al. 2019). PI3K/AKT/mTOR pathways are also important for neuronal survival. In Alzheimer, the PI3K pathway controls cell survival, neurogenesis, oxidative stress, Aβ metabolism, and tau phosphorylation. Aβ possesses a neurotoxic effect by inhibiting the PI3K signaling, whereas PI3K activator may provide neuroprotection by activating the PI3K pathway (O'Neill 2013). A study proposed the role of the PI3K/AKT/mTOR pathway in Aβ25–35-induced autophagy.

15.3.5 Anticancer Agents Can Be Repurposed for AD

Various neurotoxins like glutamate, amyloid-β, rotenone, β-N-Methylamino-L-alanine, 3-Nitropropionic acid, 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine, 1-methyl-4-phenylpyridinium, nitric oxide (NO), and ROS are responsible for neuronal injury and toxicity via different ways like apoptosis, mitochondrial dysfunction, autophagy clearance, and oxidative stress. However, some antineoplastic agents are recognized to date, which can overcome the adverse effects of these neurotoxins and aids in neuroprotection. NO is a neurotransmitter, which is essential for healthy brain functioning (Advani et al. 2020). But if it is excessively produced in the brain, it leads to neurodegenerative disease. In AD, Aβ activates NO production, which leads to mitochondrial dysfunction and neurotoxicity. Moreover, oxidative stress caused by reactive oxygen species (ROS) releases free radicals that contribute to disease by affecting various cellular functions (Niedzielska et al. 2016). The intracellular accumulation of Aβ oligomers exerts their toxic effects via tau hyperphosphorylation, proteasome dysfunction, lipid peroxidation, and endothelial cell damage (Lublin and Gandy 2010). Anticancer drugs such as bexarotene, nilotinib, tamibarotene, thalidomide, carmustin dactolisib, epothilone D, lonafarnib, paclitaxel, sunitinib, and saracatinib can decrease toxicity induced by various pathological markers of AD as summarized in Table 15.3. Moreover, another factor contributing to neuronal toxicity is the microglial cell which is phagocytic cells of the brain but depending on the environmental situation they possess neurotoxic effects. The microglial cell produced several ROS like NO, hydrogen peroxide, peroxynitrite, and superoxide that leads to oxidative damage along with this these cells are also responsible for excito-neurotoxicity by releasing glutamate (Bellozi et al. 2019).

15.3.6 Bexarotene

The two retinoid X receptor (RXR) agonist like bexarotene and tamibarotene exhibited neuroprotective properties. Bexarotene is a compound currently used to treat cutaneous T-cell lymphomas and has shown promising results in AD mouse models. Bexarotene's mechanism of action is retinoid X receptor agonist, and it induces changes in gene expression that is responsible for cellular differentiation, suppress cell growth, apoptosis, and inhibition of tumor growth along with this it

Table 15.3 Neuroprotective effects of various antineoplastic agents in AD

Drugs	Drug class	Cancer (MOA)	AD (MOA)	Pathways involved	References
Bexarotene	Retenoid X receptor agonist	Inhibits cell cycle progression, prevents multidrug resistance, inhibits angiogenesis and metastasis	Reduces Aβ	P53/p73 pathways	Dickey et al. (2017)
Imatinib	Tyrosine kinase inhibitor	Inhibits leukemogenesis by targeting downstream signaling of Abl kinase	Inhibition of γ-secretase activity	JAK-STAT, Ras/MAPK, PI3K-Akt,	Cuny (2009)
Carmustin	Alkylatin g agent, DNA crosslinking agent	Tumor growth inhibitor. Inhibit DNA replication and transcription.	Reduces Aβ production	DNA synthesis pathway	Hayes et al. (2013)
Dactolisib	PI3K and mTOR inhibitor	Inhibits autophagy, interferes with DNA repair, and stops the proliferation of cancer cells	Reduced memory impairment, decreases microglial activation, and lowers IL-10 levels	PI3K/Akt/mTOR pathway	Bellozi et al. (2019), Brinkman et al. (2020), Ediriweera et al. (2019)
Dabrafenib	Tyrosine kinase inhibitor	Inhibits MAPK signaling and causes cell cycle arrest	Inhibits amyloid-dependent microgliosis	JAK-STAT, MAPK, and PI3K-Akt pathway	Keating (2017)
Epothilone D	Microtubule-stabilizing agent	Stops cell cycle by binding to tubulin in cancer cells leading to apoptosis	Reduced axonal dystrophy and increases axonal microtubule density improving axonal transport and cognitive function	Cell cycle	Cheng and Huang (2018)
Lonafarnib	Farnesyl transferase inhibitor	Blocks post-translational modification of Ras and inactivates it	Activates lysosomes and decreases tau pathology	Rhes pathway	Hernandez et al. (2019)
Nilotinib	Tyrosine kinase receptor	Antiproliferative action by inhibiting different tyrosine kinases	Reduction of Aβ	JAK-STAT, MAPK, and PI3K-Akt pathway	Tanabe et al. (2014)

Paclitaxel	Microtub ule inhibitor, Bcl-2 inhibitor	Inhibits cell cycle progression by inducing mitotic arrest	Neuroprotecti ve, reduction in tau hyperphosphorylation	PI3K/AKT, MAPK, and EGFR pathway	Weaver (2014)
Pazopanib	Tyrosine kinase inhibitor	Inhibits Raf-MAPK/ERK pathway	Acetylcholines terase inhibitor, reduces tau hyperphosphorylation	JAK-STAT, MAPK, and PI3K-Akt pathway	Javidnia et al. (2017)
Sunitinib	Tyrosine kinase inhibitor	Stops tumor cell proliferation and angiogenesis	Acetylcholine sterase inhibitor, an angiogenesis inhibitor, inhibits nitric oxide production	JAK-STAT, MAPK, and PI3K-Akt pathway	Huang et al. (2016)
Saracatinib	Src and Bcr-Abl tyrosine-kinase inhibitor	Anti-invasive and antitumor	Rescues spatial memory deficits and synapse loss	JAK-STAT, MAPK, and PI3K-Akt pathway	Kaufman et al. (2015)
Tamibarotene	Retinoid X receptor agonist	Inhibits retinoid signaling	Reduction in Aβ, reduction in pro-inflammatory cytokines & chemokines	Retinoid signal-ing pathways	Fukasawa et al. (2012)
Thalidomide	TNF alpha inhibitor, an angiogenesis inhibitor	Inhibits angiogenesis and cytokine production. Immunomodulat ion.	Reduction of Aβ, microglial activa-tion, beta secretase 1 (BACE1) enzyme inhibition, reduction in pro-inflammatory TNF-α	Ubiquitin/Pr oteasome System	He et al. (2013)

also affects the activation of apolipoprotein E (ApoE) expression via affecting liver X receptor–retinoid X receptor complexes (Rodriguez et al. 2021). It has been reported that oral administration of bexarotene in an AD model resulted in the removal of amyloid-beta (Aβ) in an apolipoprotein E (ApoE)-dependent manner. ApoE glycoproteins are primarily expressed in the brain and liver. In the brain, microglia and astrocytes have an expression of ApoE protein. It has function as an Aβ binding protein and also increases Aβ accumulation in amyloid plaques. Bexarotene increases Aβ clearance via peroxisome proliferator-activated receptor gamma-retinoid X receptor (PPARγ-RXR) and liver X receptor-retinoid X receptor (LXR:RXR) and facilitate expression of ApoE, ATP-binding cassette transporter 1 (ABCA1), and ATP binding cassette subfamily G member 1 (ABCG1) genes. In an in-vivo study, bexarotene (300 mg) was given to two different groups, for example, ApoE noncarriers and ApoE carriers. It reduced Aβ plaque deposition in apoE4 noncarriers. The authors reported that the Aβ plaques in the ApoE4 carriers group are difficult to solubilize due to their compression (Cummings et al. 2016). A study demonstrated that age-dependent critical concentration of bexarotene could reverse brain cell damage in APP/PS1 mice (Rosenthal et al. 2016). Work on the *C. elegans* model suggested that bexarotene interfered with the primary nucleation of Aβ-42 aggregation (Habchi et al. 2016). Bexarotene is an excellent drug for further drug development in AD treatment given its high blood–brain barrier (BBB) permeability and safety profile.

15.3.7 Tamibarotene (Am80)

It is a retinoic acid receptor (RAR) α/β agonist approved in Japan for the treatment of acute promyelocytic leukemia (APL). Tamibarotene, a multiple-targeted drug, may be proven as a potential agent for the treatment of AD. In-vivo study on mice (APP23) identified that tamibarotene decreases extracellular insoluble Aβ, but no effects were seen in the soluble Aβ levels. Reduction in extracellular Aβ may be due to increased level of α-secretase transcription and phagocytosis process of microglia (Advani et al. 2020).

15.3.8 Nilotinib

In-vivo study on nilotinib demonstrated that it activates autophagy and helps to decrease Aβ and tau phosphorylation in AD brains. It blocked c-Abl tyrosine kinase and helps to regulate parkin-beclin1 communication that leads to the removal of phosphorylated tau and Aβ. Moreover, another study on embryonic stem cells of human-derived AD models demonstrated that nilotinib has the potential to improve synaptic dysfunction and elevate the expression of Ras-related protein Rab-3A (RAB3A). Additionally, clinical trail have been held at georgetown university

(2017) to explore the mechanism of nilotinib in the removal of Aβ plaques and tau tangles in the brain of the AD patient (Lonskaya et al. 2014; Nishioka et al. 2016).

15.3.9 Thalidomide

Primarily this drug is utilized as a sedative and it is majorly known for its teratogenic effects. It is approved for the treatment of severe erythema nodosum leprosum and multiple myeloma. A study has shown that 3, 6′ dithalidomide (3, 6-DT) reduced various markers of AD such as Aβ accumulation, tau phosphorylation, Aβ plaque number, and cognitive deficits in AD mice. Co-administration of both drugs for example thalidomide and 3, 6-DT decreases the activation of microglia cells as well as the release of ROS and proteolytic enzymes produced by a microglial cell that leads to increasing the processing of amyloid precursor proteins into insoluble Aβ peptide (Tweedie et al. 2012). Furthermore, the in-vitro study of thalidomide reported that thalidomide inhibits vascular changes such as cell growth, angiogenesis, and breakdown of BBB along with affecting cerebral microvasculature, thalidomide also blocked astrogliosis and decrease hippocampal neuronal loss via inhibiting TNF-α. National institute of health has conducted 24-week, randomized, double blinded, placebo-controlled phase II trial on 185 humans having mild-to-moderate AD. The result has been shown that the thalidomide at the maximum dose of 400 mg/day decreases the amyloidogenesis process. These data demonstrated that there was no significant cognitive dysfunction in the thalidomide-treated group (Decourt et al. 2017). Therefore, thalidomide may have anti-AD effects via antiangiogenic, anti-inflammatory, and neuroprotective mechanisms.

15.3.10 Imatinib (Gleevec)

Imatinib decrease Aβ levels via inhibiting the γ-secretase enzyme indirectly and by producing APP variants. It is an approved drug by FDA for the management of chronic leukemia and gastrointestinal stromal cancer, targets the Bcr–Abl complex, and binds with the ATP-binding site of c-ABL and other tyrosine kinases. Various in-vitro and in-vivo studies have suggested that it may have a therapeutic effect in AD. It reduced Aβ (β-amyloid) production in AD models when treated with imatinib along with its neuroprotective effects. Although the mild cardiotoxic effect has been demonstrated there have been no major adverse effects reported with imatinib's use in humans. The primary limitation of imatinib use for the treatment of AD is its low cerebral penetrability so that it is readily removed from the CNS by glycoprotein-p (Netzer et al. 2017). Therefore, acetylcholinesterase inhibitors are the majority of explored drugs for the treatment of AD.

15.3.11 Sunitinib

It is an antineoplastic agent approved by the FDA for the management of metastatic carcinoma of renal cells and imatinib-resistant GIT cancer, which showed success as an anti- Ach esterase drug. Furthermore, sunitinib may act as a potential drug for the treatment of AD, moreover, sunitinib have shown the significant cognitive improvement in tg2576 and 3xTgAD animal models of AD (Grammas et al. 2014). A study has been shown that in the scopolamine-induced AD mouse model, sunitinib reduces the acetylcholine esterase activity. Molecular docking analysis of sunitinib reveals the interaction with the catalytic and peripheral anion site of acetylcholine esterase (Huang et al. 2016). Moreover, it was also investigated in HIV models of neurotoxicity that sunitinib inhibited CDK5 activity and tau hyper-phosphorylation (Wrasidlo et al. 2014). Sunitinib is considered an antiangiogenic agent and can be used for AD therapeutics for neo-angiogenesis and for hypervascularization which is associated with pathological conditions of AD. Sunitinib can alter the levels of Aβ secreted from endothelial cells by inhibiting VEGF signaling (Jefferies et al. 2013).

15.3.12 Pazopanib

Pazopanib is a tyrosine kinase inhibitor and also inhibits Ach esterase leads to restoration of cognitive dysfunction that is similar to donepezil. A study has shown that pazopanib suppresses tau hyperphosphorylation and also altered astrocytes activity in the AD mouse model (Javidnia et al. 2017; Yang et al. 2019).

15.3.13 Carmustine (BCNU)

Carmustine is a FDA-approved drug for various types of cancer such as metastatic brain tumors, myelomas, and lymphomas. It is an alkylating agent, responsible for cell cycle arrest, DNA disruption, and apoptosis. A study revealed that carmustine reduced the Aβ level by altering APP trafficking and cleavage. In vivo and in vitro study of carmustine is independent of the α, β, and ϒ secretase enzymes. The prime benefit of carmustine is that there are no side effects observed with its secretase inhibitors property, and it has also a good blood–brain barrier (BBB) permeability and therefore, carmustine can be a potential anti-Aβ drug (Hayes et al. 2013).

15.3.14 Paclitaxel (Taxol)

It is a microtubule-stabilizing agent, FDA approved for the treatment of breast cancer, ovarian carcinoma, AIDS-related Kaposi's sarcoma, and non–small-cell lung cancer. Studies reported its therapeutic potential in AD and tauopathies. It altered the stability of the microtubule via binding to the β subunit of tubulin. Researches have revealed that it blocks the cell division process and induces apoptosis in cancerous cells (Brunden et al. 2011). A study by Angiotech Pharmaceuticals reported that paclitaxel has favorable effects on movement disorders. Moreover, a group of researchers conducted a study to confirm the Taxol role in neuroprotection. Additionally, further study revealed that paclitaxel decreases Aβ toxicity via preventing Aβ-induced stimulation of calpain, which decreases the proteolysis of p35 to p25 and suppresses activation of CDK5/p25 complex. The suppression of CDK5/p25 complex activity helps reduce tau phosphorylation and disease prognosis (Li et al. 2003). Phosphorylated tau (p-tau) protein decreases tau's potential to bind microtubules and increase fibrillization. Like imatinib, paclitaxel is also a glycoprotein-p substrate and has poor CNS penetration. Paclitaxel's neuroprotective effect is related to microtubule stabilization, decreasing tau phosphorylation, and improving its function along with inhibition of Aβ-induced stimulation of the cystolic cdk5–p25 complex (Advani et al. 2020)

15.4 Conclusion and Future Perspective

AD and cancer share a common mechanism of protein and genes involved in various signaling pathways. This chapter has been focused on those genes and signaling pathways that shared common pathways in the two most threatening disorders. Drug repurposing is an electrifying opportunity for a new drug development approach for AD. Recently, antineoplastic agents are seeking more attraction from researchers for drug repositioning for AD. Based on available data, antineoplastic drugs have potential neuroprotective effects using various ways such as removal of the aggregated toxic protein, inhibits neuroinflammation, and immunomodulatory effects. Some classes of drugs exhibit excellent repurposing activity for example kinase inhibitors, alkylating agents, antimetabolites, and antibodies among these, kinase inhibitors are more gaining focus to date. Additionally, repurposing of drugs significantly reduced the time and cost of drug development process because they have already been explored for their safety; therefore, there is no need of the further preclinical studies. Protein kinases have been recognized to play a central role in various pathophysiology related to AD. Moreover, animal model and cellular studies have reported the accomplishment of these for AD and have fortified their repurposing effects. Although the complete molecular pathways of these drugs in CNS disorder are still unknown and demand further investigations. Furthermore, there is a need for certain neurotoxic effects of antineoplastic drugs taken into

consideration are the biggest challenge in the drug repurposing process. But there is a need for further in vitro and in vivo studies to explore the exact mechanistic pathways, along with their adverse effect when they are used for repurposing. Furthermore, the dual nature of some antineoplastic agents is also a matter of future research to explore the neurotoxic as well as neuroprotective effects. Therefore, the repurposing of chemotherapeutic agents for AD opens new doors in context with the urgent need for drug development. There are 53 clinical trials included 58 FDA-approved agents acting on various therapeutic targets like neuroprotection, neuroinflammation, neurotransmission alteration were registered in the ClinicalTrials.gov, accounting for 39% of the overall AD pipeline. Furthermore, since 2019, the number of phase III studies targeting Aβ decrease by 20%. Therefore, there is a need of using animal as well as computational techniques along with effective clinical trial to explore the safety, tolerability and therapeutic potential of antitumor agents for AD in the nearby future.

References

Advani D, Gupta R, Tripathi R, Sharma S, Ambasta RK, Kumar P (2020) Protective role of anticancer drugs in neurodegenerative disorders: a drug repurposing approach. Neuro Int 24: 104841. https://doi.org/10.1016/j.neuint.2020.104841

Appleby BS, Nacopoulos D, Milano N, Zhong K, Cummings JL (2013) A review: treatment of Alzheimer's disease discovered in repurposed agents. Dement Geriatric Cogn Disord 35(1–2): 1–22. https://doi.org/10.1159/000345791

Baker SJ, Reddy EP (2012) CDK4: a key player in the cell cycle, development, and cancer. Genes Cancer. https://doi.org/10.1177/1947601913478972

Behrens M, Lendon C, Roe C (2009) A common biological mechanism in cancer and Alzheimer's disease? Curr Alzheimer Res. https://doi.org/10.2174/156720509788486608

Bellozi PMQ, Gomes GF, de Oliveira LR, Olmo IG, Vieira ELM, Ribeiro FM, Fiebich BL, de Oliveira ACP (2019) NVP-BEZ235 (dactolisib) has protective effects in a transgenic mouse model of Alzheimer's disease. Front Pharmacol 10:1345. https://doi.org/10.3389/fphar.2019.01345

Bonda DJ, Wang X, Perry G, Nunomura A, Tabaton M, Zhu X, Smith MA (2010) Oxidative stress in Alzheimer disease: a possibility for prevention. Neuropharmacology 59(4-5):290–294

Brinkman JA, Liu Y, Kron SJ (2020) Small-molecule drug repurposing to target DNA damage repair and response pathways. Semin Cancer Biol. https://doi.org/10.1016/j.semcancer.2020.02.013

Brunden KR, Yao Y, Potuzak JS, Ferrer NI, Ballatore C, James MJ, Hogan AML, Trojanowski JQ, Smith AB, Lee VMY (2011) The characterization of microtubule-stabilizing drugs as possible therapeutic agents for Alzheimer's disease and related tauopathies. Pharmacol Res. https://doi.org/10.1016/j.phrs.2010.12.002

Checler F, Alves Da Costa C (2014) P53 in neurodegenerative diseases and brain cancers. Pharmacol Ther. https://doi.org/10.1016/j.pharmthera.2013.11.009

Chen Y, Wu YR, Yang HY, Li XZ, Jie MM, Hu CJ, Wu YY, Yang SM, Yang YB (2018) Prolyl isomerase Pin1: a promoter of cancer and a target for therapy. Cell Death Dis 9:883. https://doi.org/10.1038/s41419-018-0844-y

Cheng H, Huang G (2018) Synthesis and antitumor activity of epothilones B and D and their analogs. Future Med Chem. https://doi.org/10.4155/fmc-2017-0320

Choi M, Kipps T, Kurzrock R (2016) ATM mutations in cancer: therapeutic implications. Mol Canc Therapeut. https://doi.org/10.1158/1535-7163.mct-15-0945

Cummings JL, Zhong K, Kinney JW, Heaney C, Moll-Tudla J, Joshi A, Pontecorvo M, Devous M, Tang A, Bena J (2016) Double-blind, placebo-controlled, proof-of-concept trial of bexarotene Xin moderate Alzheimer's disease. Alzheimers Res Ther. https://doi.org/10.1186/s13195-016-0173-2

Cuny G (2009) Kinase inhibitors as potential therapeutics for acute and chronic neurodegenerative conditions. Curr Pharmaceut Des 15:3919–3939. https://doi.org/10.2174/138161209789649330

Decourt B, Drumm-Gurnee D, Wilson J, Jacobson S, Belden C, Sirrel S, Ahmadi M, Shill H, Powell J, Walker A, Gonzales A, Macias M, Sabbagh M (2017) Poor safety and tolerability hamper reaching a potentially therapeutic dose in the use of thalidomide for Alzheimer's disease: results from a double-blind, placebo-controlled trial. Curr Alzheimer Res. https://doi.org/10.2174/1567205014666170117141330

Dickey AS, Sanchez DN, Arreola M, Sampat KR, Fan W, Arbez N, Akimov S, Van Kanegan MJ, Ohnishi K, Gilmore-Hall SK, Flores AL, Nguyen JM, Lomas N, Hsu CL, Lo DC, Ross CA, Masliah E, Evans RM, La Spada AR (2017) PPARΔ activation by bexarotene promotes neuroprotection by restoring bioenergetic and quality control homeostasis. Sci Transl Med. https://doi.org/10.1126/scitranslmed.aal2332

Domanskyi A, Geißler C, Vinnikov IA, Alter H, Schober A, Vogt MA, Gass P, Parlato R, Schütz G (2011) Pten ablation in adult dopaminergic neurons is neuroprotective in Parkinson's disease models. FASEB J. https://doi.org/10.1096/fj.11-181958

Ediriweera MK, Tennekoon KH, Samarakoon SR (2019) Role of the PI3K/AKT/ mTOR signaling pathway in ovarian cancer: biological and therapeutic significance. Semin Canc Biol. https://doi.org/10.1016/j.semcancer.2019.05.012

Folke J, Pakkenberg B, Brudek T (2019) Impaired Wnt signaling in the prefrontal cortex of Alzheimer's disease. Mol Neurobiol. https://doi.org/10.1007/s12035-018-1103-z

Fukasawa H, Nakagomi M, Yamagata N, Katsuki H, Kawahara K, Kitaoka K, Miki T, Shudo K (2012) Tamibarotene: a candidate retinoid drug for Alzheimer's disease. Biol Pharm Bull

Grammas P, Martinez J, Sanchez A, Yin X, Riley J, Gay D, Desobry K, Tripathy D, Luo J, Evola M, Young A (2014) J Alzheim Dis. https://doi.org/10.3233/JAD-2014-132057

Habchi J, Arosio P, Perni M, Costa AR, Yagi-Utsumi M, Joshi P, Chia SR, Cohen SIA, Müller MBD, Linse S, Nollen EAA, Dobson CM, Knowles TPJ, Vendruscolo M (2016) An anti-cancer drug suppresses the primary nucleation reaction that initiates the formation of toxic Aβ aggregates associated with Alzheimer's disease. Sci Adv. https://doi.org/10.1126/sciadv.1501244

Hayes CD, Dey D, Palavicini JP, Wang H, Patkar KA, Minond D, Nefzi A, Lakshmana MK (2013) Striking reduction of amyloid plaque burden in an Alzheimer's mouse model after chronic administration of carmustine. BMC Med. https://doi.org/10.1186/1741-7015-11-81

He P, Cheng X, Staufenbiel M, Li R, Shen Y (2013) Long-term treatment of thalidomide ameliorates amyloid-like pathology through inhibition of β-secretase in a mouse model of Alzheimer's disease. PLoS One. https://doi.org/10.1371/journal.pone.0055091

Hernandez I, Luna G, Rauch JN, Reis SA, Giroux M, Karch CM, Boctor D, Sibih YE, Storm NJ, Diaz A, Kaushik S, Zekanowski C, Kang AA, Hinman CR, Cerovac V, Guzman E, Zhou H, Haggarty SJ, Goate AM, Fisher SK, Cuervo AM, Kosik KS (2019) A farnesyltransferase inhibitor activates lysosomes and reduces tau pathology in mice with tauopathy. Sci Transl Med 11:eaat3005. https://doi.org/10.1126/scitranslmed.aat3005

Huang L, Lin J, Xiang S, Zhao K, Yu J, Zheng J, Xu D, Mak S, Hu S, Nirasha S, Wang C, Chen X, Zhang J, Xu S, Wei X, Zhang Z, Zhou D, Zhou W, Cui W, Han Y, Hu Z, Wang Q (2016) Sunitinib, a clinically used anticancer drug, is a potent AChE inhibitor and attenuates cognitive impairments in mice. ACS Chem Neurosci. https://doi.org/10.1021/acschemneuro.5b00329

Javidnia M, Hebron ML, Xin Y, Kinney NG, Moussa CEH (2017) Pazopanib reduces phosphorylated tau levels and alters astrocytes in a mouse model of tauopathy. J Alzheim Dis. https://doi.org/10.3233/JAD-170429

Jefferies WA, Price KA, Biron KE, Fenninger F, Pfeifer CG, Dickstein DL (2013) Adjusting the compass: new insights into the role of angiogenesis in Alzheimer's disease. Alzheimers Res Ther. https://doi.org/10.1186/alzrt230

Kaufman AC, Salazar SV, Haas LT, Yang J, Kostylev MA, Jeng AT, Robinson SA, Gunther EC, Van Dyck CH, Nygaard HB, Strittmatter SM (2015) Fyn inhibition rescues established memory and synapse loss in Alzheimer's mice. Ann Neurol 77:953–971. https://doi.org/10.1002/ana.24394

Keating GM (2017) Dasatinib: a review in chronic myeloid leukaemia and Ph+ acute lymphoblastic leukaemia. Drugs. https://doi.org/10.1007/s40265-016-0677-x

Kim EK, Choi EJ (2015) Compromised MAPK signaling in human diseases: an update. Arch Toxicol. https://doi.org/10.1007/s00204-015-1472-2

Kimura T, Ishiguro K, Hisanaga S (2014) Physiological and pathological phosphorylation of tau by Cdk5. Front Mol Neurosci. https://doi.org/10.3389/fnmol.2014.00065

Kobayashi Y, Lim SO, Yamaguchi H (2020) Oncogenic signaling pathways associated with immune evasion and resistance to immune checkpoint inhibitors in cancer. Semin Canc Biol. https://doi.org/10.1016/j.semcancer.2019.11.011

Kucheryavykh LY, Ortiz-Rivera J, Kucheryavykh YV, Zayas-Santiago A, Diaz- Garcia A, Inyushin MY (2019) Accumulation of innate amyloid beta-peptide in glioblastoma tumors. Int J Mol Sci. https://doi.org/10.3390/ijms20102482

Kumar S, Chowdhury S, Kumar S (2017) In silico repurposing of antipsychotic drugs for Alzheimer's disease. BMC Neurosci. https://doi.org/10.1186/s12868-017-0394-8

Kumar R, Harilal S, Gupta SV, Jose J, Thomas DG, Uddin MS, Shah MA, Mathew B (2019) Exploring the new horizons of drug repurposing: a vital tool for turning hard work into smart work. Eur J Med Chem. https://doi.org/10.1016/j.ejmech.2019.111602

Lanni C, Racchi M, Memo M, Govoni S, Uberti D (2012) P53 at the crossroads between cancer and neurodegeneration. Free Radic Biol Med. https://doi.org/10.1016/j.freeradbiomed.2012.02.034

Li G, Faibushevich A, Turunen BJ, Yoon SO, Georg G, Michaelis ML, Dobrowsky RT (2003) Stabilization of the cyclin-dependent kinase 5 activator, p35, by paclitaxel decreases β-amyloid toxicity in cortical neurons. J Neurochem. https://doi.org/10.1046/j.1471-4159.2003.01526.x

Li P, Lin X, Zhang J-R, Li Y, Lu J, Huang F-C, Zheng C-H, Xie J-W, Wang J-B, Huang C-M (2016) The expression of presenilin 1 enhances carcinogenesis and metastasis in gastric cancer. Oncotarget. https://doi.org/10.18632/oncotarget.7298

Liu SL, Wang C, Jiang T, Tan L, Xing A, Yu JT (2016) The role of Cdk5 in Alzheimer's disease. Mol Neurobiol. https://doi.org/10.1007/s12035-015-9369-x

Lonskaya I, Hebron ML, Desforges NM, Schachter JB, Moussa CEH (2014) Nilotinib-induced autophagic changes increase endogenous parkin level and ubiquitination, leading to amyloid clearance. J Mol Med. https://doi.org/10.1007/s00109-013-1112-3

Lublin AL, Gandy S (2010) Amyloid-β oligomers: possible roles as key neurotoxins in Alzheimer's disease. Mt Sinai J Med. https://doi.org/10.1002/msj.2016

Mottini C, Napolitano F, Li Z, Gao X, Cardone L (2019) Computer-aided drug repurposing for cancer therapy: approaches and opportunities to challenge anticancer targets. Semin Canc Biol. https://doi.org/10.1016/j.semcancer.2019.09.023

Netzer WJ, Bettayeb K, Sinha SC, Flajolet M, Greengard P, Bustos V (2017) Gleevec shifts APP processing from a β-cleavage to a nonamyloidogenic cleavage. Proc Natl Acad Sci. https://doi.org/10.1073/pnas.1620963114

Niedzielska E, Smaga I, Gawlik M, Moniczewski A, Stankowicz P, Pera J, Filip M (2016) Oxidative stress in neurodegenerative diseases. Mol Neurobiol. https://doi.org/10.1007/s12035-015-9337-5

Nishioka H, Tooi N, Isobe T, Nakatsuji N, Aiba K (2016) BMS-708163 and Nilotinib restore synaptic dysfunction in human embryonic stem cell-derived Alzheimer's disease models. Sci Rep. https://doi.org/10.1038/srep33427

O'Neill C (2013) PI3-kinase/Akt/mTOR signaling: impaired on/off switches in aging, cognitive decline and Alzheimer's disease. Exp Gerontol 48:647–653. https://doi.org/10.1016/j.exger.2013.02.025

Ogino M, Ichimura M, Nakano N, Minami A, Kitagishi Y, Matsuda S (2016) Roles of PTEN with DNA repair in Parkinson's disease. Int J Mol Sci. https://doi.org/10.3390/ijms17060954

Otto T, Sicinski P (2017) Cell cycle proteins as promising targets in cancer therapy. Nat Rev Canc. https://doi.org/10.1038/nrc.2016.138

Palomer E, Buechler J, Salinas PC (2019) Wnt signaling deregulation in the aging and Alzheimer's brain. Front Cell Neurosci 13:227. https://doi.org/10.3389/fncel.2019.00227

Pozo K, Bibb JA (2016) The emerging role of Cdk5 in cancer. Trends Canc. https://doi.org/10.1016/j.trecan.2016.09.001

Proctor CJ, Gray DA (2010) GSK3 and p53—is there a link in Alzheimer's disease? Mol Neurodegener. https://doi.org/10.1186/1750-1326-5-7

Rodriguez S, Hug C, Todorov P, Moret N, Boswell SA, Evans K, Zhou G, Johnson NT, Hyman BT, Sorger PK, Albers MW (2021) Machine learning identifies candidates for drug repurposing in Alzheimer's disease. Nat Commun 12(1):1–3. https://doi.org/10.1038/s41467-021-21330-0

Rosenthal ES, Karchmer AW, Theisen-Toupal J, Castillo RA, Rowley CF (2016) Suboptimal addiction interventions for patients hospitalized with injection drug use-associated infective endocarditis. Am J Med 129(5):481–485

Rossi G, Redaelli V, Contiero P, Fabiano S, Tagliabue G, Perego P, Benussi L, Bruni AC, Filippini G, Farinotti M, Giaccone G, Buiatiotis S, Manzoni C, Ferrari R, Tagliavini F (2018) Tau mutations serve as a novel risk factor for cancer. Can Res 78:3731–3739. https://doi.org/10.1158/0008-5472.CAN-17-3175

Savva, K., Zachariou, M., Oulas, A., Minadakis, G., Sokratous, K., Dietis, N., Spyrou, G. M., 2019. Computational drug repurposing for neurodegenerative diseases. In: Silico drug design. Elsevier Inc. doi: https://doi.org/10.1016/b978-0-12-816125-8.00004-3.

Seo J, Park M (2019) Molecular crosstalk between cancer and neurodegenerative diseases. Cell Mol Life Sci. https://doi.org/10.1007/s00018-019-03428-3

Tanabe A, Yamamura Y, Kasahara J, Morigaki R, Kaji R, Goto S (2014) A novel tyrosine kinase inhibitor AMN107 (nilotinib) normalizes striatal motor behaviors in a mouse model of Parkinson's disease. Front Cell Neurosci. https://doi.org/10.3389/fncel.2014.00050

Tweedie D, Ferguson RA, Fishman K, Frankola KA, Van Praag H, Holloway HW, Luo W, Li Y, Caracciolo L, Russo I, Barlati S, Ray B, Lahiri DK, Bosetti F, Greig NH, Rosi S (2012) Tumor necrosis factor-α synthesis inhibitor 3,6′- dithiothalidomide attenuates markers of inflammation, Alzheimer pathology and behavioral deficits in animal models of neuroinflammation and Alzheimer's disease. J Neuroinflammation. https://doi.org/10.1186/1742-2094-9-106

Weaver BA (2014) How Taxol/paclitaxel kills cancer cells. Mol Biol Cell 25:2677–2681. https://doi.org/10.1091/mbc.e14-04-0916

Wrasidlo W, Crews LA, Tsigelny IF, Stocking E, Kouznetsova VL, Price D, Paulino A, Gonzales T, Overk CR, Patrick C, Rockenstein E, Masliah E (2014) Neuroprotective effects of the anti-cancer drug sunitinib in models of HIV neurotoxicity suggests potential for the treatment of neurodegenerative disorders. Br J Pharmacol. https://doi.org/10.1111/bph.12875

Xu L, Ren Z, Chow FE, Tsai R, Liu T, Rizzolio F, Boffo S, Xu Y, Huang S, Lippa CF, Gong Y (2017) Pathological role of peptidyl-prolyl isomerase Pin1 in the disruption of synaptic plasticity in Alzheimer's disease. Neural Plast. https://doi.org/10.1155/2017/3270725

Yang J, Nie J, Ma X, Wei Y, Peng Y, Wei X (2019) Targeting PI3K in cancer: mechanisms and advances in clinical trials. Mol Cancer. https://doi.org/10.1186/s12943-019-0954-x

Zhang X, Gao F, Wang D, Li C, Fu Y, He W, Zhang J (2018) Tau pathology in Parkinson's disease. Front Neurol. https://doi.org/10.3389/fneur.2018.00809

Zhou XZ, Lu KP (2016) The isomerase PIN1 controls numerous cancer-driving pathways and is a unique drug target. Nat Rev Canc. https://doi.org/10.1038/nrc.2016.49

Chapter 16
Repurposing of Drugs for the Treatment of Microbial Diseases

Kushal Kumar Bansal, Rajat Goyal, Archana Sharma, Prabodh Chander Sharma, and Ramesh K. Goyal

Abstract The looming threat of rising antimicrobial resistance (AMR) is one of the major threats to public health, and there is an immediate unmet need of discovery and development of new antimicrobial agents. Traditional drug discovery and development involves several stages for the discovery of a new drug and to obtain marketing approval. Drug repurposing also referred to as drug repositioning has been influential in identifying new therapeutic uses for already-approved drugs. Different categories of FDA-approved drugs such as antineoplastic, anti-inflammatory, anthelmintic have been explored for their possible use as anti-infective agents. Among several anticancer drugs active against microbes, gallium nitrate has been found very effective chemical mimic to disrupt iron metabolism in microbes specially *Pseudomonas aeruginosa.* Similarly, toremifene an FDA-approved anticancer agent for treatment of advanced breast cancer is found to be effective antimicrobial candidate by preventing biofilm formation. Further, several anticancer topoisomerase inhibitors have found applications in the development of novel antibacterial drugs that are valuable against fluoroquinolone-resistant microbial pathogens. Antibiofilm activity of nonsteroidal anti-inflammatory drugs renders these agents as promising candidates for further exploration as antimicrobial agents. Closantel, a veterinary antihelminthic drug, has been found effective against antibiotic-resistant (VRSA strain VRS1) *Staphylococcus aureus.* Similarly, numerous cardiovascular agents have been reported to exhibit antimicrobial activities through different mechanisms. In this chapter, we have summarized the already-approved drugs of different categories along with possible mechanism of action for their possible use as anti-infective agents.

K. K. Bansal
Govt. Pharmacy College, Rakkar, Distt. Kangra, Matyari, Himachal Pradesh, India

R. Goyal
MM College of Pharmacy, Maharishi Markandeshwar (Deemed to be University), Mullana-Ambala, Haryana, India

A. Sharma · P. C. Sharma · R. K. Goyal (✉)
School of Pharmaceutical Sciences, Delhi Pharmaceutical Sciences and Research University, New Delhi, India

R. C. Sobti et al. (eds.), *Drug Repurposing for Emerging Infectious Diseases and Cancer*, https://doi.org/10.1007/978-981-19-5399-6_16

Keywords Infection · Antimicrobial resistance · Repurposing drug

16.1 Introduction

New and re-emerging contagious infections pose a severe threat in the twenty-first century and are dominant cause of deaths among humans worldwide (Kharb et al. 2011). The deadly microbial infections insensitive to the antimicrobial chemotherapy claimed nearly 700,000 lives per year globally and are estimated to cause mortality pace of ten million people annually by 2050. Infections associated with antimicrobial resistance raise healthcare costs as a result of extended hospital admittances, enhance costs in treatment and many time treatment failures. As per a report, more than 2.8 million multidrug-resistant microbial infections occur per year in the USA only that leads to at least 35,000 deaths and $20 billion in healthcare expenditures (de Kraker et al. 2016; Dadgostar 2019). Though some marked improvements in treatment and disease management have been made in recent years, the AMR pathogens and emerging diseases are ongoing challenges. In order to combat AMR, multiple drug therapy or combinations of antimicrobial drugs are being prescribed for severe clinical infections such as, *Mycobacterium tuberculosis*, bacteremias (Cheng et al. 2019). A series of studies have revealed the synergistic effects of two or more antibiotic combination to overcome the complex problem of antimicrobial resistance. Consequently, the persistent use of antimicrobial agents has led to the development of multidrug resistance (MDR), which is even more serious challenge regarding the optimal use of multidrug therapy (Perron et al. 2012). Since medicinal choices to manage and cure infections are increasingly being limited, the research is now being directed toward new types of antimicrobials such as peptides, nanoparticles, and combinatorial therapies. Each of these approaches has faced significant difficulties as a result of escalating microbial resistance; this increases the morbidity and mortality related with microbial diseases. Therefore, new promising alternatives to fight infections and to mitigate the spread of antimicrobial resistance have become imperative caused by MDR-resistant pathogens (Leon-Buitimea et al. 2020).

A possible solution to tackle the problem of antimicrobial resistance is drug repurposing (also known as drug repositioning). The term drug repurposing/drug repositioning has been interchangeably used with drug re-profiling, drug re-cycling, drug rescuing, drug re-tasking, drug redirection, and therapeutic switching. Drug repurposing has gained exceptional devotions recently as a result of persistent high failure rates and essential costs in new drug development process. The field has been emerged as a promising field in drug discovery that involves establishing new therapeutic use for already-known drugs, including approved, discontinued, abandoned, and experimental drugs (Pushpakom et al. 2019; Rudrapal et al. 2020). A series of studies revealed several non-antimicrobial agents that are not intentionally used as antimicrobial agents but have one or more antimicrobial properties. Some noteworthy classes of drugs such as anthelmintics, anticancer drugs, anti-inflammatory/immunomodulatory drugs, antipsychotic, and antidepressant drugs are approved for their well-defined clinical indications with reported antimicrobial

properties in preclinical and clinical studies and could be utilized successfully for drug repurposing in treatment and management of microbial infections (Miro et al. 2019). This promising approach offers numerous advantages such as enhancement in portfolio of pharmaceutical companies by reducing the time and money required for producing new antimicrobials, and also being able to utilize compounds with known pharmacological properties (Rangel-Vega et al. 2015). The conventional approach to develop a new low molecular weight antimicrobial agent usually takes around 10–12 years and costs about hundreds of millions of dollars. Moreover, process of development of novel broad-spectrum antimicrobial is growingly difficult. The development of new antimicrobial risk has been greatly amplified by the ominous threat of antimicrobial resistance; therefore, alternative approaches, such as drug repurposing, are needed to meet the challenges and resolve the complex problems of emergence of drug-resistant microbial diseases (Zheng et al. 2017).

This chapter focuses on understanding the mechanism of antimicrobial resistance and need of repurposing of drugs for the treatment of microbial diseases. The chapter also emphasizes on the current status of information on repurposing of drugs for microbial pathogens, their mechanism of action, efficacy, extent of activity against microbial agents, as well as their possible clinical use in treatment of microbial infections.

16.2 Antimicrobial Agents: Mechanism of Resistance

Antimicrobial agents are generally classified according to their molecular structure and their mechanism of action on cell components of microbial cells. These cell components are also referred to as bio-targets which comprise cell wall synthesis, protein synthesis, ribonucleic acid synthesis (RNA), deoxyribonucleic acid (DNA) synthesis, and intermediary metabolism (Tenover 2006). Figure 16.1 summarizes the cellular sites of action along with approved antimicrobial agents. Since development of resistance to many drugs has reduced their use, other synthetic derivatives have gained increasing attention in recent years. The microbes such as bacteria, viruses, fungi, and parasites have evolved a special mechanism to overcome the action of medicines for their survival. Consequently, the infection becomes challenging to treat with the risk of spreading of disease, severe illness, and death. There are many reasons that promote the antimicrobial resistance,

which can be described as increased consumption, improper or unnecessary prescription, overuse, and misuse of various common antimicrobials agents by medical practitioners on account of their choice of combination having low cost and toxicity to the patients. Knowingly or unknowingly, all these factors contribute to emergence of resistant micro-organisms that can vary depending upon the strain and susceptibility. Microbial resistance is categorized as natural (intrinsic or induced) and acquired resistance. The common source of microbial resistance is intrinsic as microbes have stable genetic property encoded in the chromosomal DNA and shared by all members of the genus. On the other hand, a change in the bacterial

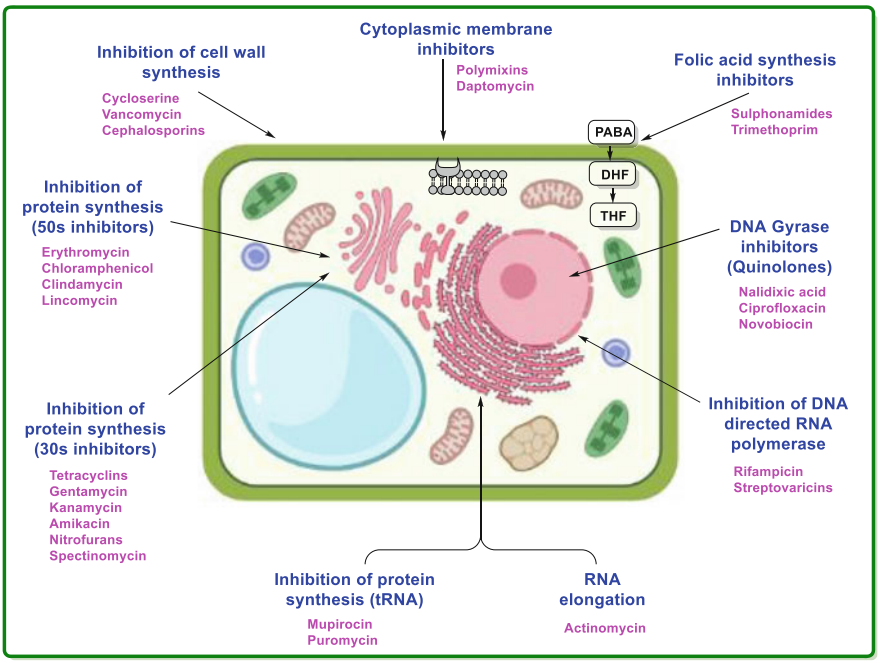


Fig. 16.1 Diagram of bacterial cell showing principle targets for approved drugs

DNA so that a new phenotypic trait can be expressed contributes to acquired resistance. Microbes have a well-elaborated machinery to replicate DNA, correct errors, and repair damage to the chromosomes. This signifies that microbes have low frequency of mutation in gene, otherwise the corrective mechanisms cannot be performed since mutations are often deleterious to the cell. Despite the low frequency, resistance mutations do survive and usually result in an altered protein that is less susceptible to the antimicrobial agents. Therefore, a mutation that confers resistance is a multistep process; the one-step mutations are relatively rare and are well documented for certain agents, such as quinolones, rifampin, streptomycin, sulfonamides, and trimethoprim. New resistance genes are transferred among bacteria from generation to generation, or stored in extra-chromosomal state on a bacterial plasmid. Plasmids carrying resistance genes are transferred via sex pili and are considered an extremely important mechanism for spreading antimicrobial resistance. Another less common mode of spreading is transduction, in which resistant genes of plasmid or chromosomal origin are carried by bacteriophages. The phenomenon of microbial resistance can be promoted by different mechanisms (Reygaert 2018). The possible mode of microbial resistance to the broad-spectrum antimicrobial agents has been described in Table 16.1 (Dever and Dermody 1991). There are different ways that microbes protect themselves and withstand with the antimicrobial agents. Most known method is to modify the antibiotic which is accomplished by introducing different chemical groups to antibiotics. On account

Table 16.1 Antimicrobial agents: Site of action and mode of resistance

Sr. no.	Class of drug	Site of action	Mode of resistance
1	Penicillin and cephalosporins	Inhibition of bacterial cell-wall synthesis	Enzymatic inactivation by ß-lactamase
2	Monobactams	Inhibition of peptidoglycan synthesis process	
3	Carbapenem	Inhibition of bacterial cell-wall synthesis	
4	Vancomycin	Inhibition of bacterial cell-wall formation by the inhibition of peptidoglycan synthesis	Inhibition of glycopeptide access
5	Trimethoprim	Inhibition of dihydrofolate reductase by blocking sequential steps in the folate synthesis pathway	Increased production of dihydrofolate reductase
6	Sulfonamides		Increased production of *p*-aminobenzoic acid; increased production of pteridine; increased production of sulfonamide-insensitive dihydropteroate synthetase
7	Aminoglycosides	Inhibition of initiation of protein synthesis and disrupt polypeptide chain elongation by inducing the incorporation of incorrect amino acids	Enzymatic modification by acetylation, phosphorylation, and nucleotidylation; ribosomal alteration
8	Chloramphenicol	Inhibition of protein synthesis by binding to the 50s ribosomal subunit	Enzymatic inactivation by acetylation; decreased drug permeability
9	Macrolides		Enzymatic modification by esterase; alteration of 23 s ribosomal RNA
10	Lincosamide		Enzymatic modification by nucleotidylation or phosphorylation; alteration of 23 s ribosomal RNA
11	Tetracyclin	Inhibition of bacterial protein synthesis by binding to the 30s ribosomal subunit	Active efflux preceded by chemical modification
12	Quinolones	Inhibition of DNA gyrase	Alteration of subunit A of DNA gyrase

of production of certain enzymes by microbes, they are capable of changing the nature of antimicrobial agents so that they cannot penetrate into cell wall. Second, the antibiotic penetrates the cell wall and reaches the target site but is unable to produce the activity due to modification in structure of the target or alterations in the primary site of action (due to mutations in microbial DNA). Expression of alternative proteins, reprogramming of target also render the drug to bypass the target. Genes located on chromosomes, plasmids, or transposons encode specific resistance

to a variety of antimicrobial agents. Above all, mutation is a spontaneous event that occurs regardless of whether antibiotic is present and allows bacteria to survive in the face of selection. Thus, it is evident that microbes develop resistance by either new mutations or the exchange of genetic information from generation to generations (Tenover 2006; Hawkey 1998; McManus 1997).

16.3 Need of Repurposing of Drugs for Microbial Diseases

Antimicrobial agents have been the fundamental allies in the treatment and management of microbial infections for almost 80 years. As discussed earlier, microbes are widely acknowledged to develop antimicrobial resistance and are continue to threaten the public health in every geographic region. With the emergence of multidrug-resistant microbial strains have created an urgent need for the development and discovery of robust antimicrobials. Development of new antimicrobial agents and vaccines usually takes 10–15 years and utilizes immense sources. This seems to be a critical situation in hand and creates deep necessity for the identification of novel strategies to develop antimicrobials to deal with this challenging health issue (Kamurai et al. 2020; Zheng et al. 2018). The ultimate goal of antimicrobial drug development is to identify molecules with the desired effect in the human body and to establish its quality, safety, and efficacy for treating patients. The conventional drug discovery or de novo approach involves stimulating the 3D structure of a ligand and protein receptor via computational aid. The process is usually time-consuming, laborious, high-risk that costs an average of US$2.6 billion and it takes a molecule to market in nearly 17 years. While, new technologies such as recombinant DNA, genomics and combinatorial chemistry, drug discovery offers considerable decrease in the time, and overall cost of new drug development. The above factors have rendered the medicinal chemists to practice drug repurposing as an alternative approach for new drug development. For example the existing drug auranofin has been approved as antirheumatic agent and has been repurposed for complex infectious disease amoebiosis. Another example comprises amphotericin B recommended for visceral leishmaniasis, whereas it was initially developed for management of fungal infections. Some other examples include dapsone, doxycycline which is recommended for malaria, whereas these were approved for pulmonary tuberculosis and as antibacterial activities, respectively (Thangamani et al. 2015a). Most recently, commercially available drugs used to treat other diseases such as HIV and malaria are being carefully evaluated as treatments for COVID-19. Multiple types of drugs such as antimalarials, antivirals, and antibiotics are being considered as potential treatments for COVID-19 either individually or in combination. Many are undergoing clinical trials to be evaluated for safety and efficacy in humans. An early example in research was the use of hydroxychloroquine (HCQ) in combination with azithromycin, which generated deep interest in the potential of chloroquine (CQ) and its derivatives for consideration as a potential treatment for COVID-19. There are several examples of drugs that can be employed as alternative

approach for treatment and management of diseases previously meant for other diseases. The approach is gaining popularity since it leads to significant improvement in patients and reduces side effects and is called drug repositioning. As the Nobel laureate pharmacologist James Black once said: "The most fruitful basis for the discovery of a new drug is to start with an old drug." Drug repositioning advocates for shorter development timeline, no need to repeat the safety assessment, use of already formulation development protocols and offers a positive outcome in orphan drug development. At present, medical fraternity lacks successful drugs for treatment and cure of severe diseases such as many types of cancer, life-threatening multi-resistant microbial infectious, neurodegenerative diseases, and many others.

16.4 Repurposing of Drugs as Antimicrobial Agents

This has been well accepted that most small-molecule drugs interact with more than one target protein. Taking the advantage of the situation, new technologies such as drug repurposing could be the major tool in order to accelerate the drug discovery process by keeping overall fruitful outcomes related to process advancing the progress in drug discovery field (Low et al. 2020; Gil and Martinez 2021; Nishimura and Hara 2018). Thus, alternative approaches, such as drug repurposing, are needed to meet the challenges of outbreaks and the emergence of drug-resistant infectious diseases. A systematic approach has revealed (Fig. 16.2) that many representatives drugs originated from the class of anticancer, anthelmintic, antihyperlipidemic, anti-inflammatory, cardiovascular, psychoactive, etc. are credited with antimicrobial potential.

16.4.1 Repurposing of Anticancer Drugs for Microbial Diseases

Both cancer cells and microbes share some common features in terms of fast proliferation, high metabolic rates, capability to metastases, cell-to-cell communication behavior, resistance against medicinal agents, and reliance for eradication on an active immune system. Cancer cells instead of dying multiply continuously to reproduce more and more abnormal cells. While microbes possess complex outer structures which, enables the microbe to escape from the immune system to improve survival rate. For instance, Gram-negative bacteria namely *Escherichia coli*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Neisseria meningitidis* have been enclosed with polysaccharide capsule which acts like shield on the membranes against the immune system and prevent them from phagocytosis. Unfortunately, most of the drugs employed in treatment and management of cancer and infection are incapable of penetration into cells and complete eradication of these

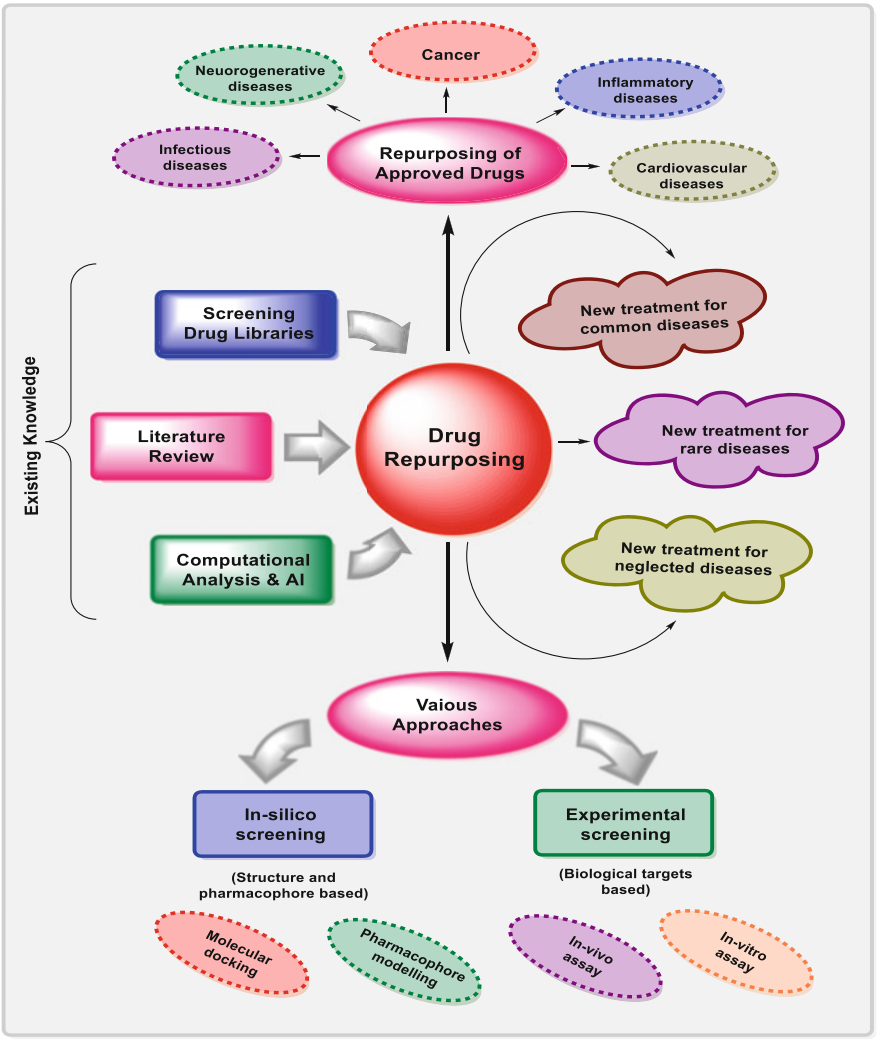


Fig. 16.2 Drug repurposing as an alternative approach for treatment of microbial infections

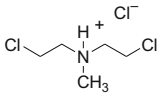
cells (Al-Hilu and Al-Shujairi 2020; Quezada et al. 2020). Hence, it is expected that at least some drugs specifically designed to inhibit cancer progression are also functional as antimicrobials, as it has been found for several anticancer compounds (Table 16.2).

Chemotherapeutic agent namely mitomycin C has been approved by the FDA for treatments of wide range of cancers such as bladder, gastric, and pancreatic cancers. The drug is a prototype which is converted into a highly reactive bis-electrophilic intermediate, passively diffuses into cells that alkylates cellular nucleophiles and causes cross-linking of DNA. The mitomycin C has been produced by

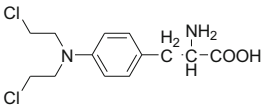
Table 16.2 Some anticancer drugs, their mode of action, and indications in treatment of diseases

Class of Drug	Mechanism of Action	Indication
Alkylating Agents; Examples; Busulfan, Carmustine, Chlorambucil, Diaziquone, Lomustine, Mechlorethamine, Mitomycin C, Streptozotocin, ThioTEPA	Alkylates DNA to form crosslinks, this DNA damage interferes with DNA synthesis and transcription	Chronic myelogenous leukemia Brain tumors, Hodgkin's and non-Hodgkin's lymphomas Multiple myeloma Metastatic cancer in pancreatic islet cells, Bladder, breast, and ovarian cancers, malignant pleural, Apericardial and peritoneal effusions
Antimetabolites; Examples; Azacitidine, 5-Fluorouracil, Gemcitabine, Mercaptopurine, Methotrexate, Thioguanine	Impairing purine and pyrimidine synthesis	Myelodysplastic syndromes Multiple myeloma Breast, ovarian, and pancreatic Acute lymphoblastic leukemia Acute lymphoblastic leukemia Acute non-lymphocytic leukemia
Antibiotics; **Examples**; Dactinomycin, Daunorubicin, Doxorubicin, Bleomycin	Inhibit DNA replication and synthesis	Hodgkin's & non-Hodgkin's lymphoma testicular cancer, ovarian cancer breast cancer, bladder cancer, Kaposi's sarcoma and cervical cancers

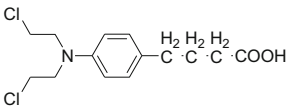
sp. *Streptomyces caespitosus* and proven effective in killing a broad-range of bacterial persister cells including spirochete *Borrelia burgdorferi*, causative pathogen of Lyme disease which results in formation of biofilms and wounds (Kwan et al. 2015). Like mitomycin C, cisplatin is another FDA-approved chemotherapeutic agent that also acts by cross-linking DNA but primarily on the same strand of DNA with intrastrand cross-linking between purines at adjacent guanines, at AG sequences, and at GNG sequences where N can be any nucleotide. Cisplatin (cis-diamminodichloroplatinum (II) is recommended in treatment of testicular, ovarian, bladder, and head and neck cancers and eradicates *Escherichia coli* K-12 persister cells through a growth-independent mechanism. In comparison to mitomycin C, cisplatin is more effective at killing *Pseudomonas aeruginosa* persister cells and against clinical isolates of *S. aureus* via forming interstrand DNA cross-linking (Chowdhury et al. 2016). The other examples of alkylating agents those found to possess antimicrobial properties include nitrogen mustards such as busulfan, chlorambucil, mechlorethamine, nitrosoureas such as, carmustine, lomustine, streptozotocin, and thiotepa.



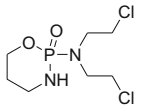
Meclorethamine Hydrochloride (Mustargen)



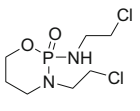
Melphalan (Alkeran)



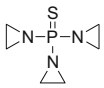
Chlorambucil (Leukeran)



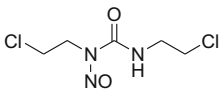
Cyclophosphamide
(Cytoxan)



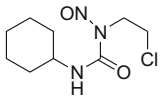
Ifosfamide
(Iflex)



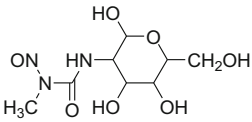
Thiotepa
(Thioplex)



Carmustine

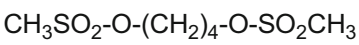


Lomustine

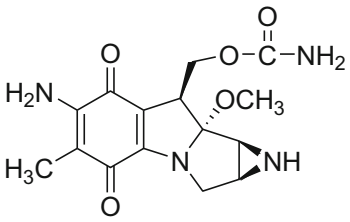


Streptozocin

Nitrosoureas



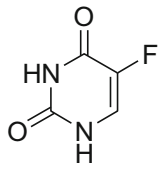
Busulfan
(Myleran)



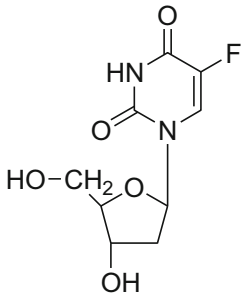
Mitomycin C

All are recommended for treatment of cancer and have been proven to cause interruption of DNA synthesis and stimulate eradication of microbial cells (Soo et al. 2017). Antimetabolites being indicated in cancer treatment affect the DNA synthesis by acting as a substitute to the actual metabolites which is required in normal metabolism. Among them 3-bromopyruvate has been recently identified as a potent and selective antistaphylococcal agent. This chemotherapeutic agent demonstrated bactericidal activity against *Staphylococcus aureus* (20 μg/ml) and several human and veterinary strains (MIC; 20 and 80 μg/ml), including MDR isolates. Most notably, 3-bromopyruvate exhibited synergistic effect with gentamicin (Visca et al. 2018). The other chemotherapeutic agent from antimetabolites such as 5-fluorouracil (5-FU) previously approved for treatment of human colon cancer has demonstrated biofilm inhibitory activity against the isolate of *P. aeruginosa* through interfering with quorum-sensing (QS) pathways, and was found nontoxic to *P. aeruginosa*

(Kang et al. 2019). FDA-approved anticancer drugs such as streptozotocin (recommended in pancreatic islet cell cancer) and floxuridine (mostly recommended in colorectal cancer) are also known for their antimicrobial activity. The drugs were reported as antivirulent against important Gram-positive human pathogen *Staphylococcus aureus* USA300 (the SaeRS two-component system). In vivo data showed that a single administration of the drugs was sufficient to protect mice from staphylococcal intraperitoneal infection. Among both anticancer drugs, floxuridine was effective in comparison to streptozotocin in repressing SaeRS promoters and protecting human neutrophils from *S. aureus*-mediated killing at a dose of 0.5 and 0.4 μM (Dylag et al. 2013).

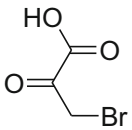


Fluorouracil (Adrucil)



Floxuridine (FUDR)

In another study, Kang et al. reported in vitro and in vivo inhibitory activity of 3-bromopyruvate on NDM-1 (New Delhi metallo-β-lactamase-1) target. The catalytic activity studies demonstrated that the scaffold was capable of inhibiting NDM-1 (IC_{50}; 2.57 μM) in a dose and time-dependent manner. Additionally, the analog effectively restored the activity of β-lactam antibiotics resulting in two- to eightfold reduction in MIC (Ueda et al. 2009). By taking the same pyruvic analog namely 3-bromopyruvate (3-BP), Dylag et al. inspected antifungal activity against on several strains of yeast and filamentous fungi. The lowest possible minimal inhibitory concentration and low toxicity was found against the human pathogenic strain *Cryptococcus neoformans* (MIC; 0.12–0.15 mM) (Yeo et al. 2018).



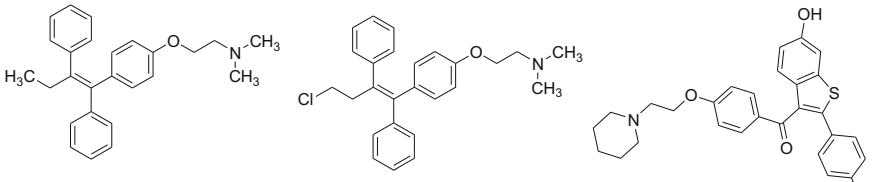
3-Bromopyruvic Acid

Tamoxifen has been approved as a selective estrogen receptor modulator and recommended in the treatment of breast cancer. Besides anticancer activity

tamoxifen also promotes biosynthesis of sphingolipid, resulting essentially in activation of pro-inflammatory pathways via regulation of neutrophil activity. The production of neutrophil extracellular trap (NET) occurs via a ceramide/PKCζ-mediated pathway, and NET synthesis can be promoted with treatment of synthetic ceramide. Conversely, in vitro pretreatment of neutrophils with tamoxifen improves neutrophil bactericidal activity against virulent human pathogens and enhances clearance of the methicillin-resistant *Staphylococcus aureus* in vivo (Corriden et al. 2015).

A structurally similar analog of tamoxifen is toremifene widely known FDA-approved anticancer agent for treatment of advanced breast cancer in postmenopausal women. Recently, toremifene has been identified for antibacterial capacity against oral bacteria namely *Porphyromonas gingivalis* and *Streptococcus mutans*. The drug toremifene depicted inhibitory activity and prevent biofilm formation at a dose range from 12.5 to 25 μM. Additionally, the drug was able to eradicate previously form biofilms at 25–50 μM dose (Gerits et al. 2016).

The other drug candidate raloxifene approved for prevention of osteoporosis and reduction of the risk of breast cancer in postmenopausal women is known to have anti-inflammatory properties and associated with production of neutrophil extracellular trap (NET) similar to tamoxifen. Raloxifene prevents neutrophil cell death in response to the classical activator phorbol 12-myristate 13-acetate (a mediator that increase DNA-based neutrophil extracellular traps). Thus, raloxifene inhibited PMA-induced, NET-based killing of human pathogen methicillin-resistant *Staphylococcus aureus* (Flores et al. 2016).

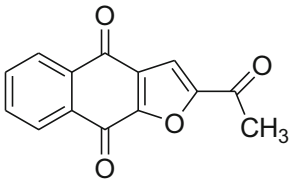


Tamoxifen (Nolvadex) **Toremifene (Fareston)** **Raloxifene (Evista)**

One of the most promising anticancer drugs to become repurposed as an antibacterial is gallium nitrate. Microbes such as bacteria require iron as essential constituent for enzymes-mediated DNA synthesis, electron transport, oxidative stress defense, and other functional processes. The iron can be successfully replaced with identical property carrying metal gallium that in turn disrupt iron metabolism in such microbes. The study indicated that gallium can act as a chemical mimic to disrupt *P. aeruginosa* iron metabolism at μM of gallium in sputum samples from patients with cystic fibrosis. Gallium showed synergistic effect with certain antibiotics but resistance developed slowly; however, gallium did not diminish the antibacterial activity of host macrophages (Goss et al. 2018).

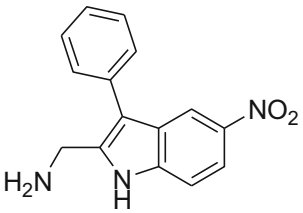
Napabucasin occurs as natural compound and known orally available Stat3 and cancer cell stemness inhibitor. Napabucasin demonstrated good antimicrobial

activity against oral streptococcal planktonic isolates (*Streptococcus mutans*, *Streptococcus gordonii*, and *Streptococcus sanguinis*) in MIC range from 0.49 to 3.91 μg/ml and MBC range from 0.98 to 15.63 μg/ml than chlorhexidine (MIC range; 0.49–1.95 μg/ml, MBC range; 3.91–7.81 μg/ml). However, the natural compound napabucasin demonstrated lower cytotoxicity as compared to the reference drug (Kuang et al. 2020).



Napabucasin

Song et al. identified a potent indigoid derivative; 5-nitro-3-phenyl-1*H*-indol-2-yl-methylamine hydrochloride (NPIMA) that kills *Escherichia coli* persister cells more effectively than DNA-cross-linker cisplatin by damaging their membranes. NPIMA exhibited excellent eradication capability toward *Pseudomonas aeruginosa* and carry moderate eradication of *Staphylococcus aureus* persister cells at MIC values of 0.1–0.25 μg/ml as compared to cisplatin (MIC; 0.15–1 μg/ml). NPIMA on several wound models was also proved to be effective in reducing *P. aeruginosa* and *S. aureus* cell with no resistance found after 1 week (Song et al. 2019).



(NPIMA)

Cisplatin has been recommended in plethora of cancers including testicular, ovarian, cervical, breast, bladder, head–neck, esophageal, lung, mesothelioma, brain tumors, and neuroblastoma. The drug shows binding with DNA and interfere with normal transcription or replication and results in cytotoxicity. Yuan et al. repurposed antineoplastic drug cisplatin for its potential antimicrobial activity against the nosocomial pathogen *Pseudomonas aeruginosa*. Cisplatin exhibits antimicrobial activity against *P. aeruginosa* PAO1 lab strain MIC) of 6.25 μM *P. aeruginosa* lab strain PA14 and a *P. aeruginosa* mucoid multiple drug-resistant (MDR) CF 57388A at MIC 6.25 μM (Yuan et al. 2018) (Fig. 16.3).

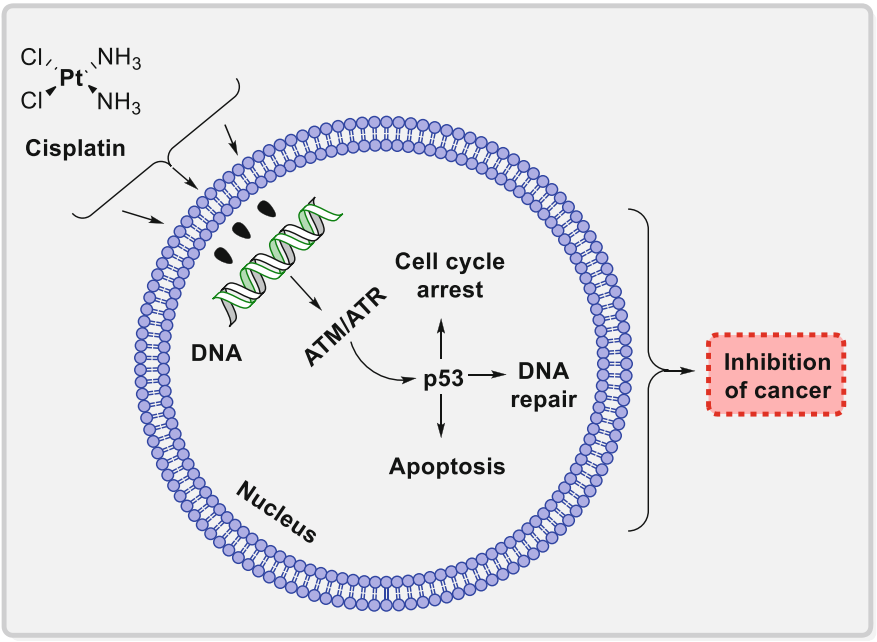
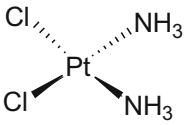


Fig. 16.3 Mechanism of action of cisplatin

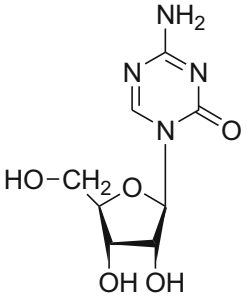
A naturally occurring pyrimidine nucleoside analog of cytidine, azacytidine was approved for acute myelogenous leukemia. It has been well documented that introduction of azacytidine into the DNA of human or pathogenic cells such as *Escherichia coli* results in hypomethylation of DNA and inhibits DNA methyltransferase (MTases) which causes inhibition of protein and DNA synthesis. The antimicrobial effect of azacytidine was demonstrated by Yadav et al. against *S. pneumoniae* (a pathogen causing pneumonia, otitis media, meningitis, and sepsis). At 15 mM concentration, the analog caused inhibition up to 7.5% with a 13% decrease in biomass of biofilms. At 100 mM concentration, 23% inhibition was observed with 54% less biomass, while at 500 mM concentration, 34% cells were inhibited and 70% of the biofilms were detected (Yadav et al. 2012).

From the class of antimetbolites, gemcitabine is a known cytotoxic nucleoside analog approved for treatment of numerous cancers including testicular cancer, breast cancer, ovarian cancer, non–small-cell lung cancer, pancreatic cancer, and bladder cancer. Jordheim et al. demonstrated antimicrobial activity of this cytotoxic analog against multi-resistant *Staphylococcus aureus* strains. Gemcitabine and its analog namely CP-4126 have shown significant antimicrobial activities against *S. aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA) and glycopeptide-intermediate *S. aureus* (GISA) at MIC values between 0.06 mg/L and 4.22 mg/L. The analogs showed bactericidal activity and exhibited synergistic effect with gentamicin. Moreover, mutations in the nucleoside kinase gene SadAK have been observed indicating its role in gemcitabine activity (Jordheim et al. 2012).

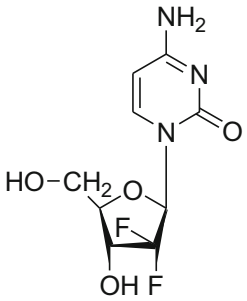


Cisplatin (Platinol)

Kruszewska et al. assayed several nonantibiotic drugs for their antimicrobial activity against microbial strains: *S. aureus*, *E. coli*, *P. aeruginosa*, and *C. albicans*. Among the screened analogs, methotrexate (A folate analog approved for antineoplastic and immunosuppressant activities) demonstrated noteworthy antimicrobial properties against *S. aureus* (MSSA strain) at MIC range 10–20 μg/ml. On the other hand, *S. aureus* (MRSA strains), MIC range was found 10–<100 μg/ml (Kruszewska et al. 2000).

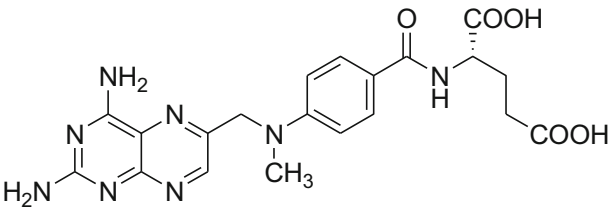


Azacytidine (Vidaza)

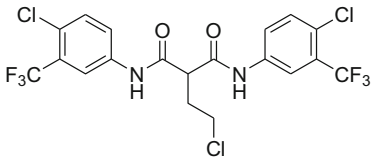


Gemcytabine (Gemzar)

Beside the antineoplastic agents, another nonantibiotic drug sorafenib has been approved as a tyrosine protein kinase inhibitor for cancer therapy and exhibits a strong cytotoxic effect. Chang et al. developed several sorafenib-based analogs and evaluated for anti-MRSA (*Staphylococcus aureus* and *Staphylococcus epidermidis*) potency. **SC5005** has been identified as most potent lead compound as anti-MRSA with an MIC_{90} of 0.5 mg/L and with low cytotoxicity against all human cancer cells with the IC_{50} ranging from 15 to 20 mg/L by increased selectivity ratios of up to 40 (Chang et al. 2016). Crunkhorn et al. suggested that sorafenib-based analogs target two essential bacterial proteins namely demethylmenaquinone methyltransferase and signal peptidase IB that killed *S. aureus*, reduced persisters, and established biofilms and did not induce in vitro resistance (Crunkhorn 2020).



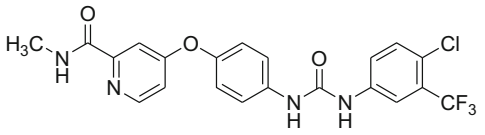
Methotrexate
(Trexall)



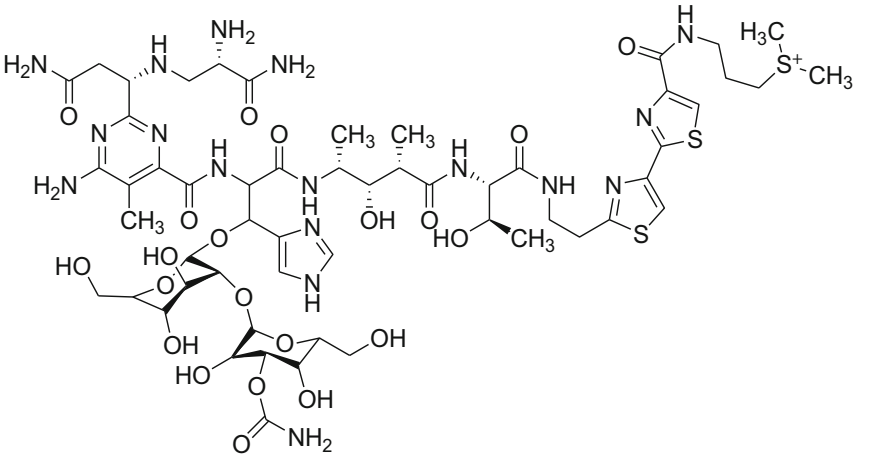
SC5005

Chemotherapeutic agents such as bleomycin and doxorubicin are known to exert their cytotoxic activity through the oxidative cleavage of DNA and recommended in Hodgkin's lymphoma, non-Hodgkin's lymphoma, testicular cancer, ovarian cancer, breast cancer, bladder cancer, Kaposi's sarcoma, and cervical cancers. These cancer medicines have been reported to inhibit growth of microbial pathogens including enterobacteriaceae, *Staphylococcus aureus*, and *Corynebacterium* spp. Bleomycin showed inhibition of both *S. pseudintermedius* and *E. coli*, while doxorubicin inhibited only staphylococcal isolates (Campbell et al. 2019).

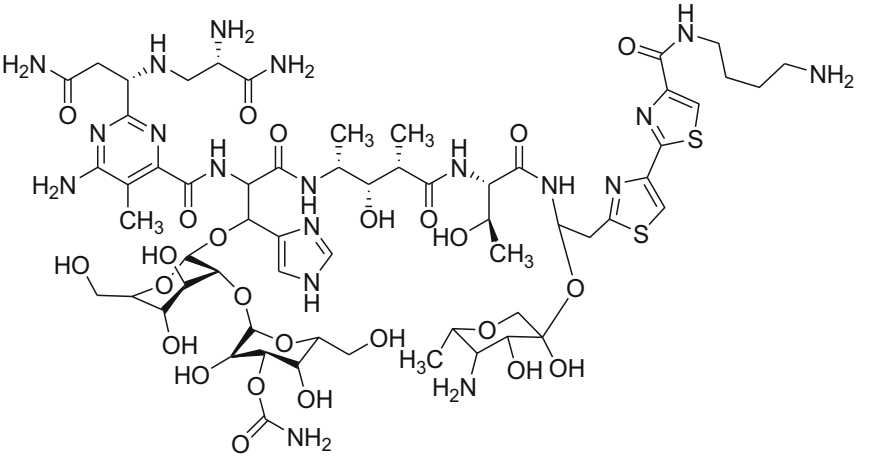
In a similar study, Andros et al. revealed antimicrobial properties of bleomycin and tallysomycin S10b against *B. subtilis*, *K. pneumoniae*, and *E. coli* with an average zone of inhibition of 14.92 ± 7.88 mm and 15.17 ± 7.88 against Gram-negative bacteria, and for Gram-positive strains 18.93 ± 6.83 mm and 2.36 ± 7.74 mm, respectively (Andros et al. 2015).



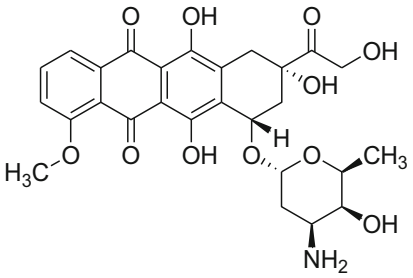
Sorafenib
(Nexavar)



Bleomycin
(Blenoxane)

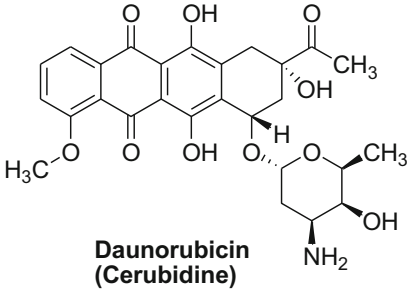


Tallysomycine S10b



Doxorubicin (Adriamycin)

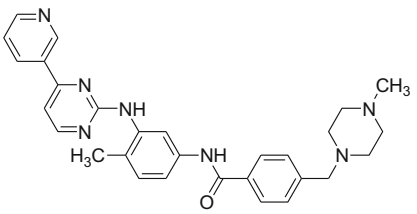
In the past decades, protein kinase inhibitors have been identified as novel antimicrobial candidates in treatment and management of HIV, tuberculosis, and malaria. Tyrosine kinases include wide varieties of enzymes that activate many proteins by signal transduction. Tyrosine kinases inhibitors are typically anticancer drugs that are recommended in chronic myelogenous leukemia and other diseases, such as idiopathic pulmonary fibrosis. Imatinib (targets ABL1/2), gefitinib (targets EGFR), and ibrutinib (targets BTK) are some examples of tyrosine kinases inhibitors that exhibit indirect anti-infective activities possibly improving the host cell capacity to fight against infections (Cheng et al. 2017; Sharma et al. 2020). Of different analogs, imatinib demonstrated synergistic effects in combination with first-line antitubercular drugs like rifampicin and rifabutin (Napier et al. 2011).



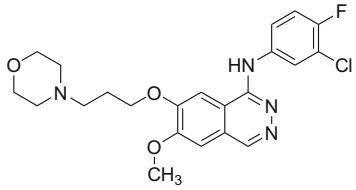
Daunorubicin (Cerubidine)

Nuclear DNA topoisomerases essential human enzyme that regulates transcription, replication and recombination processes. Inhibitors of DNA topoisomerases such as anthracyclin derivatives (daunorubicin, doxorubicin, epirubicin, and idarubicin) are widely used cancer chemotherapy and known to stimulate DNA cleavage (Binaschi et al. 1995). Recently topoisomerase inhibitors found applications in the development of novel antibacterial drugs that are valuable against fluoroquinolone-resistant microbial pathogens (Hiasa 2018; Biswas et al. 2013). Gajadeera et al. suggested that daunorubicin and idarubicin showed *Mtb* DnaG inhibitory activity at low IC_{50} values 7.2 ± 0.3 μM and 8.2 ± 1.1 μM, respectively. Aloe-emodin, a plant origin compound with cathartic and mild anticancer activities

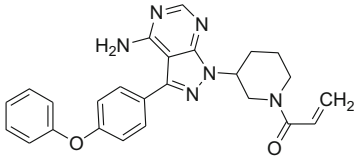
demonstrated inhibition of *Mtb* DnaG enzyme with IC_{50} value 19 ± 2 μM. Another DNA intercalator namely mitoxantrone (a derivative of an anticancer drug) inhibited *Mtb* DnaG about twofold more weak potency with IC_{50} value of 38 ± 1 μM than aloe-emodin (Gajadeera et al. 2015). Mitoxantrone (an anthraquinone derivative) itself demonstrated inhibitory activity against *Mycobacterium tuberculosis* PknB (an essential receptor-like protein kinase involved in cell growth control) and capable of preventing mycobacterial growth. The drug showed antimycobacterial activity against *M. tuberculosis* $H_{37}Rv$, *M. smegmatis*, and *M. aurum* in MIC range 25–400 μM (Wehenkel et al. 2006; Ali et al. 2018).



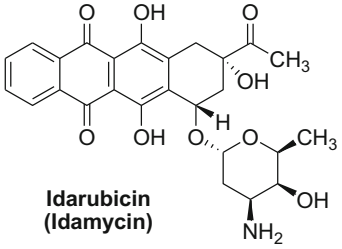
Imatinib (Gleevec)



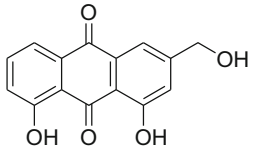
Gefitinib (Iressa)



Ibrutinib (PCI-32765)



Idarubicin (Idamycin)



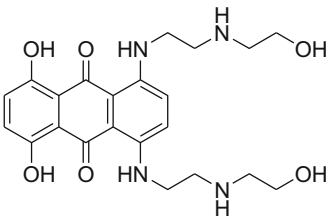
Aloe-emodin (Rhabarberone)

16.4.2 Repurposing of Anti-inflammatory Drugs for Microbial Diseases

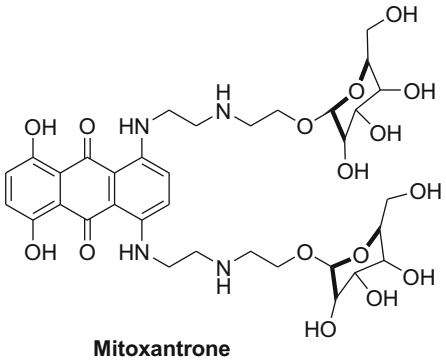
Nonsteroidal anti-inflammatory drugs (NSAIDs) are recommended in the treatment of inflammation and known to act through inhibition of cyclooxygenases thereby

decreasing the synthesis of the pro-inflammatory mediator prostaglandin. A series of studies have revealed antibiofilm activity of nonsteroidal anti-inflammatory drugs (NSAIDs). Some known approved anti-inflammatory drugs such as ibuprofen, diclofenac, and acetylsalicylic acid can be employed as adjunctive therapies against infections related to formation of biofilm. It has been anticipated that NSAIDs exert an antimicrobial or antibiofilm action by inhibiting hemolysis, blocking AgrA-regulated virulence, disrupting staphyloxanthin synthesis, low expression of biofilm formation genes fnbA and icaA (Leme and da Silva 2021).

Among NSAIDs, carprofen, bromfenac, and vedaprofen showed antibacterial activity against *Escherichia coli* by inhibiting DNA polymerase IIIb subunit. Besides *E. coli*, the drugs were also screened for their antibacterial activities against *acinetobacter baylyi*, *Staphylococcus aureus*, and *Bacillus subtilis*. MIC values of these NSAIDs are given in Table 16.3. The *S. aureus* and *B. subtilis* (Gram-positive) demonstrated higher susceptibility toward NSAIDs used in study than against *E. coli* and *A. baylyi* (Gram-negative) (Yin et al. 2014).



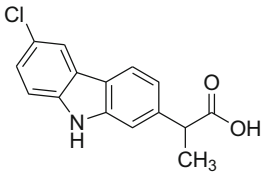
Mitoxantrone (Novantrone)



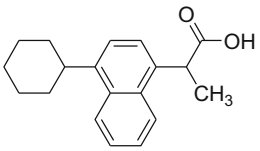
Mitoxantrone derivative

Table 16.3 MIC values of some NSAIDs

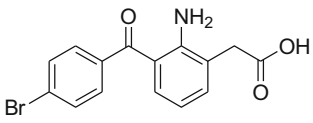
NSAID	*E. coli* (MIC; μg/ml)	*A. baylyi* (MIC; μg/ml)	*S. aureus* (MIC; μg/ml)	*B. subtilis* (MIC; μg/ml)
Carprofen	680	340	85	85
Vedaprofen	1410	705	44	44
Bromfenac	835	1670	835	418
Flufenamic acid	>1400	1400	175	88
Tolfenamic acid	>1300	1300	163	82



Carprofen (Rimadyl)



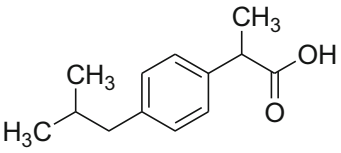
Vedaprofen



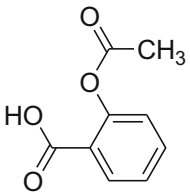
Bromfenac (Bromday)

Kroesen et al. pointed beneficial effect of NSAIDs as adjunct to TB therapy, mediated by host-immune reaction (Kroesen et al. 2017). To evaluate the effect of anti-inflammatory agents, Vilaplana et al. showed anti-TB effect of ibuprofen in an experimental animal model. The treatment of animals with ibuprofen demonstrated statistically significant reduction in the size and number of lung lesions, significant reduction in the bacillary load, and increased survival of animals in comparison to untreated animals. The results encouraged that incorporation of ibuprofen in the standard tuberculosis therapy for the treatment of the humans infected with *Mycobacterium tuberculosis* could produce better results in resolution of TB. It is also anticipated that administering ibuprofen in case of MDR or XDR strains could also contribute to effective treatment of TB (Vilaplana et al. 2013).

On the other hand, a popularly known salicylic acid derivative aspirin was found to induce antibiotic resistance in various bacterial species. Aspirin (anti-inflammatory drug) demonstrated antagonistic effect on isoniazid treatment of murine pulmonary tuberculosis, whereas the nonsalicylate ibuprofen did not show this effect (Byrne et al. 2007). Schaller et al. analyzed the effect of salicylate on the on a range of antituberculosis (anti-TB) drugs such as isoniazid, rifampin, ethambutol, streptomycin, and *p*-aminosalicylate. Salicylate-induced resistance was more pronounced for *p*-aminosalicylate, streptomycin, and ethambutol but was not apparent for isoniazid and rifampin when salicylate and the anti-TB agents were incorporated simultaneously (Schaller et al. 2002).



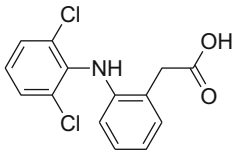
Ibuprofen (Advil)



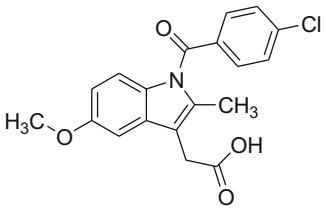
Aspirin

In addition to the potent anti-inflammatory activity, diclofenac sodium was found to possess effective antibacterial activity against clinical isolates of *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, and *Mycobacterium* spp. (both drug-sensitive and drug resistant). Noteworthy, the drug showed inhibitory activity against the different isolates of *Mycobacterium tuberculosis*, and other mycobacteria at a dose range between 10 and 25 μ/ml concentrations. Diclofenac sodium demonstrated bactericidal activity against both *E. coli* and *L. monocytogenes* by inhibiting the DNA synthesis. The drug was found to possess antiplasmid activity and identified as helper compound that act synergistically with streptomycin against *E. coli* and *Mycobacterium* or in combination with gentamicin against *Listeria* (Dutta et al. 2004; Mazumdar et al. 2009). AL-Janabi et al. also reported antibacterial activity of diclofenac, indomethacin, and mefenamic acid. *Staphylococcus aureus* was found susceptible toward diclofenac and mefenamic acid. Notably, diclofenac showed lowest MIC value at 2.5 mg/ml against seven isolated strains (*E. coli, Sal. Typhi, Ent. Cloacae, Ent. Aerogenes, S. aureus, B. subtilis, P. yeei*), while all the isolates were observed to exhibit resistance to indomethacin up to 5 mg/ml (AL-Janabi AAH. 2009).

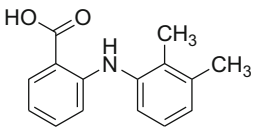
Sukul et al. screened metal complexes of indomethacin with cobalt, copper, manganese, and zinc for their analgesic, antimicrobial, cytotoxic, and antioxidant activities. In particular, indomethacin and its complexes with cobalt, copper, and manganese exhibited mild-to-moderate antimicrobial activity (zone inhibition diameter 8–9 mm) and the indomethacin–manganese complex also displayed highest cytotoxicity (Sukul et al. 2014).



Diclofenac
(Cataflam)



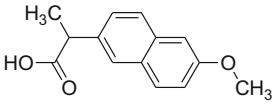
Indomethacin
(Indocid)



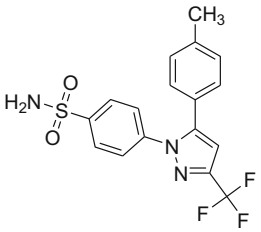
Mefenamic acid
(Ponstel,)

Another propionic acid derivative naproxen has been approved for the cure of many injuries or pain associated with inflammation. Recently, naproxen except for anti-inflammatory activity has been identified as anticancer and antimicrobial agent. Diclofenac, aspirin, and naproxen showed inhibitory activity on fungal growth. Diclofenac revealed significant antifungal activity against dermatophyte strain *T. mentagrophytes* at MIC 700 μg/ml for and against *E. floccosum* at 280 μg/ml. Aspirin and naproxen also showed significant activity against dermatophytes, but less than diclofenac. Celecoxib exhibited 50% of inhibition against *T. mentagrophytes* at MIC value of 500 μg/ml than meloxicam at MIC 1000 μg/ml (Hussein and AL-Janabi 2011). A series of study has also reported the antimicrobial properties of celecoxib. Celecoxib showed inhibitory activities against *Histoplasma capsulatum*, *Francisella tularensis*, *F. novicida*, *S. aureus*, and *S. epidermidis*. Beside antimicrobial activity, celecoxib contributed to the sensitivity of bacteria to various antibiotics, such as ampicillin, kanamycin, ciprofloxacin, and chloramphenicol by inhibiting multidrug efflux pumps in *Mycobacterium smegmatis* and *S. aureus*. In particular, celecoxib sensitizes intracellular *S. aureus* to antibiotic through modulation of SIRT1 in macrophages infected with *S. aureus*, thereby

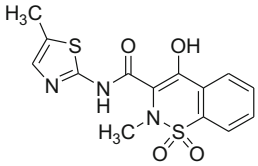
limiting bacterial survival and infection (Thangamani et al. 2015b; Annamanedi and Kalle 2014).



Naproxen (Aleve)



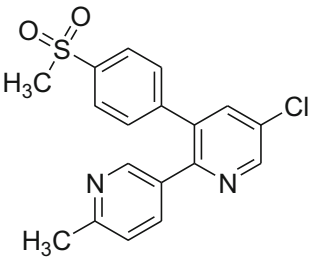
Celecoxib (Celebrex)



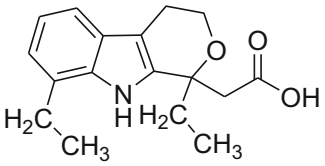
Meloxicam (Mobic)

Mishra et al. demonstrated antimicrobial activity of etoricoxib at a dose range of 20–100 μg/ml. This broadly used anti-inflammatory drug has been screened against the isolates of *S. aureus*, *P. aeruginosa*, *E. coli*, *S. typhi*, *C. albicans*. Among the isolates, *S. aureus* showed comparable sensitivity toward etoricoxib and exhibited zone of inhibition of 10, 15, 18 mm at MIC value of 100 μg/ml (Mishra et al. 2010).

Etodolac (a nonsteroidal anti-inflammatory drug, NSAID) was reported to show antibacterial and antibiofilms formation activities against ESKAPE (*E. faecium*, *S. aureus*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, *Enterobacter* sp.) and *E. faecalis* and *E. coli* pathogens at 10 and 1 mM concentrations. The drug demonstrated biological activity against all tested Gram-positive bacterial isolates and antibiofilm activity particularly against *E. faecium*, while the Gram-negative isolates have null influence except *A. baumannii* regarding inhibition of biofilm formation (Pereira et al. 2018).



Etoricoxib (Arcoxia)

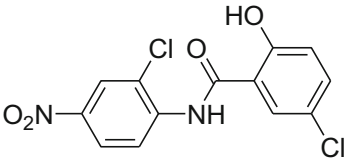


Etodolac
(Lodine)

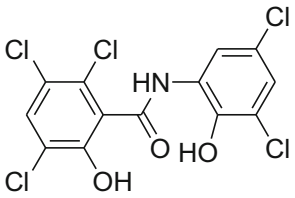
16.4.3 Repurposing of Anthelmintic Drugs for Microbial Diseases

Anthelmintics are some of the most widely used drugs in treatment and management of infections caused by helminths (the parasitic worms). Some commonly known parasitic worms include roundworms (nematodes), flukes (trematodes), and tapeworms (cestodes). An anthelmintic drug exhibits its action either by killing the worms or by causing their eradication from the host body without any significant side effects (Abongwa et al. 2017; Partridge et al. 2020).

Anthelmintics depict their actions through different modes such as rapid and selective neuromuscular transmission thereby causing spastic paralysis or acting as GABA agonist on nematode muscles thereby causing flaccid paralysis. Some drugs are also known to act by opening of glutamate-gated chloride (GluCl) channels (results in paralysis) or increasing permeability of calcium or binding selectively to beta-tubulin thereby inhibiting microtubule formation (Martin 1997). Besides their actions as anthelmintics, some drugs have also been investigated for their antimicrobial properties against the microbial pathogens. Niclosamide (an FDA-approved drug recommended in tapeworm infestations) and oxyclozanide (a veterinary medicine) were reported to show antimicrobial activity by Rajamuthiah et al. Both drugs demonstrated strong in vivo and in vitro antimicrobial activities against methicillin-resistant *S. aureus* at MIC values 0.125 and 0.5 μg/ml, respectively, with MEC $\leq$ 0.78 μg/ml. Against Gram-positive isolate namely *E. faecium*, both niclosamide and oxyclozanide showed activity at MIC values 0.25 and 2 μg/ml, respectively. On the other hand, against Gram-negative isolates namely *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, and *E. aerogenes*, no antimicrobial activity was observed. In vitro antimicrobial activity results showed that niclosamide and oxyclozanide exhibited activity at MICs at 0.0625–0.5 and 0.125–2 μg/ml, respectively, against methicillin-resistant vancomycin, linezolid, or daptomycin *S. aureus* isolates (Rajamuthiah et al. 2015).

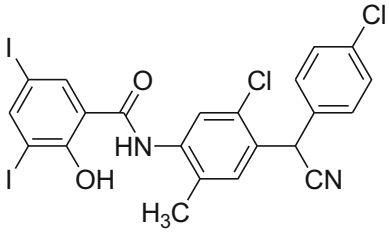


Niclosamide (Niclocide)



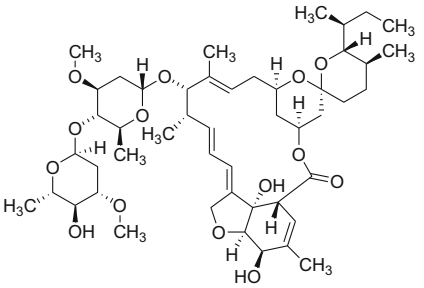
Oxyclozanide

Many studies have documented antihelminthic properties of closantel (a veterinary antihelminthic drug). Recently, closantel was found active against vancomycin-resistant *Staphylococci*. The drug depicted in vitro activity against other bacteria namely *E. coli*, *B. subtilis*, *E. faecalis*, and *Enterococcus faecium* at a very low MIC. Against antibiotic-resistant (VRSA strain VRS1) *S. aureus* isolate, the drug exhibited lowest MIC value at 0.78 μg/ml in comparison to oxacillin or vancomycin (MIC >256 μg/ml) (Hlasta et al. 1998; Rajamuthiah et al. 2014).

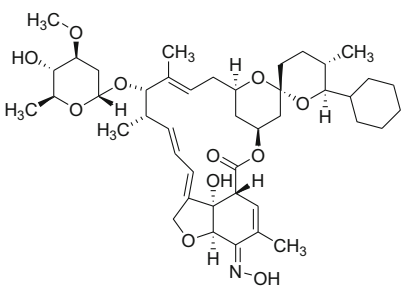


Closantel

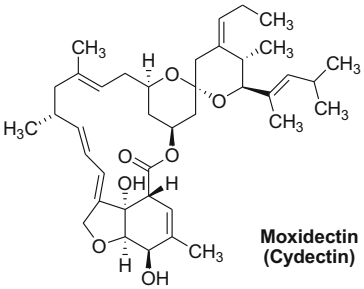
Avermectins are class of macrolides widely recommended in treatment of helmintic infections in humans and animals. Some approved drugs from this class such as ivermectin, selamectin, and moxidectin have been reported to show bactericidal activity against multidrug-resistant and widely drug-resistant clinical isolates of *M. tuberculosis*. Ivermectin, selamectin, and moxidectin demonstrated antimycobacterial activity against *M. tuberculosis* isolates at MIC_{90} range from 0.5 to 8 μg/ml (Lim et al. 2013). Similarly, Omansen et al. showed that ivermectin and moxidectin exhibited dose-dependent killing and inhibition of growth of *M. ulcerans* at MIC value range 4–8 μg/ml. Rifampicin (0.1 μg/ml) and ivermectin (8 μg/ml) combination demonstrated synergistic killing effect (Omansen et al. 2015).



Ivermectin (Stromectol)

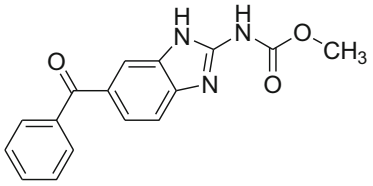


Selamectin (Revolution)

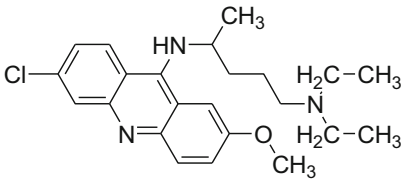


Moxidectin (Cydectin)

Miro-Canturri et al. reported antifungal activity of mebendazole (indicated in number of parasitic worm infestations) against *Cryptococcus neoformans* and *Cryptococcus gattii* and antibiofilm properties against *C. neoformans*. Mebendazole in combination with amphotericin B demonstrated twofold fungicidal activity against *C. neoformans* than single-dose administration of amphotericin B. Additionally, quinacrine (antiprotozoal, antirheumatic) in combination with caspofungin or amphotericin B depicted synergistic antifungal activity against *C. albicans* (Miro-Canturri et al. 2019).



Mebendazole (Vermox)



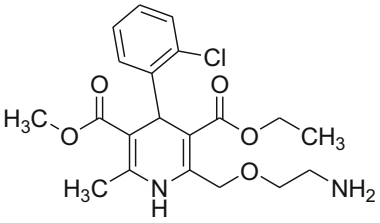
Quinacrine (Atabrine)

16.4.4 *Repurposing of Cardiovascular Drugs for Microbial Diseases*

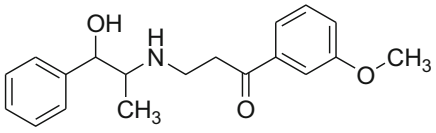
Cardiovascular drugs are indicated widespread incidences of cardiovascular diseases such as hypertension, hyperlipidemia, arrhythmias, heart failure, and coronary artery disease. Antiplatelets and anticoagulants are also included in cardiovascular drugs to prevent cardiogenic embolism. Among these therapeutics, calcium channel blockers are approved for treating angina or cardiac dysrhythmias and known to act by reducing calcium flux into cells thereby causing dilation of arteries, and lowering of blood pressure. Numerous cardiovascular agents have been reported to exhibit antimicrobial activities (Patil et al. 2016). As per the recent findings, amlodipine which is employed as calcium channel blockers in treatment and management of high blood pressure and coronary artery disease also revealed antimicrobial properties (Kelly 2021; Elliott and Ram 2011).

Kumar et al. demonstrated antimicrobial activity of amlodipine against several Gram-positive and Gram-negative bacterial strains. The drug showed sensitivity against screened clinical isolates in MIC range 50–200 μg/ml earlier which was found at 1–5 mg/ml on treatment with common antibiotics. The activity follows the decreasing order *S. aureus*, *V. cholerae*, *V. parahemolyticus*, *Shigella* spp., *Salmonella* spp., *Bacillus* spp. The drug revealed bactericidal properties when studied against *S. aureus*, *V. cholerae*, and Sh boydii. Additionally, amlodipine showed resistance toward *E. coli*, *Klebsiella* spp., and *P. aeruginosa* at low concentration (Kumar et al. 2003).

A vasodilator and β adrenoreceptor agonist oxyfedrine approved for angina pectoris showed inhibitory activity against several strains of Gram-positive and Gram-negative isolates in MIC range from 50 to 200 μg/ml. Furthermore, the drug depicted significant activity against enterobacteria at MIC at 25–100 μg/ml and possessed insignificant activity against *E. coli* and *Klebsiella* spp. (Mazumdar et al. 2003).



Amlodipine (Norvasc)



Oxyfedrine

Statins are also known as 3-hydroxy-3-methyl-glutaryl (HMG)-CoA reductase inhibitors that find applications in treatment of hyperlipidemia. Besides their action

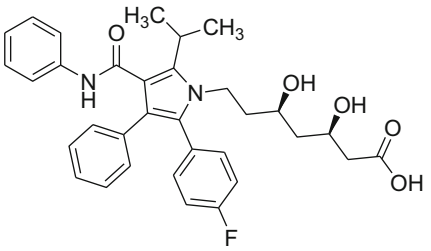
as antihyperlipidemic, these are also acknowledged with immunomodulatory, antioxidative, and anticoagulant activities. Beyond well-known antihyperlipidemic effect, a series of studies have also revealed their antimicrobial action against a range of standard bacterial strains. Simvastatin approved for treatment of hypercholesterolemia and atherosclerotic cardiovascular disease and demonstrated broad-spectrum antibacterial activity against methicillin-resistant *S. aureus* (MRSA). Thangamani et al. suggested that simvastatin exhibited MIC_{90} and MIC_{50} at concentration of 32 μg/ml against Gram-positive bacteria except *Streptococcus pneumoniae* (MIC_{90} & MIC_{50} of 64 μg/ml) and against *Bacillus anthracis* (MIC_{90} & MIC_{50} of 16 μg/ml). On the other hand, against the Gram-negative bacteria, the MIC range was found 8–32 μg/ml (Thangamani et al. 2015c).

Masadeh et al. compared the antimicrobial effect of atorvastatin, simvastatin, and rosuvastatin. Atorvastatin and simvastatin depicted significant antimicrobial properties against methicillin-sensitive *S. aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA), vancomycin-susceptible *Enterococci* (VSE), vancomycin-resistant *Enterococcus* (VRE), *A. baumannii*, *S. epidermidis*, and *E. aerogenes*. Atorvastatin also depicted activity against *E. coli*, *P. mirabilis*, and *E. cloacae* (Masadeh et al. 2012).

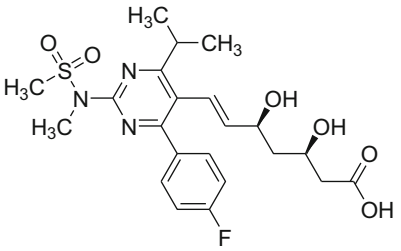
Graziano et al. also reported antimicrobial activity of atorvastatin, pravastatin, and simvastatin against the isolates of *S. aureus*, *P. aeruginosa*, *E. coli*, and *E. faecalis*. Among them, simvastatin depicted lowest MIC value for *S. aureus* at 15.65 μg/ml and 31.25 μg/mL for the other strains of *S. aureus* (Graziano et al. 2015).

Gupta et al. have reported potentiating killing effect of verapamil (calcium channel blocker) when given as adjunctive with bedaquiline and clofazimine on *M. tuberculosis* by 8- to 16-fold. By potentiating the killing of bedaquiline and clofazimine, verapamil allows shorter duration of treatment and may accelerate clearance of *M. tuberculosis* of both drug-susceptible and drug-resistant strains (Gupta et al. 2013, 2015).

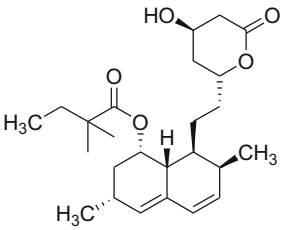
Similarly, Haschka et al. reported bacteriostatic activity of nifedipine (calcium channel blocker) against intracellular bacterium *Salmonella typhimurium* and found to show synergistic antimicrobial potential when used in combination with antibiotics (Haschka et al. 2021). The drug showed in vitro and in antibacterial activity against Gram-positive and Gram-negative isolates by exhibiting MIC in range 25–200 μg/ml (Dutta et al. 2006).



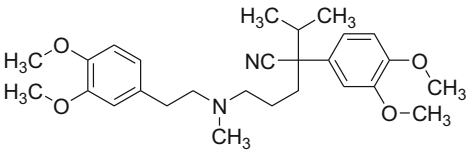
Atorvastatin
(Lipitor)



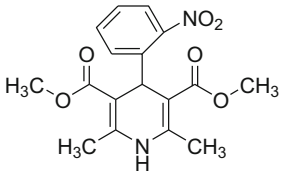
Rosuvastatin
(Crestor)



Simvastatin
(Zocor)

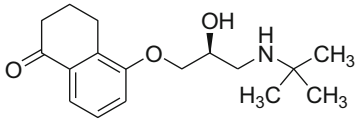


Verapamil
(soptin)

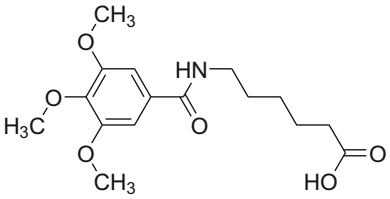


Nifedipine
(Adalat)

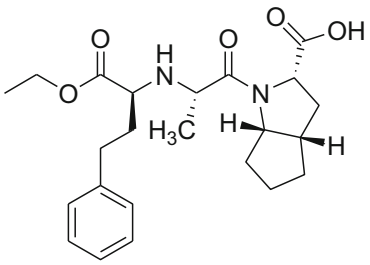
Some other cardiovascular agents, nonantibiotic molecules have also been reported as novel treatments against microbial pathogenic strains namely *Mycobacterium abscessus* and *Mycobacterium chelonae*. Levobunolol (β-adrenoceptor blocker), capobenic acid (coronary vasodilator), ramipril (ACE inhibitor), labetalol (α-adrenergic blocker), diltiazem (calcium channel blocker) exhibited antimicrobial activity against *Mycobacterium chelonae*, at MIC values 4.1, 4.07, 1.3, 0.3, 0.18 mg/L, respectively (Chopra et al. 2011).



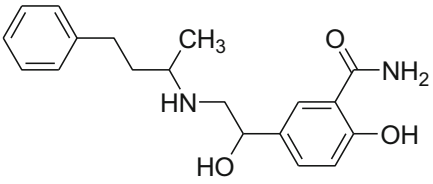
Levobunolol
(AKBeta)



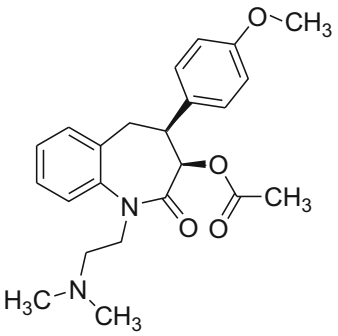
Capobenic acid



Ramipril
(Altace)



Labetalol
(Normodyne)



Diltiazem
(Cardizem)

16.4.5 Repurposing of Antipsychotic and Antidepressant Drugs for Microbial Diseases

Patients affected by mental illness or mood disorders are often under treatment with various classes of psychotropic drugs which include antipsychotics, antidepressants,

mood stabilizers, and anticonvulsants. Some psychotropic drugs are known to act by blocking specific receptors in the brain such as anticholinergics or β-blockers (Schulz and Steimer 2000; Frank et al. 2005).

It has been well documented that many known psychotherapeutic drugs other than antibiotics and chemotherapeutic agents demonstrated antimicrobial activity at high concentrations. The antimicrobial effect of these drugs was found independent of their antidepressant, antihistaminic, neuroleptic, and antihypersecretory effect (Kristiansen 1990).

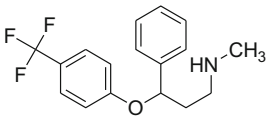
Munoz-Bellido et al. have described antimicrobial activity of selective serotonin re-uptake inhibitors (SSRI), namely sertraline, fluoxetine, and paroxetine. SSRIs act by modifying the 5-HT (serotonin) in the synapse cleft. SSRIs have a significant antimicrobial activity against *H. influenzae*, *M. catarrhalis*, and *C. jejuni*. The drugs showed synergistic effect in combination with antibiotics against resistant isolates such as *Corynebacterium urealyticum* (Munoz-Bellido et al. 2000).

Sertraline demonstrated antimicrobial activity against *S. aureus*, *E. coli*, and *P. aeruginosa* at MIC values of 20, 40, and 60 μg/ml, respectively, while 55.5% inhibition of *S. aureus* and 50% inhibition of *E. coli* observed at 20 and 60 μg/ml, respectively. On the other hand, 60% inhibition of *A. niger* and *A. fumigatus* were observed at 40 and 80 μg/ml, respectively (Ayaz et al. 2015a).

Fluoxetine demonstrated antibacterial activity against standard and resistant strains of *S. aureus* at MIC values 256 and 102 μg/ml, respectively. Against both standard and resistant isolates of *P. aeruginosa* (MIC value at 161 μg/ml) and against *E. coli* (MIC value at 102 μg/ml) fluoxetine depicted significant antibacterial activity. A combination of fluoxetine with gentamicin and erythromycin revealed synergistic effects against *P. aeruginosa* and *E. coli* (de Sousaa et al. 2018).

Sertraline and fluoxetine demonstrated significant time- and dose-dependent antifungal activity against *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus terreus*, and *Candida parapsilosis* (Lass-Florl et al. 2001).

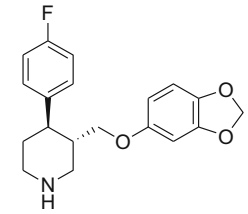
In another study by Costa Silva et al., fluoxetine showed the antifungal activity against fluconazole-resistant *Candida* spp. In MIC ranges of 20–160 μg/ml, for sertraline MIC in range 10–20 μg/ml, and for paroxetine the MIC range from 10 to 100.8 μg/ml (Table 16.4) (Costa Silva et al. 2017).



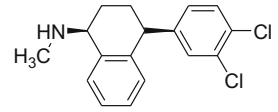
Fluoxetine (Prozac)

Table 16.4 Activity of fluoxetine, paroxetine, and sertraline against fluconazole-resistant *Candida* spp.

Fungal isolates	Fluoxetine (MIC; μg/ml)	Paroxetine (MIC; μg/ml)	Sertraline (MIC; μg/ml)
Candida albicans AB861478	40	63.5	20
Candida albicans AB861479	127	80	20
Candida albicans KJ740174	63.5	80	20
Candida tropicalis AB861493	40	80	20
Candida tropicalis AB861490	80	80	20
Candida parapsilosis AB861486	80	40	20
Candida parapsilosis AB861488	80	80	10
Candida parapsilosis AB861485	20	10	10
Candida glabrata AB861484	80	63.5	15.9



Paroxetine (Paxil)

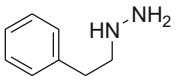


Sertraline (Zoloft)

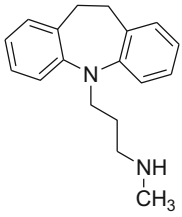
A series of studies have reported antimicrobial effect of antidepressants on the gut microbiome (McGovern et al. 2019; Caldara and Marmiroli 2021). Ait Chait et al. described antimicrobial activity of antidepressants namely phenelzine, venlafaxine, desipramine, bupropion, aripiprazole, and (S)-citalopram against common isolates of human gut microbiota. The test analogs from antidepressants class depicted noteworthy activity against *Akkermansia muciniphila* and *Clostridium leptum*, while

Lactobacillus rhamnosus was most resistant against tested drugs. Desipramine (tricyclic antidepressants) and aripiprazole being most effective against all the tested strains exhibited inhibition zone range from 13 to 35 mm and 15 to 31 mm, respectively, followed by phenelzine and (s)-citalopram (inhibition zone range 9–19 mm) (Ait Chait et al. 2020).

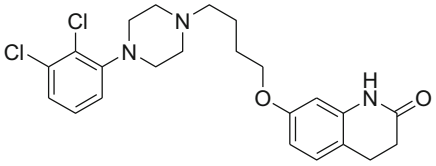
Citalopram also exhibited intrinsic activity against Gram-positive bacteria (*S. aureus*) with an inhibition zone of 5, 8, and 14 mm at doses 1250, 2500, and 5000 μg/ml, respectively. Against *E. coli* at doses 2500 and 5000 μg/ml zone diameters of 5 and 12 mm, while against *P. aeruginosa* at 5000 μg/ml exhibited inhibitory zone of 13 mm. The drug depicted minimum inhibitory concentrations (MICs) at 4, 5, and 4 mg/ml against *S. aureus*, *E. coli*, and *P. aeruginosa*, respectively (Ayaz et al. 2015b).



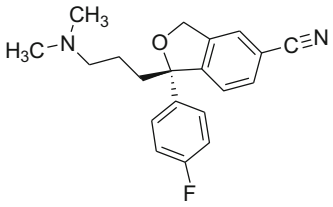
Phenelzine
(Nardil)



Desipramine
(Norpramin)

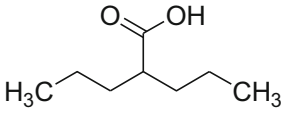


Aripiprazole
(Abilify)



(S)-Citalopram
(Celexa)

Anticonvulsant drugs are recommended in seizures in patient with epilepsy. Some approved drugs namely carbamazepine, primedase, lobazam, lonazep, gabapentine, valproic acid, lametec and valparin alka have been assayed for their antimicrobial activities by Nathiya et al. Among the tested drugs, valproic acid was found to be effective in vitro against *Staphylococcus aureus* and *Proteus vulgaris* at MIC concentration of 100 μg/ml (Nathiya et al. 2015). Valproic acid also demonstrated potent activity against the yeast strains in a dose-dependent manner by exhibiting MIC values in range 10–20 μg/ml (Esiobu and Hoosein 2003).



valproic acid
(Depakote)

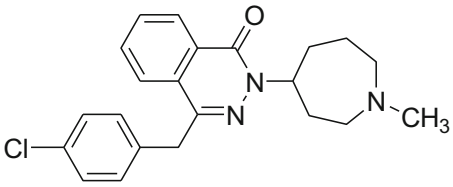
16.4.6 Repurposing of Antihistaminic Agents for Microbial Diseases

Antihistaminics are referred to the drugs that antagonize the actions of histamine (a key mediator in allergic reactions) at H_1 receptor. This class of drug includes a number of aminoalkyl ethers: ethylenediamines, piperazines, propylamines, phenothiazines, and dibenzocycloheptene. The clinical efficacy, safety, and pharmacology of antihistaminics have been well documented. H_1 antihistaminics are choice of drugs in the treatment of allergic rhinitis, allergic conjunctivitis, urticaria, etc. (Block and Beale 2004; Simon and Simons 2008).

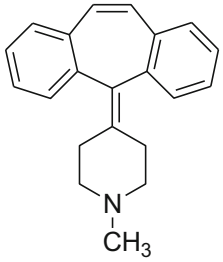
Antihistaminics are employed for various indications in microbial infection. El-Nakeeb et al. inspected bacteriostatic activity of 10 antihistaminics against multi-resistant Gram-positive and Gram-negative strains. A new generation phthalazinone derivative, azelastine showed significant bacteriostatic activity against the test Gram-positive strains. Furthermore, the study reported that screened drugs

azelastine, cyproheptadine, mequitazine, and promethazine demonstrated significant bacteriostatic activity against *S. aureus*, *S. epidermidis*, *E. faecium*, *E. coli*, *Klebsiella* spp. in a MIC range of 62.5–250 μg/ml (El-Nakeeb et al. 2011).

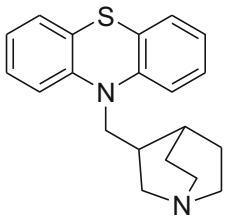
The antiallergic drug cetirizine depicted moderate in vitro antimicrobial activity against Gram-positive and Gram-negative strains at MIC ranged from 200 to 2000 μg/ml (Maji et al. 2017).



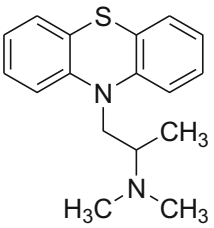
Azelastine (Optivar)



Cyproheptadine (Periactin)



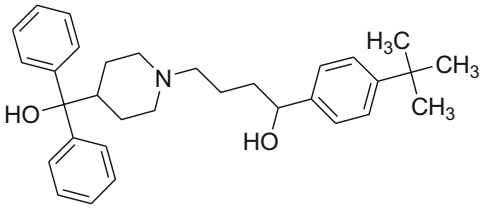
Mequitazine (Primalan)



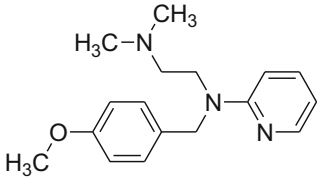
Promethazine
(Phenergan)

Other antihistamine terfenadine was reported to exhibit antimicrobial efficacy against the isolates of *S. aureus* and other bacterial pathogens, including *Mycobacterium tuberculosis* probably through inhibition of the bacterial type II topoisomerases. The terfenadine and its analogs showed antibacterial activity against methicillin-resistant (MRSA), vancomycin-intermediate (VISA), vancomycin-resistant (VRSA) *S. aureus*, and flouroquinolone-resistant strains at MIC 16 μg/ml (for terfenadine) and 1–4 μg/ml (for analogs), respectively (Perlmutter et al. 2014).

Mepyramine (a first generation antihistamine) in combination with colistin antibiotic exhibited reduction in MIC from 8.0 to 1.0 μg/ml against *E. coli* and in combination with florfenicol MIC value decreased from 8.0 to 2.0 μg/ml (Bruer et al. 2019).



Terfenadine
(Seldane)

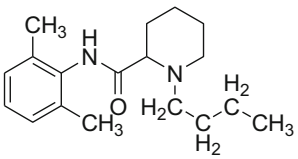


Mepyramine
(Pyrilamine)

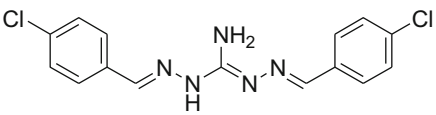
16.4.7 Other Drugs Repurposed Against Microbial Infections

The drug repositioning for microbial infections has gained exceptional devotions in recent years due to the looming threat of antimicrobial resistance and lack in development of new antimicrobial drugs. The drugs other than those mentioned above are also known to exhibit antimicrobial properties against microbes and are well documented. Bupivacaine, a local anesthetic depicted marked antibacterial activity against *S. epidermidis*, *S. pyogenes*, and *S. pneumonia* at MIC value 2.5 mg/ml, while morphine (opioid analgesics) showed no activity at same concentration against any of the strains (Rosenberg and Renkonen 1985). Robenidine (recommended for treatment of coccidiosis) in combination with ethylenediaminetetraacetic acid (EDTA) or polymyxin B nonapeptide (PMBN) showed enhanced antimicrobial activity with 2- to 2048-fold against Gram-negative and Gram-positive strains. Robenidine exhibited bactericidal action against *Acinetobacter baumannii* (MIC value 8 μg/ml) and *Acinetobacter calcoaceticus* (MIC 2 μg/ml) (Khazandi et al. 2019). Amoxapine belongs to a tricyclic antidepressant and approved for treatment of symptoms of depression. This nonantibiotic drug showed marked antimicrobial activity against *Y. pestis*, *C. difficile*, and *K. pneumonia.* In vivo infection models also showed activity against *K. pneumoniae* and *A. baumannii (*Andersson et al. 2017*)*. Recently, pentamidine (antiprotozoal drug) has been used in clinics for trypanosomiasis, antimony-resistant leishmaniasis, and pneumocystis carinii pneumonia (Sands et al. 1985). Pentamidine in combination with gentamicin, tobramycin, amikacin, tigecycline, rifampicin, or doripenem exhibited synergistic action against carbapenemase-producing *Klebsiella pneumoniae*, one carbapenemase-producing *Escherichia coli*, and two colistin-resistant *Enterobacter cloacae*. The MIC range for pentamidine was 200–800 μg/ml (Cebrero-Cangueiro et al. 2018). Pentamidine with antibiotics produced effective drug combination against a wide range of Gram-negative pathogens and *Acinetobacter baumannii* in vivo (Stokes et al. 2017). Ebselen (organoselenium compound), though not a FDA-approved drug, has investigated in clinical trials for the treatment of bipolar disorder and ischemic stroke. The drug exhibited potent antibacterial and significant antibiofilm activity against multidrug-resistant *Staphylococcus aureus*, methicillin- and vancomycin-resistant *S. aureus* (MRSA and VRSA) at MIC values <1 mg/L (Miro et al. 2019; Thangamani et al. 2015d). Most notably, ebselen was most active against *A. baumannii* at MIC 32 μM by inhibiting its TonB-dependent transport processes (Nairn et al. 2017). Vitamin D or calcitriol is a steroid hormone that can modulate the innate and adaptive immune responses to fight against bacterial infections. Calcitriol significantly increased M1 macrophage polarization and their bactericidal activity against *P. aeruginosa (*Nouari et al. 2015*)*. Metformin being an oral antidiabetic drug used as first-line drug of choice for the treatment of type 2 diabetes. Metformin showed antibacterial activity with a zone inhibition diameter 12–15 mm with a maximum zone inhibition against *Escherichia coli* at a dose 500 μg ml. At the same dose, drug exhibited zone inhibition diameter 17 mm against fungal strain *Aspergillus niger (*Meherunisa and

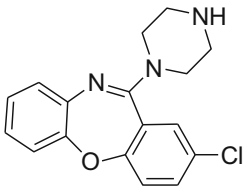
Seth 2018). Metformin in combination with antibiotic such as levofloxacin, chloramphenicol, rifampicin, ampicillin, and doxycycline exhibited potent antimicrobial activity against highly resistant bacteria (MRSA and *P. aeruginosa*) having low cytotoxicity on mammalian cells (Masadeh et al. 2021).



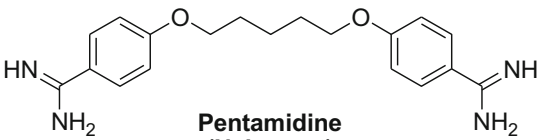
Bupivacaine (Marcaine)



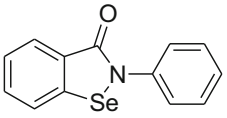
Robenidine



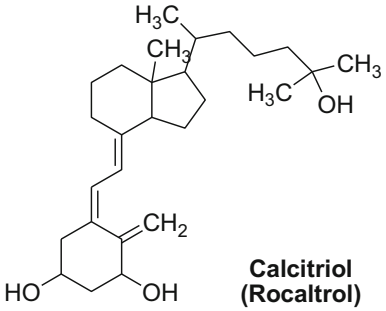
Amoxapine (Asendin)



Pentamidine (Nebupent)



Ebselen
(PZ 51)



Calcitriol
(Rocaltrol)

16.5 Conclusion

The global burden of life-threatening microbial infection is exacerbated by the lack of safe, effective antibiotics vital to their prevention and treatment. The usefulness of current antimicrobial therapies is being increasingly threatened by newly emerging pathogens and widely spread microbial resistance. Therefore, the need for effective therapeutic strategies to combat old emergent and re-emergent microbial pathogens has become the prime task for the medicinal researchers. Among anticancer drugs, several approved drugs namely 5-fluorouracil, gallium nitrate, DNA cross-linker mitomycin C, azacitidine, gemcitabine, mercaptopurine, methotrexate, hormonal modulators raloxifene, tamoxifen, radiomimetic compounds bleomycin, topoisomerase inhibitors daunorubicin, doxorubicin, etoposide, etc., have found to possess clinical efficacy to combat bacterial and fungal infections. Independent of antimicrobial activity, some approved drugs from categories such as anti-inflammatory (ibuprofen, aspirin, carprofen, bromfenac, vedaprofen, etc.), antipsychotic, antidepressant (fluoxetine, paroxetine, sertraline, etc.), and cardiovascular drugs (amlodipin, verapamil, nefidepin, etc.) also have the ability to interfere with microbial pathogenesis and alter host immune response to enhance pathogen killing and clearance. These findings suggested that drug repurposing can be successfully utilized in the discovery and development of new antimicrobial drugs with novel and effective therapeutic indications. For better drug repositioning, more in-depth understanding are required to be executed with integrated approaches between

computational and experimental methods to ensure high success rates of repositioned drugs.

References

Abongwa M, Martin RJ, Robertson AP (2017) A brief review on the mode of action of antinematodal drugs. Acta Vet (Beogr) 67(2):137–152

Ait Chait Y, Mottawea W, Tompkins TA, Hammami R (2020) Unravelling the antimicrobial action of antidepressants on gut commensal microbes. Sci Rep 10:17878

Al-Hilu SA, Al-Shujairi WH (2020) Dual role of bacteria in carcinoma: stimulation and inhibition. Int J Microbiol 4639761:1–15

Ali MT, Blicharska N, Shilpi JA, Seidel V (2018) Investigation of the anti-TB potential of selected propolis constituents using a molecular docking approach. Sci Rep 8:12238

AL-Janabi AAH. (2009) Comparison of the disc diffusion assay with spectrophotometer technique for antibacterial activity of diclofenac sodium, indomethacin and mefenamic acid. Asian J Pharmaceut 3(2):148–152

Andersson JA, Sha J, Kirtley ML, Reyes E, Fitts EC, Dann SM, Chopra AK (2017) Combating multidrug-resistant pathogens with host-directed nonantibiotic therapeutics. Antimicrob Agent Chemother 62(1):e01943–e01917

Andros CC, Dubay RA, Mitchell KD, Chen A, Holmes DE, Kennedy DR (2015) A novel application of radiomimetic compounds as antibiotic drugs. J Pharm Pharmacol 67(10): 1371–1379

Annamanedi M, Kalle AM (2014) Celecoxib sensitizes *Staphylococcus aureus* to antibiotics in macrophages by modulating SIRT1. PLoS One 9(6):e99285

Ayaz M, Subhan F, Ahmed J, Khan A, Ullah F, Ullah I, Ali G, Syed N-H, Hussain S (2015a) Sertraline enhances the activity of antimicrobial agents against pathogens of clinical relevance. J Biol Res (Thessalon) 22(1):4

Ayaz M, Subhan F, Ahmed J, Khan A, Ullah F, Sadiq A, Syed N, Ullah I, Hussain S (2015b) Citalopram and venlafaxine differentially augments antimicrobial properties of antibiotics. Acta Pol Pharmaceut Drug Res 72(6):1269–1278

Binaschi M, Zunino F, Capranico G (1995) Mechanism of action of DNA topoisomerase inhibitors. Stem Cell 13(4):369–379

Biswas T, Resto-Roldan E, Sawyer SK, Artsimovitch I, Tsodikov OV (2013) A novel non-radioactive primase–pyrophosphatase activity assay and its application to the discovery of inhibitors of *Mycobacterium tuberculosis* primase DnaG. Nucleic Acid Res 41(4):e56

Block JH, Beale JM (2004) Wilson and Gisvold's textbook of organic medicinal and pharmaceutical chemistry, 11th edn. Lippincott Williams & Wilkins. Chapter-21, pp. 696

Bruer GG, Hagedorn P, Kietzmann M, Tohamy AF, Filor V, Schultz E, Mielke-Kuschow S, Meissner J (2019) Histamine H_1 receptor antagonists enhance the efficacy of antibacterials against *Escherichia coli*. BMC Vet Res 15:55

Byrne ST, Denkin SM, Zhang Y (2007) Aspirin antagonism in isoniazid treatment of tuberculosis in mice. Antimicrob Agent Chemother 51(2):794–795

Caldara M, Marmiroli N (2021) Antimicrobial properties of antidepressants and antipsychotics-possibilities and implications. Pharmaceut 14(9):915

Campbell O, Gagnon J, Rubin JE (2019) Antibacterial activity of chemotherapeutic drugs against *Escherichia coli* and *Staphylococcus pseudintermedius*. Lett Appl Microbiol 69:353–357

Cebrero-Cangueiro T, Alvarez-Marin R, Labrador-Herrera G, Smani Y, Cordero-Matia E, Pachon J, Pachon-Ibaiez ME (2018) *In vitro* activity of pentamidine alone and in combination with aminoglycosides, tigecycline, rifampicin, and doripenem against clinical strains of

carbapenemase-producing and/or colistin-resistant *Enterobacteriaceae*. Front Cell Infect Microbiol 18(8):363

Chang H-C, Huang Y-T, Chen C-S, Chen Y-W, Huang Y-T, Su J-C, Teng L-J, Shiau C-W, Chiu H-C (2016) *In vitro* and *in vivo* activity of a novel sorafenib derivative SC5005 against MRSA. J Antimicrob Chemother 71:449–459

Cheng Y, Schorey JS, Zhang CC, Tan X (2017) Protein kinase inhibitors as potential antimicrobial drugs against tuberculosis, malaria and HIV. Curr Pharm Des 23:4369–4389

Cheng Y-S, Williamson PR, Zheng W (2019) Improving therapy of severe infections through drug repurposing of synergistic combinations. Curr Opin Pharmacol 48:92–98

Chopra S, Matsuyama K, Hutson C, Madrid P (2011) Identification of antimicrobial activity among FDA-approved drugs for combating *Mycobacterium abscessus* and *Mycobacterium chelonae*. Antimicrob Chemother 66:1533–1536

Chowdhury N, Wood TL, Martinez-Vazquez M, Garcia-Contreras R, Wood TK (2016) DNA-crosslinker cisplatin eradicates bacterial persister cells. Biotechnol Bioeng 113(9): 1984–1992

Corriden R, Hollands A, Olson J, Derieux J, Lopez J, Chang JT, Gonzalez DJ, Nizet V (2015) Tamoxifen augments the innate immune function of neutrophils through modulation of intracellular ceramide. Nat Commun 6:8369

Costa Silva RA, da Silva CR, Joa BAN, Silva RA, da Silva CR, de Andrade Neto JB, da Silva AR, Campos RS, Sampaio LS, do Nascimento FB, da Silva Gaspar B, da Cruz Fonseca SG, Josino MA, Grangeiro TB (2017) *In vitro* anticandida activity of selective serotonin reuptake inhibitors against fluconazole-resistant strains and their activity against biofilm-forming isolates. Microb Path 107:341–348

Crunkhorn S (2020) Sorafenib analogue combats MRSA. Nat Rev Drug Discov 19(2):92

Dadgostar P (2019) Antimicrobial resistance: Implications and costs. Infect Drug Resist 12:3903–3910

de Kraker MEA, Stewardson AJ, Harbarth S (2016) Will 10 million people die a year due to antimicrobial resistance by 2050? PLoS Med 13(11):e1002184

de Sousaa AK, Rocha JE, de Souza TG, de Freitas TS, Ribeiro-Filho J, Coutinho HDM (2018) New roles of fluoxetine in pharmacology: antibacterial effect and modulation of antibiotic activity. Microb Path 123:368–371

Dever LA, Dermody TS (1991) Mechanisms of bacterial resistance to antibiotics. Arch Intern Med 151:886–895

Dutta NK, Kumar KA, Mazumdar K, Dastidar SG (2004) *In vitro* and *in vivo* antimycobacterial activity of antiinflammatory drug, diclofenac sodium. Indian J Exp Biol 42:922–927

Dutta NK, Mazumdar K, Gupta AD (2006) Assessment of antibacterial activity of the cardiovascular drug nifedipine. Orient Pharm Exp Med 6(2):126–133

Dylag M, Lis P, Niedzwiecka K, Ko YH, Pedersen PH, Goffeau A, Ulaszewski S (2013) 3-Bromopyruvate: a novel antifungal agent against the human pathogen *Cryptococcus neoformans*. Biochem Biophys Res Commun 434(2):322–327

Elliott WJ, Ram CVS (2011) Calcium channel blockers. J Clin Hypertens (Greenwich) 13(9): 687–689

El-Nakeeb MA, Abou-Shleib HM, Khalil AM, Omar HG, El-Halfawy OM (2011) *In vitro* antibacterial activity of some antihistaminics belonging to different groups against multi-drug resistant clinical isolates. Braz J Microbiol 42(3):980–991

Esiobu N, Hoosein N (2003) An assessment of the *in vitro* antimicrobial effects of two antiepileptic drugs-sodium valproate and phenytoin. Antonie Van Leeuwenhoek 83(1):63–68

Flores R, Dohrmann S, Schaal C, Hakkim A, Nizet V, Corriden R (2016) The selective estrogen receptor modulator raloxifene inhibits neutrophil extracellular trap formation. Front Immunol 7: 566

Frank RG, Conti RM, Goldman HH (2005) Mental health policy and psychotropic drugs. Milbank Q 83(2):271–298

Gajadeera C, Willby MJ, Green KD, Shaul P, Fridman M, Garneau-Tsodikova S, Posey JE, Tsodikov OV (2015) Antimycobacterial activity of DNA intercalator inhibitors of *Mycobacterium tuberculosis* primase DnaG. J Antibiot (Tokyo) 68:153–157

Gerits E, Defraine V, Vandamme K, De Cremer K, De Brucker K, Thevissen K, Michiels J (2016) Repurposing toremifene for treatment of oral bacterial infections. Antimicrob Agent Chemother 61(3):e01846–e01816

Gil C, Martinez A (2021) Is drug repurposing really the future of drug discovery or is new innovation truly the way forward? Expert Opin Drug Discov 16(8):1–3

Goss CH, Kaneko Y, Khuu L, Anderson GD, Ravishankar S, Aitken ML, Singh PK (2018) Gallium disrupts bacterial iron metabolism and has therapeutic effects in mice and humans with lung infections. Sci Trans Med 10(460):eaat7520

Graziano TS, Cuzzullin MC, Franco GC, Schwartz-Filho HO, de Andrade ED, Groppo FC, Cogo-Muller K (2015) Statins and antimicrobial effects: simvastatin as a potential drug against *Staphylococcus aureus* biofilm. PLoS One 10(5):e0128098

Gupta S, Cohen KA, Winglee K, Maiga M, Diarra B, Bishai WR (2013) Efflux inhibition with verapamil potentiates bedaquiline in *Mycobacterium tuberculosis*. Antimicrob Agent Chemother 58(1):574–576

Gupta S, Tyag S, Bishai WR (2015) Verapamil increases the bactericidal activity of bedaquiline against *Mycobacterium tuberculosis* in a mouse model. Antimicrob Agent Chemother. 59(1): 673–676

Haschka D, Grander M, Eibensteiner J, Dichtl S, Koppelstatter S, Weiss G (2021) Nifedipine potentiates susceptibility of *Salmonella Typhimurium* to different classes of antibiotics. Antibiotica 10(10):1200

Hawkey PM (1998) The origins and molecular basis of antibiotic resistance. British Med J 317(7159):657–660

Hiasa H (2018) DNA topoisomerases as targets for antibacterial agents. Method Mol Biol 1703:47–62

Hlasta DJ, Demers JP, Foleno BD, Fraga-Spano SA, Guan J, Hilliard JJ, Macielag MJ, Ohemeng KA, Sheppard CM, Sui Z, Webb GC, Weidner-Wells MA, Werblood H, Barrett JF (1998) Novel inhibitors of bacterial two-component systems with gram positive antibacterial activity: pharmacophore identification based on the screening hit closantel. Bioorg Med Chem Lett 8: 1923–1928

Hussein AA, AL-Janabi AAH (2011) Investigation of anti-dermatophytic effects of non-steroidal anti-inflammatory drugs on *Trichophyton mentagrophytes* and *Epidermophyton floccosum*. Iranian J Pharma Res 10(3):547–552

Jordheim LP, Larbi SB, Fendrich O, Ducrot C, Bergeron E, Dumontet C, Freney J, Doleans-Jordheim A (2012) Gemcitabine is active against clinical multiresistant *Staphylococcus aureus* strains and is synergistic with gentamicin. Int J Antimicrob Agent 39:444–447

Kamurai B, Mombeshora M, Mukanganyama S (2020) Repurposing of drugs for antibacterial activities on selected ESKAPE bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Inter J Microbiol 8885338:1–9

Kang PW, Su JP, Sun LY, Gao H, Yang KW (2019) 3-Bromopyruvate as a potent covalently reversible inhibitor of New Delhi metallo-β-lactamase-1 (NDM-1). Eur J Pharm Sci 142:105161

Kelly MA (2021) Chapter 29: Neurological complications of cardiovascular drugs. Handb Clin Neurol 177:319–344

Kharb R, Shaharyar M, Sharma PC (2011) Insight view on the chemistry and anti-infective spectrum of triazole scaffold. Curr Med Chem 18:3265–3297

Khazandi M, Pi H, Chan WY, Ogunniyi AD, Sim JXF, Venter H, Garg S, Page SW, Hill PB, McCluskey A, Trott DJ (2019) In vitro antimicrobial activity of robenidine, ethylenediaminetetraacetic acid and polymyxin B nonapeptide against important human and veterinary pathogens. Front Microbiol 10:837

Kristiansen JE (1990) The antimicrobial activity of psychotherapeutic drugs and stereo-isomeric analogues. Danish Med Bull 37(2):165–182

Kroesen VM, Groschel MI, Martinson N, Zumla A, Maeurer M, van der Werf TS, Vilaplana C (2017) Non-steroidal anti-inflammatory drugs as host-directed therapy for tuberculosis: a systematic review. Front Immunol 8:772

Kruszewska H, Zareba T, Tyski S (2000) Antimicrobial activity of selected non-antibiotics--activity of methotrexate against *Staphylococcus aureus* strains. Acta Pol Pharm 57:117–119

Kuang X, Yang T, Zhang C, Peng X, Ju Y, Li C, Zhou X, Luo Y, Xu X (2020) Repurposing napabucasin as an antimicrobial agent against oral streptococcal biofilms. Biomed Res Int 8379526:1–9

Kumar KA, Ganguly K, Mazumdar K, Dutta NK, Dastidar SG, Chakrabarty AN (2003) Amlodipine: a cardiovascular drug with powerful antimicrobial property. Acta Microbiol Pol 52(3):285–292

Kwan BW, Chowdhury N, Wood TK (2015) Combatting bacterial infections by killing persister cells with mitomycin C. Environ Microbiol 17(11):4406–4414

Lass-Florl C, Dierich MP, Fuchs D, Semenitz E, Jenewein I, Ledochowski M (2001) Antifungal properties of selective serotonin reuptake inhibitors against *Aspergillus* species *in vitro*. J Antimicrob Chemother 48(6):775–779

Leme RCP, da Silva RB (2021) Antimicrobial activity of non-steroidal anti-inflammatory drugs on biofilm: current evidence and potential for drug repurposing. Front Microbiol 12:707629

Leon-Buitimea A, Garza-Cardenas CR, Garza-Cervantes JA, Lerma-Escalera JA, Morones-Ramírez JR (2020) The demand for new antibiotics: antimicrobial peptides, nanoparticles, and combinatorial therapies as future strategies in antibacterial agent. Design Front Microbiol 11:1669

Lim LE, Vilcheze C, Ng C, Jacobs WR Jr, Ramon-Garcia S, Thompson CJ (2013) Anthelmintic avermectins kill *Mycobacterium tuberculosis*, including multidrug-resistant clinical strains. Antimicrob Agent Chemother. 57:1040–1046

Low ZY, Farouk IA, Lal SK (2020) Drug repositioning: new approaches and future prospects for life-debilitating diseases and the COVID-19 pandemic outbreak. Viruses 12:1058

Maji HS, Maji S, Bhattacharya M (2017) An exploratory study on the antimicrobial activity of cetirizine dihydrochloride. Indian J Pharm Sci 79(5):751–757

Martin RJ (1997) Modes of action of anthelmintic drugs. Vet J 154(1):11–34

Masadeh M, Mhaidat N, Alzoubi K, Al-azzam S, Alnasser Z (2012) Antibacterial activity of statins: a comparative study of atorvastatin, simvastatin, and rosuvastatin. Ann Clin Microbiol Antimicrob 11:13

Masadeh MM, Alzoubi KH, Masadeh MM, Aburashed ZO (2021) Metformin as a potential adjuvant antimicrobial agent against multidrug resistant bacteria. Clin Pharmacol 13:83–90

Mazumdar K, Ganguly K, Kumar KS, Dutta NK, Chakrabarty AN, Dastidar SG (2003) Antimicrobial potentiality of a new non-antibiotic: the cardiovascular drug oxyfedrine hydrochloride. Microbiol Res 158:259–264

Mazumdar K, Dastidar SG, Park JH, Dutta NK (2009) The anti-inflammatory non-antibiotic helper compound diclofenac: an antibacterial drug target. Eur J Clin Microbiol Infect Dis 28:881–891

McGovern AS, Hamlin AS, Winter G (2019) A review of the antimicrobial side of antidepressants and its putative implications on the gut microbiome. Australian New Zealand J Psych 53(12): 1151–1166

McManus MC (1997) Mechanisms of bacterial resistance to antimicrobial agents. American J Health-System Pharm 54(12):1420–1433

Meherunisa JS, Seth V (2018) Study of metformin effect on antimicrobial property. Int Arch BioMed Clin Res 4(3):85–87

Miro-Canturri A, Ayerbe-Algaba R, Smani Y (2019) Drug repurposing for the treatment of bacterial and fungal infections. Front Microbiol 10:41

Miro-Canturri A, Ayerbe-Algaba R, Smani Y (2019) Drug repurposing for the treatment of bacterial and fungal infections. Front Microbiol 10(41):1–12

Mishra D, Kumar PS, Panda PK (2010) Evaluation of antimicrobial activity of selective COX-2 inhibitor. Rasayan J Chem 3(3):532–538

Munoz-Bellido JL, Munoz-Criado S, Garcia-Rodriguez JA (2000) Antimicrobial activity of psychotropic drugs selective serotonin reuptake inhibitors. Int J Antimicrob Agent 14:177–180
Nairn BL, Eliasson OS, Hyder DR, Long NJ, Majumdar A, Chakravorty S, McDonald P, Roy A, Newton SM, Klebba PE (2017) Fluorescence high-throughput screening for inhibitors of TonB action. J Bacteriol 199:e889–e816
Napier RJ, Rafi W, Cheruvu M, Powell KR, Zaunbrecher MA, Bornmann W, Salgame P, Shinnick TM, Kalman D (2011) Imatinib-sensitive tyrosine kinases regulate mycobacterial pathogenesis and represent therapeutic targets against tuberculosis. Cell Host Microb 10:475–485
Nathiya R, Mahalingam G, Purushothaman I, Vidya A, Sangeetha K (2015) Evaluation of *in vitro* antibacterial activity of anticonvulsant drugs. J Pharm Res 4(1):13–15
Nishimura Y, Hara H (2018) Drug repositioning: current advances and future perspectives. Front Pharmacol 9. https://doi.org/10.3389/fphar.2018.01068
Nouari W, Ysmail-Dahlouk L, Aribi M (2015) Vitamin D3 enhances bactericidal activity of macrophage against *Pseudomonas aeruginosa*. Int Immunopharmacol 30:94–101
Omansen TF, Porter JL, Johnson PD, vander Werf TS, Stienstra Y, Stinear TP (2015) *In-vitro* activity of avermectins against *Mycobacterium ulcerans*. PLoS Negl Trop Dis 9:e0003549
Partridge FA, Forman R, Bataille CJR, Wynne GM, Nick M, Russell AJ, Else KJ, Sattelle DB (2020) Anthelmintic drug discovery: target identification, screening methods and the role of open science. Beilstein J Org Chem 16:1203–1224
Patil TR, Patil S, Patil A, Patil ST (2016) Antimicrobial actions of antihypertensives. Inter J Toxicol Pharmacol Res 8(6):445–449
Pereira SG, Domingues VS, Theriaga J, Chasqueira MDJ, Paixao P (2018) Non-antimicrobial drugs: Etodolac as a possible antimicrobial or adjuvant agent against ESKAPE pathogens. Open Microbiol J 12(1):288–296
Perlmutter JI, Forbes LT, Krysan DJ, Ebsworth-Mojica K, Colquhoun JM, Wang JL, Dunman PM, Flaherty DP (2014) Repurposing the antihistamine terfenadine for antimicrobial activity against *Staphylococcus aureus*. J Med Chem 57(20):8540–8562
Perron GG, Kryazhimskiy S, Rice DP, Buckling A (2012) Multidrug therapy and evolution of antibiotic resistance: when order matters. Appl Environ Microbiol 78(17):6137–6142
Pushpakom S, Iorio F, Eyers PA, Escott KJ, Hopper S, Wells A et al (2019) Drug repurposing: progress, challenges and recommendations. Nat Rev Drug Discov 18:41–58
Quezada H, Martinez-Vazquez M, Lopez-Jacome E, Gonzalez-Pedrajo B, Andrade A, Fernandez-Presas AM, Tovar-Garcia A, Garcia-Contreras R (2020) Repurposed anti-cancer drugs: the future for anti-infective therapy? Expert Rev Antiinfect Ther 18(7):609–612
Rajamuthiah R, Fuchs BB, Jayamani E, Kim Y, Larkins-Ford J, Conery A, Ausubel FM, Mylonakis E (2014) Whole animal automated platform for drug discovery against multi-drug resistant *Staphylococcus aureus*. PLoS One 9(2):e89189
Rajamuthiah R, Fuchs BB, Conery AL, Kim W, Jayamani E, Kwon B, Ausubel FM, Mylonakis E (2015) Repurposing salicylanilide anthelmintic drugs to combat drug resistant *Staphylococcus aureus*. PLoS One 10(4):e0124595
Rangel-Vega A, Bernstein LR, Mandujano-Tinoco EA, Garcia-Contreras SJ, Garcia-Contreras R (2015) Drug repurposing as an alternative for the treatment of recalcitrant bacterial infections. Front Microbiol 6:282
Reygaert WC (2018) An overview of the antimicrobial resistance mechanisms of bacteria. AIMS Microbiol 4(3):482–501
Rosenberg PH, Renkonen OV (1985) Antimicrobial activity of bupivacaine and morphine. Anesthesiology 62(2):178–179
Rudrapal M, Khairnar SJ, Jadhav AG (2020) Drug repurposing (DR): an emerging approach in drug discovery. In: Drug repurposing—hypothesis, molecular aspects and therapeutic applications. Intechopen. https://doi.org/10.5772/intechopen.93193
Sands M, Kron MA, Brown RB (1985) Pentamidine: a review. Rev Infect Dis 7(5):625–634

Schaller A, Sun Z, Yang Y, Somoskovi A (2002) Salicylate reduces susceptibility of *Mycobacterium tuberculosis* to multiple antituberculosis drugs. Antimicrob Agent Chemother 46(8): 2636–2639

Schulz P, Steimer T (2000) Psychotropic medication, psychiatric disorders, and higher brain functions. Dialogue Clin Neurosci 2(3):177–182

Sharma PC, Bansal KK, Sharma A, Sharma D, Deep A (2020) Thiazole-containing compounds as therapeutic targets for cancer therapy. Eur J Med Chem 188:112016

Simon FER, Simons KJ (2008) H_1-Antihistamines: current status and future directions. World Allergy Organ J 1(9):145–155

Song S, Gong T, Yamasaki R, Kim J, Wood TK (2019) Identification of a potent indigoid persister antimicrobial by screening dormant cells. Biotechnol Bioeng. https://doi.org/10.1002/bit.27078

Soo VWC, Kwan BW, Quezada H, Castillo-Juarez I, Perez-Eretza B, Garcia-Contreras SJ, Martinez-Vazquez M, Wood TK, Garcia-Contreras R (2017) Repurposing of anticancer drugs for the treatment of bacterial infections. Curr Top Med Chem 17(10):1157–1176

Stokes JM, MacNair CR, Ilyas B, French S, Cote J, Bouwman C, Farha MA, Sieron AO, Whitfield C, Coombes BK, Brown ED (2017) Pentamidine sensitizes Gram-negative pathogens to antibiotics and overcomes acquired colistin resistance. Nat Microbiol 2:17028

Sukul A, Das SC, Saha SK, Rahman SMA (2014) Screening of analgesic, antimicrobial, cytotoxic and antioxidant activities of metal complexes of indomethacin. Dhaka Univer J Pharma Sci 13(2):175–180

Tenover FC (2006) Mechanisms of antimicrobial resistance in bacteria. American J Med 119(6A): S3–S10

Thangamani S, Mohammad H, Younis W, Seleem MN (2015a) Drug repurposing for the treatment of staphylococcal infections. Curr Pharm Des 21(16):2089–2100

Thangamani S, Younis W, Seleem MN (2015b) Repurposing celecoxib as a topical antimicrobial agent. Front Microbiol 6:750

Thangamani S, Mohammad H, Abushahba MFN, Hamed MI, Sobreira TJP, Hedrick VE, Paul LN, Seleem MN (2015c) Exploring simvastatin, an antihyperlipidemic drug, as a potential topical antibacterial agent. Sci Rep 5(1):16407

Thangamani S, Younis W, Seleem M (2015d) Repurposing ebselen for treatment of multidrug-resistant *Staphylococcal* infections. Sci Rep 5:11596

Ueda A, Attila C, Whiteley M, Wood TK (2009) Uracil influences quorum sensing and biofilm formation in *Pseudomonas aeruginosa* and fluorouracil is an antagonist. Microb Biotech 2(1): 62–74

Vilaplana C, Marzo E, Tapia G, Diaz J, Garcia V, Cardona P-J (2013) Ibuprofen therapy resulted in significantly decreased tissue bacillary loads and increased survival in a new murine experimental model of active tuberculosis. J Infect Dis 208(2):199–202

Visca P, Pisa F, Imperi F (2018) The antimetabolite 3-bromopyruvate selectively inhibits *Staphylococcus aureus*. Int J Antimicrob Agent. https://doi.org/10.1016/j.ijantimicag.2018.11

Wehenkel A, Fernandez P, Bellinzoni M, Catherinot V, Barilone N, Labesse G, Jackson M, Alzari PM (2006) The structure of PknB in complex with mitoxantrone, an ATP-competitive inhibitor, suggests a mode of protein kinase regulation in mycobacteria. FEBS Lett 580:3018–3022

Yadav MK, Chae S-W, Song J-J (2012) Effect of 5-azacytidine on *in vitro* biofilm formation of *Streptococcus pneumonia*. Microb Patho 53:219–226

Yeo WS, Arya R, Kim KK, Jeong H, Cho KH, Bae T (2018) The FDA-approved anti-cancer drugs, streptozotocin and floxuridine, reduce the virulence of *Staphylococcus aureus*. Sci Rep 8:2521

Yin Z, Wang Y, Whittell LR, Jergic S, Liu M, Harry E, Dixon NE, Kelso MJ, Beck JL, Oakley AJ (2014) DNA replication is the target for the antibacterial effects of nonsteroidal anti-inflammatory drugs. Chem Biol 21(4):481–487

Yuan M, Chua SL, Liu Y, Drautz-Moses DI, Yam JKH, Aung TT, Beuerman RW, Salido MMS, Schuster SC, Tan C-H, Givskov M, Yang L, Nielsen TE (2018) Repurposing the anticancer drug cisplatin with the aim of developing novel *Pseudomonas aeruginosa* infection control agents. Beilst J Org Chem 14:3059–3069

Zheng W, Sun W, Simeonov A (2017) Drug repurposing screens and synergistic drug-combinations for infectious diseases. British J Pharmacol 175(2):181–191

Zheng W, Sun W, Simeonov A (2018) Drug repurposing screens and synergistic drug-combinations for infectious diseases. British J Pharmacol 175:181–191

Chapter 17
Repurposing Anti-inflammatory Agents in the Potential Treatment of SARS-COV-2 Infection

Ansab Akhtar, Jaspreet Kaur, Mei Nee Chiu, and Sangeeta Pilkhwal Sah

Abstract SARS-Cov-2 is the novel coronavirus with predominantly inflammatory pathogenesis. The inflammation can be initiated and finally aggravated through a number of interconnected inflammatory pathways such as NF-κB, JAK-STAT, MAPK TLRs, iNOS, COX, etc. In the current chapter, these signaling pathways which instigate inflammation in SARS-Cov-2 are discussed. Moreover, drugs inhibiting these pathways in other inflammatory conditions or diseases are either in clinical use as COVID-19 therapy, or have been proposed as potential future therapeutic interventions in this chapter. These repurposing strategies can halt the COVID-19 symptoms as well as disease progression. This was demonstrated by establishing a link between the regulatory actions of these molecules or drugs in the inflammatory pathway like cytokine release against the COVID-19-related inflammatory havoc. Hence, the chapter will provide profound insights in the inflammatory control pertaining to COVID-19 severity and complications.

Keywords SARS-Cov-2 · COVID-19 · Inflammation · Cytokines · Anti-inflammatory drugs

Abbreviations

ACE2	Angiotensin-converting enzyme-2
ALRs	AIM2-like receptors
AP-1	Activator protein-1
ASC	Apoptosis-associated speck-like protein containing a CARD domain
BAFFR	B-cell activation factor
CD	Cluster of differentiation
CLRs	C-type lectin receptors
COX	Cyclooxygenase

A. Akhtar · J. Kaur · M. N. Chiu · S. P. Sah (✉)
University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh, India

R. C. Sobti et al. (eds.), *Drug Repurposing for Emerging Infectious Diseases and Cancer*, https://doi.org/10.1007/978-981-19-5399-6_17

COX-2	Cyclo-oxygenase-2
DAMPS	Damage-associated molecular patterns
DMARDs	Disease-modifying antirheumatic drugs
ERK	Extracellular-signal-regulated kinase
G-CSF	Granulocyte- Colony stimulating factor
IKK	IκB kinase
IL-1R	Interleukin-1 receptor
iNOS	Inducible nitric oxide synthase
IRF3	Interferon regulatory factor 3
JAK	Janus Kinase
MAPK	Mitogen-activated protein kinase
MCP-1	Monocyte chemoattractant protein
MIP-1α	Macrophage inflammatory product 1 alpha
NF-κB	Nuclear factor κ light chain enhancer of activated B cells
NIK	NF-κB-inducing kinase
NLRs	NOD-like receptors
NSAIDs	Non-steroidal anti-inflammatory drugs
PAMP	Pathogen-associated molecular pattern
PGG2	Prostaglandin G2
PLA2	Phospholipase-A2
PRRs	Pattern recognition receptors
RANK	Receptor activator for nuclear factor kappa B
RCT	Randomized controlled trial
RLSs	RIG-I-like receptors
ROS	Reactive oxygen species
SARS-Cov-2	Severe acute respiratory syndrome Coronavirus-2
STAT	Signal Transducer and Activator Protein
TLRs	Toll-like receptors
TNFR	Tumor necrosis factor receptor
TXA2	Thromboxane

17.1 Introduction

Severe acute respiratory syndrome coronavirus-2 (SARS-Cov-2) is the novel and one of the most infectious categories of coronaviruses emerged in Wuhan, China in December 2019, hence the disease is named as COVID-19. The virus has crown-type appearance over their surface and spread through respiratory tract primarily affecting the lungs. Fever, cough, sore throat, fatigue, etc. are the major symptoms. Loss of smell and taste can be the additional symptoms. The virus binds to ACE2 receptors to propagate its invasive chain (Luan et al. 2020). The inflammatory cascade is the first and foremost response following SARS-CoV-2 entrance in the human body through respiratory tract (Fu et al. 2020). Storm of pro-inflammatory cytokines, chemokines, inflammasomes, etc. constitute the core picture which could

be pathological endpoint of several overlapping and connected pathways (Fu et al. 2020) such as NF-κB overactivation, JAK-STAT pathway, MAPK dysregulation and involvement of TLRs, iNOS, COX, etc. (Masiello et al. 2020; Talukdar et al. 2020). Hence, modulating these systems and pathways in a corrective way to minimize the deteriorating influence of inflammation can overwhelmingly be adopted in potential COVID-19 therapy. The major categories of the anti-inflammatory drugs which can be repurposed to combat the inflammatory pathogenesis of COVID-19 are counted as NF-κB inhibitors like dexamethasone, JAK-STAT pathway regulator tofacitinib, MAPK inhibitor pirfenidone, COX inhibitor NSAIDs (ibuprofen, aspirin), and iNOS inhibitor L-arginine. Hence, the current chapter gives an overview that the strategies by targeting inflammatory cascade through numerous pathways can be the useful alternatives and potential future symptomatic relief options or curative measures for the treatment of COVID-19.

17.2 Epidemiology of COVID-19

The cases of disastrous COVID-19 pandemic were initially reported in the Chinese city of Wuhan, which kept spreading almost to the every corner of the globe like a wildfire. By now, almost 250 million cases and over 5 million deaths have occurred worldwide with a slight proportion remaining unreported. Several variants and subvariants named as alpha, beta, gamma, delta, delta-plus, and most recently omicron (McIntosh 2021) with variable infectious rate and severity kept coming into the picture, out of which delta plus and omicron are regarded as variant of concern. Even though its origin was suspected to be China, either from bat or accidental leak from laboratory with no confirmatory evidence, still in initial phase it dominated in European countries such as Italy, Germany, and Spain. Later, USA, UK, and Brazil become the epicenter with 2–3 waves. India also grappled with two waves in which the second one was deadlier and far more catastrophic. Even though mortality rate due to COVID-19 is around 2–3%, still containment was thought to be extremely essential due to its exceptionally high contagious feature, comorbidity, and possible long-lasting damage incurred in the human body and mind (Kiang et al. 2020).

17.3 Inflammatory Reaction in Pathophysiology of COVID-19

In terms of inflammatory reaction, SARS-CoV-2 first binds to ACE2 receptors present in pulmonary cells, instigating the series of cytokine bombardment in the form of interleukins and TNF-α. Interleukins predominantly include IL-1, IL-6, and IL-8. Additionally, chemokines, interferons, and C-reactive protein can also lead the

way to inflammation (Fu et al. 2020). These cytokines and other pro-inflammatory factors generate the signals to attract defensive cells like T-helper cells and neutrophils, which becomes the basis of inflammatory damage in the pulmonary tissue. G-CSF, MCP-1, and MIP-1α further exacerbate the phenomenon. Types of CD cells present in the lungs get sequestered with these inflammatory markers to induce lung injury (Yang et al. 2020). The spread of the injury can often extend to length and breadth of other vital organs like heart, kidney, and even brain.

17.4 Pathways Involved in Inflammation

There are several pathways which play a critical role in the generation of inflammation in the body through diverse mechanisms. These pathways can be intertwined and influence each other's upregulation or downregulation depending on the trigger they receive (Fig. 17.1). At the end, they can be the potential targets of various anti-inflammatory agents which are already been utilized against numerous other

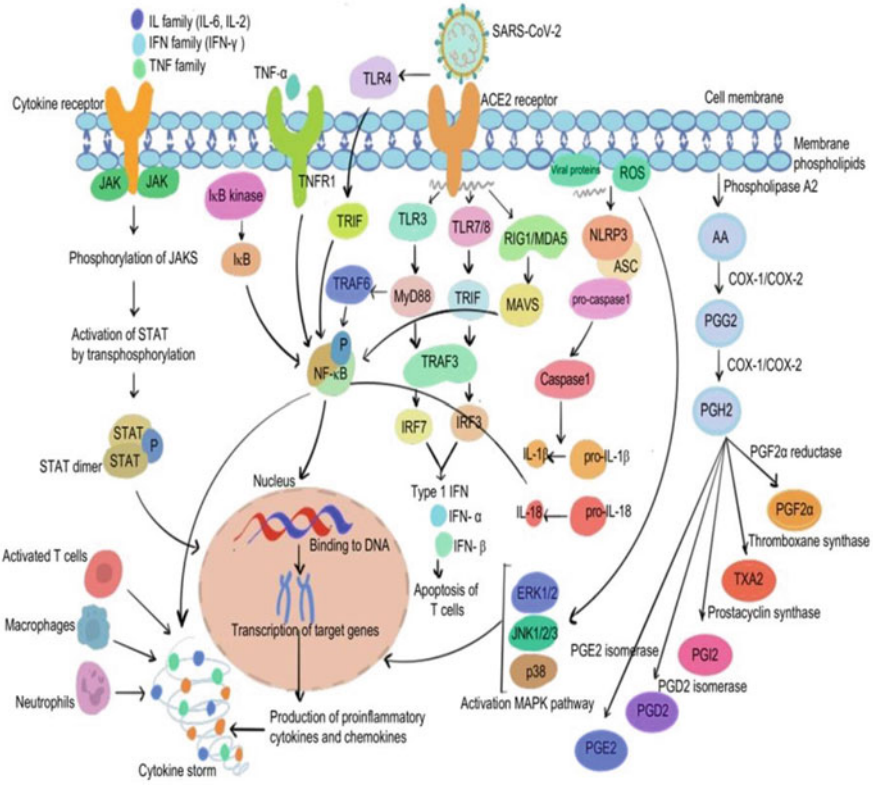


Fig. 17.1 Involvement and interlinkage of different inflammatory pathways with each other

inflammatory conditions or diseases. A few worth-mentioning pathways in this category as potential targets in COVID 19 are as follows.

17.4.1 JAK/STAT Pathway

The Janus kinase signal transducer and activator transcription (JAK/STAT) pathway constitutes the main regulatory signaling pathways involved in cytokine activation and progression to cytokine storm (Satarker et al. 2021). Recently, it is also called the IL-6 signaling pathway due to its stimulation by IL-6 cytokine. This pathway plays a significant role in the regulation of multiple cellular processes, such as immune responses, differentiation, apoptosis, and cell proliferation, such that any abnormalities in the pathway can lead to inflammatory, autoimmune diseases, and cancer growth (Montero et al. 2021). The JAK cytokine-receptor-bound tyrosine kinase family chiefly comprises of four members: JAK1, JAK2, JAK3, and TYK2, whereas the STAT family located in the cytoplasm consists of seven members: STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, and STAT6 (Banerjee et al. 2017). The JAK/STAT mechanism involves intracellular transduction in response to extracellular signals received in the form of various cytokines, growth factors, interferons, and growth hormones. The stimulation of receptor-associated JAKs begins with the attachment of extracellular factors to receptors, followed by the tyrosine autophosphorylation of the intracellular tail of receptors, which in turn serves as docking sites for STATs. These STATs recruited are trans-phosphorylated, dimerized, and translocated into the nucleus, where they trigger transcription of various target genes including cytokines (Bagca and Avci 2020). The inhibition of the JAK/STAT pathway can be a promising therapeutic strategy to control the inflammatory and immune complications caused due to SARS-CoV-2 infection.

17.4.2 NF-κB Pathway

The nuclear factor κ light chain enhancer of activated B cells (NF-κB) constitutes a transcription factor family present in nearly all animal cells. Performing significant roles in different processes of inflammation, immune response, cell proliferation and apoptosis, NF-κB signaling especially regulates the expression of inflammatory cytokines involved in cytokine storm characterized by elevated levels of IL-6, IL-10, IFN-γ, and TNF-α (Ahmed et al. 2017; Bhandari et al. 2021). The family comprises of five related transcription factors, specifically, NF-κB1, NF-κB2, RelA (p65), RelB, c-Rel, where NF-κB1 and NF-κB2 are proteolytically processed to p50 and p52, from their precursors, p105 and p100, respectively (Hoesel and Schmid 2013). The NF-κB activation involved in inflammatory and immune responses are based on two separate pathways: the canonical and noncanonical (also named as an alternative) pathways. The canonical pathway is stimulated through Toll-like

receptors (TLR), tumor necrosis factor receptor (TNFR), pattern recognition receptors (PRRs) various cytokine receptors like the interleukin-1 receptor (IL-1R) and antigen receptors. Initially, NF-κB present in the cytoplasm is inhibited by IκB proteins (Chen et al. 2018). The stimulation of receptors results in the activation of IκB kinase (IKK) complex, consisting of two kinase subunits, IKKα and IKKβ and a regulatory subunit, IKKγ that eventually leads to the phosphorylation of IκB by IKKβ. IκB phosphorylation causes its proteasomal degradation, resulting in nuclear translocation of NF-κB members, mainly the p50/RelA and p50/cRel complexes as well as the regulation of gene transcription (Liu et al. 2017). The noncanonical or alternative pathway, on the other hand, is stimulated by a subset of TNFR family members such as lymphotoxin β-receptor (LTβR), B-cell activation factor (BAFFR), CD40, and receptor activator for nuclear factor kappa B (RANK). Alternative pathway, in contrast to the canonical pathway, however, is independent of both IKKβ and IKKγ activity and involves NF-κB-inducing kinase (NIK) that by activating IKKα, induces the phosphorylation of p100 targeting it for ubiquitination and processing to p52 (Lawrence 2009). Hyperactivation of NF-κB pathway is involved in the pathogenesis of the severe/critical COVID-19 phenotype (Hariharan et al. 2021).

17.4.3 Toll-like Receptor Pathway

TLRs or toll-like receptors belong to a family of pattern recognition receptors (PRRs) among receptors like NOD-like receptors (NLRs), RIG-I-like receptors (RLSs), C-type lectin receptors (CLRs), AIM2-like receptors (ALRs), and inflammasomes (Mohan and Gupta 2018). These regulatory receptors play an important role in the innate immune system by recognizing the pathogen-associated molecular pattern (PAMP) as well as damage-associated molecular patterns (DAMPS). Eleven types of TLRs are identified in humans, which are able to recognize a distinct PAMP, are either membrane-bound or intracellular in nature (Manik and Singh 2021). TLRs such as TLR1, TLR2, TLR4, and TLR6 are present in the cell membrane, while TLR3, TLR7, TLR8, and TLR9 are endosomal (Kumar 2020). Triggering of TLR pathway gives rise to inflammatory responses in the form of production of inflammatory cytokines including TNFα, IL-6, chemokines like IL-8, MIP-2, and type 1 interferons. TLR signaling is also involved in the regulation of the adaptive immune response through the stimulation of costimulatory molecules, T cell, and B cell, resulting in the release of inflammatory cytokines such as IL-6, IFN-γ, IL-12, and TNF. Myeloid differentiation primary response 88 (MyD88) mediates the transmission of PAMPS and DAMPS for the transduction of TLR's signals. It results in the nuclear translocation of transcription factors, like nuclear factor-kB (NF-kB), activator protein-1 (AP-1) and interferon regulatory factor 3 (IRF3) and activation of MAPK phosphorylation, and the subsequent production of type 1 IFN and pro-inflammatory cytokines including IL-1, IL-6, TNF-α, and IL-12 (Khanmohammadi and Rezaei 2021; Manes and Nita-Lazar 2021).

17.4.4 MAPK Pathway

Another pathway with a vital role in inflammatory responses is the mitogen-activated protein kinases (MAPK) pathway. Belonging to a family of protein kinases which are serine/threonine protein kinases that communicate intracellular signaling on receiving various extracellular stimuli, like osmotic stress, mitogens, heat shock, and inflammatory cytokines (including IL-1, TNF-α, and IL-6) (Chen et al. 2018). The MAPK signaling also plays a critical role in the regulation of different processes such as cell proliferation, differentiation, stress response, immune function, and apoptosis. In mammalian cells, the MAPKs cascade largely constitutes the extracellular-signal-regulated kinase ERK1/2, p38 MAPK, and c-Jun NH2-terminal kinases (JNK1/2/3) (Mohanta et al. 2020). The initiation of the MAPK cascade occurs in successive phosphorylations, where each MAPK signaling module is composed of: MAPK, MAP2K, and MAP3K. The activation of the cascade involves the MAP3K to phosphorylate and activate MAP2K, which in turn phosphorylate and activate MAPK. While mitogens or growth factors, hormones, and pro-inflammatory stimuli are responsible for activating ERKs1/2, different stresses along with pro-inflammatory stimuli play the part in the activation of JNK1/2/3 and p38 MAPK (Soares-Silva et al. 2016). Therefore, MAPKs activation, including activated ERK1/2, JNK, results in the phosphorylation and activation of p38 transcription factors, found in the cytoplasm or nucleus, and induction of pro-inflammatory cytokines (IL-6, TNF-α, and IL-1β) that can lead to cytokine storm in COVID-19 (Grimes and Grimes 2020; Asiedu et al. 2021).

17.4.5 COX Pathway

A crucial part of the inflammation responses in COVID-19 disease is the cyclooxygenase (COX) pathway, where COX enzymes catalyze the synthesis of prostanoids. Activated by various physical, chemical, and biological stimuli, arachidonic acid (AA), a polyunsaturated fatty acid released from membrane phospholipids through phospholipase-A2 (PLA2), is the predominant substrate in prostaglandin or thromboxane (TXA2) synthesis in the COX pathway (Mahesh et al. 2021). First, COX catalyzes the oxidation of AA to form unstable prostaglandin G2 (PGG2), followed by its reduction via peroxidation into stable prostaglandin H2 (PGH2). Consequently, PGH2 is isomerized by terminal synthases or isomerase enzymes into a range of prostanoids including PGE2, PGD2, PGI2, PGF2α that play a role in the inflammation cascade (Ripon et al. 2021). There are two isoforms of COX: constitutive COX-1 and inducible COX-2, out of which COX-2 plays a vital part in the synthesis of PGs present in inflammation (Mitchell and Kirkby 2019). It is also proposed that COX-2 has an important role in the hyperinflammatory and immune process in COVID-19 pathophysiology and can be assessed in patients with COVID-19 (Smeitink et al. 2020). Since the COX

pathway is an integral component in the inflammatory response, it can also be a promising therapeutic target for anti-inflammatory drugs.

17.4.6 Inflammasome

Pattern recognition receptors (PRRs) particularly, nucleotide-binding oligomerization domain (NOD)-like receptor family proteins (NLRs) and absent in melanoma 2 (AIM2), respond to specific pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs) like lipopolysaccharide (LPS) in the form of macromolecular multiprotein complexes called as inflammasomes (Lee et al. 2020). The multimeric complex, in turn, stimulates the maturation of pro-inflammatory cytokines such as IL-1β and IL-18 and also leads to inflammatory cell death or pyroptosis. Inflammasome comprises of an NLR sensor, the adaptor protein apoptosis-associated speck-like protein containing a CARD domain (ASC) and caspase 1 protein. On its activation, caspase-1 causes cleavage of pro-IL-1β and pro-IL-18, leading to the production of their matured forms as well as the cleavage of gasdermin D (GSDMD) that induces pyroptotic cell death (Platnich and Muruve 2019). There exists five canonical inflammasomes: NLRP1, NLRP3, NLRC4, PYRIN, and AIM2 inflammasome and a noncanonical inflammasome, caspase 4 (caspase 11 in mice) that recognizes the presence of cytosolic LPS. Inflammasome activation is a two-stage process involving priming step and activation step (Carty et al. 2021). In the first step, priming leads to the transcription of pro-IL-1β and NLRP3, through activation of PRRs like toll-like receptors (TLRs) or NOD2, followed by NF-κB signaling activation. The second step involves the activation of sensory protein and varies for all five inflammasomes. NLRP3 inflammasome activation, in particular, is associated with an array of PAMPS, DAMPS, or pathogens via molecular or cellular events such as K^+ or Cl^- efflux, Ca2+, mitochondrial dysfunction, and reactive oxygen species (ROS) production (Zhao et al. 2021).

17.5 Inhibitors/Targeting Agents of Inflammatory Pathways of JAK-STAT, NF-κB, MAPK, COX, iNOS, etc.

For the sake of checking the deteriorating consequences of inflammation arising from the overactivation or dysregulation of inflammatory pathways, there are anti-inflammatory agents used for other diseases that can be repurposed in the therapeutic interventions of COVID-19. Hence, we gathered some of them which can potentially halt the havoc created by SARS-CoV-2.

17.5.1 JAK-STAT Inhibitors/Targeting Agents

17.5.1.1 Tofacitinib

Tofacitinib, the first JAK inhibitor which got licensed for use in humans to treat autoimmune disorders (Kotyla 2018). It primarily inhibits JAK1 and JAK 3, with JAK2 and TYK 2 being inhibited to a lesser extent. JAK inhibition, particularly JAK1 and JAK3, inhibits the signaling of various interleukins, resulting in a reduction in the inflammatory cascade (Dowty et al. 2014). Tofacitinib has been approved for the treatment of adults with moderate-to-severe rheumatoid arthritis (RA) who have had a poor response or intolerant to methotrexate (MTX), disease-modifying antirheumatic drugs (DMARDs), or biologics, like TNF-inhibitors. For the treatment of moderate-to-severe RA, it can be used alone or in combination with MTX or other nonbiologic disease-modifying antirheumatic medications (Azevedo and Torres 2018). Tofacitinib is now being studied in clinical trials for COVID-19 because of its efficacy as a JAK inhibitor (Satarker et al. 2021).

17.5.1.2 Baricitinib

With IC50s of 5.9 and 5.7 nmol/L, respectively, baricitinib is an ATP competitive kinase inhibitor that inhibits JAK1 and JAK2 selectively, efficiently, and reversibly (Fridman et al. 2010). In patients with moderate COVID-19, baricitinib is certainly the safer option, with inhibitory effects on cytokine release as an anti-inflammatory drug and SARS-CoV-2 virions endocytosis. It also helps to prevent viral reproduction by inhibiting proteins in the host cell, and as an anti-inflammatory medication, it helps to reduce inflammation in ARDS patients (Satarker et al. 2021).

17.5.1.3 Ruxolitinib

Ruxolitinib, the first FDA-approved jakinib, is a strong inhibitor of JAK1 and JAK2 (O'Shea et al. 2015). The drug ruxolitinib was developed to treat polycythemia vera and intermediate- and high-risk primary myelofibrosis (Banerjee et al. 2017). Ruxolitinib inhibits cytokine signaling and can be used to treat severe COVID-19 disease associated with ARDS. Several clinical trials are currently being conducted to determine the role of ruxolitinib in COVID-19 patients (Satarker et al. 2021).

17.5.1.4 Other Jakinibs

Other jakinibs are peficitinib, fedratinib, upadacitinib, filgotinib, etc. In cellular experiments, oclacitinib mostly inhibits the function of JAK1-dependent cytokines while simultaneously inhibiting the function of JAK2-dependent cytokines

(Gonzales et al. 2014; Gonzales et al. 2016). Filgotinib (GLPG0634) is an oral JAK1 inhibitor that is powerful and highly selective (Nakayamada et al. 2016; D'Amico et al. 2018) and is being studied for the treatment of RA and inflammatory bowel disease (Namour et al. 2015; Namour et al. 2016; Taylor et al. 2017). Upadacitinib (ABT-494) is a selective JAK1 inhibitor being developed as an oral disease-modifying medication to treat RA and other autoimmune and inflammatory diseases (Mohamed et al. 2016; Klunder et al. 2018). Peficitinib (also known as ASP015K) is a once-daily JAK inhibitor that is currently being developed for the treatment of RA (Takeuchi et al. 2016). Fedratinib (SAR302503/TG101348) is a JAK2 (as opposed to other JAK family kinases)-specific inhibitor (Zhou, Georgeon et al. 2014). Pacritinib is a JAK2 and fms-like tyrosine kinase-3 inhibitor that also inhibits interleukin 1 receptor-associated kinase 1 to suppress the IL-1-triggered inflammatory pathway (Singer et al. 2016). In humans and mice, momelotinib (MMB; GS-0387; CYT387), a JAK1 and JAK2 inhibitor has therapeutic activity in myelofibrosis (Pardanani et al. 2013; Tefferi 2016; Tefferi et al. 2018). The mechanisms, side effects, and uses of jakinibs are discussed in Table 17.1.

17.5.2 COX Inhibitors/Targeting Agents

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most widely prescribed medications, with a wide range of applications. Nonselective cyclooxygenase (COX) inhibitors such as ibuprofen, aspirin (acetylsalicylate), diclofenac, and naproxen, as well as selective COX2 inhibitors such as celecoxib, rofecoxib, etoricoxib, lumiracoxib, and valecoxib are examples of NSAIDs (Organization 2020). NSAIDs inhibit the synthesis of inflammatory mediators by blocking the cyclooxygenase enzymes (COX-1/COX2). Concerns have been raised that using NSAIDs in individuals with acute viral respiratory infections, such as COVID-19, may increase the risk of severe effects (Russell et al. 2020). While studying the severity of COVID-19 symptoms in individuals with asthma and hypertension, Fang et al. linked the SARS-CoV-2 to the downregulation of angiotensin-converting enzyme-2 (ACE2), which instead is upregulated by ibuprofen (Fang 2020). As reported by Amici et al., at a concentration of 1 mg/kg, indomethacin, which is commonly used to treat gout and arthritis, inhibited human virus SARS-CoV multiplication (Amici et al. 2006). However, some studies have cast doubt on the efficacy of NSAIDs, particularly ibuprofen, in COVID-19 patients. The justification for this is that long-term use of NSAIDs has been linked to a variety of side effects, including gastrointestinal and cardiovascular problems, nephrotoxicity, and so on. According to Voiriot et al., NSAIDs were linked to serious consequences such as respiratory tract infection and peritonsillar abscess after a respiratory tract infection (Voiriot et al. 2019). Some authors and even governments objected to the use of NSAIDs during the early stages of the current pandemic, claiming that they could worsen the sickness and further recommending the use of paracetamol, a non-anti-inflammatory NSAID (Little 2020; Willsher 2020). Some studies have

Table 17.1 Inflammatory pathways, targeting agents, and their mechanisms, adverse reactions, and uses

S. No.	Target pathways	Anti-inflammatory agent	Mechanism (Targets)	Adverse reactions	Uses
1.	JAK-STAT inhibitors/targeting agents	Tofacitinib	JAK1 & JAK3	Abdominal pain, acne vulgaris, anemia, angioedema, diarrhea, dehydration, dyspepsia, headache, hepatotoxicity, hyperlipidemia, hepatitis, lymphoma, lymphopenia, nausea, neutropenia, pulmonary embolism, rashes, vomiting, blood clots, GI perforations, hepatotoxicity, hyperlipidemia, hepatitis, lymphoma, lymphopenia (Harigai 2019)	Psoriasis and RA
		Baricitinib	JAK1 & JAK2	Patients taking Baricitinib frequently have adverse symptoms such as headache, upper respiratory tract infection (UTI), and nasopharyngitis. Long-term usage of Baricitinib has been linked to an increased risk of major infections and thromboembolic events. (Jorgensen et al. 2020) Immunosuppressants taken at the same time as Baricitinib made patients more susceptible to infection. Fungal infections (candidiasis), pneumocystis, bacterial, viral infections, pneumonia, Herpes zoster, UTI, acute histoplasmosis, cryptococcosis, and other adverse effects have been reported. (Zhang et al. 2020)	RA
		Ruxolitinib	JAK1 & JAK2	In two SARS-CoV-2 patients, the drug Ruxolitinib caused purpuric sores on the limbs' skin and erythrodermic rashes. In many trials and studies anemia was also	Myelofibrosis and Polycythemia vera

(continued)

Table 17.1 (continued)

S. No.	Target pathways	Anti-inflammatory agent	Mechanism (Targets)	Adverse reactions	Uses
				diagnosed, increased the chances for opportunistic infections (Gaspari et al. 2020) Myelosuppression, GI toxicity, immunosuppression, bladder pain, dizziness, headache, sore throat, skin rashes, weight gain, flatulence, and other common side effects include myelosuppression, GI toxicity, immunosuppression, bladder pain, dizziness, headache, sore throat, skin rashes, weight gain, flatulence, and other common side effects. (Blum et al. 2016)	
		Peficitinib	JAK1 & JAK3	Most frequent: Nasopharyngitis, herpes zoster infection, blood CK increase, lymphopenia; Occasional: Pneumonia, pharyngitis, epipharyngitis, URTI, bronchitis, influenza, cystitis; Rare: Gastrointestinal perforation, sepsis (Markham and Keam 2019)	RA
		Fedratinib	JAK2	Anemia, gastrointestinal symptoms, and increased levels of liver transaminases, serum creatinine, and pancreatic enzymes (Pardanani et al. 2015)	Primary and secondary myelofibrosis
		Upadacitinib	JAK1	Upper respiratory tract infections (URTI), nausea, elevated liver enzymes, fever, cough (Genovese et al. 2018)	RA
		Filgotinib	JAK1		RA and Crohn's disease

				Nausea, upper respiratory tract infection, urinary tract infection, dizziness (Dhillon and Keam 2020)	
2.	COX cyclooxygenase inhibitors/ targeting agents	Ibuprofen	Nonselective COX inhibition	GI bleeding, peptic ulceration, hemorrhagic cerebrovascular accident, renal impairment, wheezing, and rash (Silverstein et al. 2000)	RA, osteoarthritis, fever, mild to moderate pain, dysmenorrhea, headache, migraine
		Aspirin	Nonselective COX inhibition		Prevention of cardiovascular events, mild pain, and inflammation
		Diclofenac	Nonselective COX inhibition		RA, osteoarthritis, fever, mild to moderate pain, dysmenorrhea, migraine
		Naproxen	Nonselective COX inhibition		Gouty arthritis, mild to moderate pain, tendonitis, fever, rheumatoid disorders, osteoarthritis, dysmenorrhea, migraine prevention
		Celecoxib	Selective COX2 inhibition	Cardiovascular risk, including the increased risk of heart attacks and strokes. Gastrointestinal (GI) effects, including bleeding, ulceration, and perforation of the stomach and intestines. New or worsening hypertension, fluid retention in patients with congestive heart failure, renal toxicity, liver toxicity, anaphylactic reactions, and skin changes ranging from a nonsevere rash to Stevens–Johnson syndrome (Cohen and Preuss 2021)	Osteoarthritis, ankylosing spondylitis, RA, acute pain, dysmenorrhea
		Rofecoxib	Selective COX2 inhibition	Cardiovascular risk, risk of a heart attack (Sibbald 2004)	Chronic arthritis, musculoskeletal pain
		Etoricoxib	Selective COX2 inhibition	Mild side effects like nausea, indigestion, diarrhea, high blood pressure, dizziness, and headache (Schumacher et al. 2002)	Rheumatoid arthritis, psoriatic arthritis, osteoarthritis, ankylosing spondylitis, chronic low back pain, acute pain and gout
		Lumiracoxib			Osteoarthritis, RA, acute pain

(continued)

Table 17.1 (continued)

S. No.	Target pathways	Anti-inflammatory agent	Mechanism (Targets)	Adverse reactions	Uses
			Selective COX2 inhibition	Gastrointestinal safety profiles of COX-2 inhibitors have improved, the cardio-nephrotoxic adverse effects are significant based on their COX-2 selectivity (Harirforoosh et al. 2013)	
		Valdecoxib	Selective COX2 inhibition		Osteoarthritis, RA, painful menstruation
3.	MAPK inhibitors/ targeting agents	Pirfenidone	p38 MAPK	nausea, rash, abdominal pain, upper respiratory tract infection, diarrhea, fatigue, headache, dyspepsia, dizziness, vomiting, anorexia, gastroesophageal reflux disease, sinusitis, insomnia, weight decreased, and arthralgia (Lancaster et al. 2017)	Idiopathic pulmonary fibrosis (IPF)
		SB 203580	p38 MAPK		Inflammation, arthritis diseases, septic shock and myocardial injury
		Losmapimod	p38 MAPK	Headache, Nausea, lethargy, dry mouth, neuralgia, and nasopharyngitis were also reported. (Barbour et al. 2013)	Atherosclerosis, COPD, CVD, depression
		VX-702	p38 MAPK	Gastroenteritis, nausea/vomiting, rash, and renal impairment (Ding 2006)	Acute coronary disease, RA, Crohn's disease, psoriasis
		PH-797804	P38 MAPK	COPD exacerbation, rash and nasopharyngitis (MacNee et al. 2013)	COPD, Neuropathic pain associated with post-herpetic neuralgia, RA
		Trametinib	MEK1/2	Rash, diarrhea, peripheral edema, fatigue, and dermatitis acneiform (Flaherty et al. 2012). Also have unique cardiac and ophthalmologic side effects(Renouf et al. 2012)	Melanoma, NSCLC, thyroid cancer
		Selumetinib	MEK1/2		Neurofibroma, NSCLC
		Binimetinib	MEK1/2		Melanoma, NSCLC
		Cobimetinib	MEK1/2		Melanoma, NSCLC
4.	NF-kB inhibitors/ targeting agents	Resveratrol		thyroid disruptor and a goitrogen (Giuliani et al. 2014)	

			Inhibit the phosphorylation of IKK-β		Anti-oxidant, anti-inflammatory, anti-tumor, anti-virus, and cardiovascular disorders
		SAR 113945	Inhibit the phosphorylation of IKK-β	Immunodeficiencies, hepato-toxicity and a potentially increased risk of malignancies arising from tissues such as the liver and the skin, reflecting the essential roles of NF-κB in innate and adaptive immune responses and tissue homeostasis (Greten et al. 2007; Hsu et al. 2011; DiDonato et al. 2012)	Osteoarthritis
		IKK-16	Inhibit the phosphorylation of IKK-β		Reduce the induction of cytokine and chemokine transcripts in human or mouse keratinocytes by IL-1α, tumor necrosis factor-α, and phorbol myristate acetate
		TPCA-1	Inhibit the phosphorylation of IKK-β		Anti-inflammatory
		BAY11-7082	Inhibit the phosphorylation of IKK-β		Antitumor effects
		BAY-7085	Inhibit the phosphorylation of IKK-β		Antitumor effects
		SC-514	Inhibit the phosphorylation of IKK-β		Anti-cancer effects
		ML-120B	Inhibit the phosphorylation of IKK-β		Anti-cancer effects
		Vinpocetine	Inhibit the phosphorylation of IKK-β		Acute ischemic stroke, cerebrovascular diseases
5.	iNOS inhibitors targeting agents	L-Arginine	iNOS	Abdominal pain, bloating, low blood pressure, nausea, vomiting, and diarrhea	Hypertension, preeclampsia, burns, critical trauma, cancer, erectile dysfunction
		L-NIL	iNOS		Arthritis, Asthma

(continued)

Table 17.1 (continued)

S. No.	Target pathways	Anti-inflammatory agent	Mechanism (Targets)	Adverse reactions	Uses
		GW273629	iNOS	Chronic low-grade inflammation in cardiovascular, metabolic and kidney disorders (Zamora et al. 2000)	Acute migraine
		GW274150	iNOS		In preclinical studies of endotoxemia, inflammatory and neuropathic pain, arthritis, migraine, cancer, graft-versus-host disease and other disorders
		1400W	iNOS		
		SC-51	iNOS		
		ONO01714	iNOS		
		AR-C102222	iNOS		

recommended paracetamol because it has a lower risk of side effects and because other NSAIDs have been linked to delayed diagnosis and a higher rate of consequences in patients with respiratory tract infections (de Girolamo et al. 2020; Little 2020; Sestili and Stocchi 2020). This creates a paradox between COVID-19 treatment modalities and pathophysiology, because over-the-counter medications that can be used to alleviate symptoms in stages 1 and 2 of the disease (e.g., paracetamol) have no benefit in halting disease progression because they lack anti-inflammatory action, which is critical for keeping the inflammatory state under control. Although paracetamol may provide symptomatic relief for patients, it has little effect on disease development in COVID-19, and without anti-inflammatory effects, it risks masking symptoms. A rising number of researches are contradicting this approach, including a recent cohort that suggests their simultaneous usage may be possibly hazardous (FitzGerald 2020; Lund et al. 2020). COX1 inhibition may not be a good idea because it reduces antiviral immunity and has no effect on cytokine storm. Celecoxib's therapeutic potential has also been demonstrated in studies using molecular library searches (Gimeno et al. 2020; Ke et al. 2020). The findings from an experimental study suggested that Celebrex medication at a standard dose (0.2 g twice a day) could help people recover from COVID-19 in both mild and severe cases (Hong et al. 2020). There is no evidence currently of severe adverse events, long-term survival, acute health care utilization, or quality of life in patients with COVID-19, as a consequence with the use of NSAIDs (Organization 2020).

17.5.3 MAPK Inhibitors/Targeting Agents

The MAPK pathway is well known to be activated in viral infections, because viruses are ultimately dependent on the host cell for their life cycle. Viruses require cellular signaling pathways for replication, translation, transport across the nuclear membrane, capsid assembly, and dissemination, as well as the reactivation of virus latency (Kumar et al. 2018). Intriguingly, the MAPK cascade is also involved in immune response regulation (Gaur et al. 2011) and apoptosis (Bian et al. 2011) in virus infected cells. Previous research has revealed that the coronavirus SPIKE protein can activate the MAPK pathway and subsequent inflammatory responses via ACE2 (Chen et al. 2010). Other evidence suggests that MAPK controls ACE2 (Gallagher et al. 2008). SB203580, a p38 MAPK inhibitor, decreased the effective phosphorylation of HSP-27, CREB, and eIF4E in SARS-CoV-infected cells, indicating that it could be a new class of antiviral medicines (Mizutani et al. 2004). A number of direct p38 MAPK inhibitors are now in clinical trials for various reasons and could be repurposed for randomized clinical trials in individuals at risk of serious COVID-19 infection (Grimes and Grimes 2020). Losmapimod is clinically the most well-studied p38 inhibitor with a good safety profile. A single IV infusion of 3 mg of losmapimod followed by a 15 mg oral dose was shown to be safe and well tolerated in 12 healthy volunteers. The only side effect that was mentioned more than once was headache. Nausea, lethargy, dry mouth, neuralgia, and nasopharyngitis

were also reported. The sole adverse event thought to be linked to the medication was nausea (Barbour et al. 2013). Another p38 inhibitor, dilmapimod, was previously evaluated in 77 patients at risk of acute lung injury after trauma in a clinical trial, with no significant safety findings (Christie et al. 2015). Findings from a study by Zhou et al. suggest that MEK inhibitors as a class suppress host SARS-CoV-2 infectivity factors such as ACE2 and TMPRSS2, and that there is innate immune system activity, as well as suppression of inflammatory cytokines and stimulation of natural killer cell activity, when used alone or in combination with remdesivir (Zhou et al. 2020). MEKi has previously been shown to lower systemic inflammation and improve immunological response in vivo, according to earlier studies. For example, MEKi inhibits endotoxin-induced kidney damage in mice in the setting of sepsis (Smith et al. 2015). In a cecal puncture-sepsis model, MEKi trametinib decreased cytokines as well as kidney, liver, and muscle injuries in vivo (Smith et al. 2016). In a Lewis lung cancer model, MEKi selumetinib was found to lower IL-6 levels however did not protect against cachexia (Au et al. 2016).

17.5.4 NF-κB Inhibitors/Targeting Agents

NF-κB is a master regulator of a variety of signaling pathways involved in development, cellular physiology, neuroplasticity, cell survival mechanisms, and immune functions (Mattson and Camandola 2001; Lawrence 2009; Oeckinghaus and Ghosh 2009).

The use of dexamethasone as a treatment for COVID-19 patients has been shown to have a high rate of recovery (Kaddoura et al. 2020; Tomazini et al. 2020). Dexamethasone is a corticosteroid used to reduce fever and block the release of pro-inflammatory molecules in a variety of clinical situations (Bunim et al. 1958; Barnes 2006; Ramamoorthy and Cidlowski 2016). Dexamethasone has also been shown to upregulate IκBα, which is a potent NF-kB signaling blocker (Auphan et al. 1995; Scheinman et al. 1995; Oeckinghaus and Ghosh 2009). Dexamethasone has also been shown to prevent p65 from moving into the nucleus (Scheinman et al. 1995; Yamamoto and Gaynor 2001). Considering the facts mentioned above, the therapeutic benefits of dexamethasone in COVID-19 conditions may be largely due to NF-κB signaling inhibition. While abnormal NF-κB signaling was previously identified during a prior SARS-CoV outbreak, its activation could possibly be linked to the current SARS-CoV-2-induced cytokine storm and tissue damage (Liao et al. 2005; DeDiego et al. 2014; Hirano and Murakami 2020). The complex of three subunits known as IKKβ, IKKα, and NF kappa B essential modulator (NEMO) are the key downstream effectors of the NF-kB (Solt and May 2008; Oeckinghaus and Ghosh 2009). Activation of NF-kB signaling pathways is aided by the phosphorylation of IKKβ followed by its breakdown (Santoro et al. 2003; Gilmore and Herscovitch 2006; Lawrence 2009; Oeckinghaus and Ghosh 2009; Li et al. 2020). As a result, preventing IKKβ-phosphorylation has been viewed as a critical step in inactivating NF-kB and so giving therapeutic benefit against a variety of

inflammatory conditions. PS-1145, SAR113945, IKK-16, TPCA-1, BAY11-7082, BAY11-7085, SC-514, TBK-1, ML-120B, BMS-345541, vinpocetine, and resveratrol are only a few of the pharmacological inhibitors that have been discovered to prevent the phosphorylation of IKK-β. (Gilmore and Herscovitch 2006; Jeon et al. 2010; Ren et al. 2013; Bhatti et al. 2019).

17.5.5 iNOS Inhibitors/Targeting Agents

Nitric oxide (NO) is an important molecule in the immunological defense against pathogens (Stamler et al. 1997; Saxena et al. 2001; Akerstrom et al. 2005; Klingstrom et al. 2006; Greaves and Chamberlain 2007). iNOS-produced NO is a toxic agent and an essential immunoregulatory mediator of the host's immune system against infectious organisms. NO can also control the function, proliferation, and death of immune cells like macrophages, neutrophils, T cells, and natural killer (NK) cells (Coleman 2001). Several substances, including microbial lipopolysaccharides and cytokines, promote iNOS expression (Asano et al. 1994; Sarawar and Doherty 1994; Guo et al. 1995). Unlike eNOS and nNOS, iNOS remains active after it has been expressed. As a result, iNOS produces high levels of NO indefinitely to chemically neutralize invading pathogens, and this level of synthesis lasts for hours or days, depending on how long the enzyme is present in cells or tissue (Coleman 2001). NO can freely move through cell membranes to neighboring cells and viruses without the use of a receptor (Burrack and Morrison 2014). NO also inhibits viral replication by acting on a range of viral targets, and cell specificity is determined by its concentration, chemical reactivity, proximity to target cells, and the way target cells are programmed to respond (Coleman 2001). Moreover, unlike antiviral lymphocytes, the NO effect is independent of immune recognition of the infected cell. This impact could be crucial in virus-infected cells where the major histocompatibility complex, which is required for adaptive immunity, is limited and/or underregulated (Burrack and Morrison 2014). The quest for effective antivirals against SARS-CoV-2 is critical in light of the recent global CoV outbreak. In vitro, NO donors were found to suppress the SARS-CoV replication cycle by impacting spike (S) protein and ACE2 fusion or by reducing viral RNA load in the early stages of replication (Akerstrom et al. 2005). Although the antiviral benefits of NO against SARS-CoV-2 have yet to be determined, data suggest that it has a lot of potential (Guimaraes et al. 2021).

Although NO protects against viral infection, it can potentially contribute to COVID-19 immunopathology. NO production is a regulated process, however pathophysiological circumstances that disrupt this regulation result in the production of reactive oxygen species (ROS) (Davis et al. 2001; Westphal et al. 2008; Kellner et al. 2017). ROS produced by the endothelium and epithelium, as well as leukocytes, plays a key role in the pathogenesis of ARDS and lung damage. ROS increases oxidative stress in pulmonary duct tissues and airways, further altering the inflammatory state. ROS also positively regulates the expression of

pro-inflammatory cytokines and adhesion molecules, causing endothelial and epithelial dysfunctions, as well as increasing oxidative stress in pulmonary duct tissues and airways, further altering the inflammatory state (Kahn et al. 2012; Kamiyama et al. 2014; Kellner et al. 2017). During the ARDS process, lung cells release a high amount of inflammatory substances, which stimulate iNOS synthesis in alveolar macrophages, neutrophils, and the bronchial epithelium, resulting in excessive NO for release into lung tissues (Matsuo 1999; Wallet et al. 2013). Furthermore, in ARDS patients, airway stress can cause bronchial obstruction and increase inflammation, causing lung tissues to release more NO (Ciprandi et al. 2013). Excessively produced NO when reacts with superoxide, it damages cell components and promotes the development of peroxynitrite, which can nitrate and oxidize proteins, lipids, and nucleotides (van der Vliet et al. 1999). When plasma NO levels rise, the interaction between NO and superoxide to create peroxynitrite speeds up to nearly three times the rate of superoxide breakdown by superoxide dismutase (Korhonen et al. 2005). Elevated peroxynitrite generation can impair mitochondrial respiration, cause protein malfunction, deplete cellular energy, and damage cell membranes and DNA (van der Vliet et al. 1999), as well as contribute to anti-inflammatory drug resistance (Barnes 2016). Although reactive nitrogen species (RNS) are more commonly mentioned, both ROS and RNS can oxidize or nitrose proteins, DNA, or lipids (Rehberg et al. 2010). The pathogenesis of lung damage is complicated by NO-mediated oxidative stress. High levels of NO, as measured by a rise in its stable metabolites nitrate and nitrite, can exacerbate lipid peroxidation, promote lung epithelial cell necrosis and denaturation, aggravate inflammation, and trigger the onset of ARDS (Tang et al. 2017). High concentrations of NO, nitrate, and nitrite metabolites in the bronchoalveolar lavage were found in a clinical study, not only in ARDS patients but also in patients at risk for ARDS, suggesting that the oxidative stress detected at the start of ARDS begins when patients are at risk, before the clinically defined syndrome is recognized (Sittipunt et al. 2001). ARDS intrapulmonary diseases should be mitigated by specific iNOS inhibitors (Enkhbaatar et al. 2003).

17.6 Conclusion

In a nutshell, the entire chapter can be concluded that anti-inflammatory drugs already showing effectiveness in other inflammatory diseases can be brought about with advanced research to elicit some responses in relation to anti-inflammatory mechanisms of the drugs. These agents could further be investigated in preclinical and clinical studies to find out whether or not they are eliciting any reliable impacts. However, other factors involved in the infection and pathogenesis of COVID-19 could not be ruled out and merely targeting the inflammatory pathways would not suffice the complete eradication of the virulence and severity and hence utilization of these medicines could be limited, which further enforces the necessities of the exposure of other avenues pertaining to the COVID-19 pathology.

References

Ahmed SMU, Luo L et al (2017) Nrf2 signaling pathway: pivotal roles in inflammation. Biochim Biophys Acta (BBA)-Mol Basis Dis 1863(2):585–597

Akerstrom S, Mousavi-Jazi M et al (2005) Nitric oxide inhibits the replication cycle of severe acute respiratory syndrome coronavirus. J Virol 79(3):1966–1969

Amici C, Di Caro A et al (2006) Indomethacin has a potent antiviral activity against SARS coronavirus. Antiviral Ther 11(8):1021

Asano K, Chee CB et al (1994) Constitutive and inducible nitric oxide synthase gene expression, regulation, and activity in human lung epithelial cells. Proc Natl Acad Sci U S A 91(21):10089–10093

Asiedu SO, Kwofie SK et al (2021) Computational identification of potential anti-inflammatory natural compounds targeting the p38 Mitogen-Activated Protein Kinase (MAPK): implications for COVID-19-induced cytokine storm. Biomolecules 11(5):653

Au ED, Desai AP et al (2016) The MEK-inhibitor selumetinib attenuates tumor growth and reduces IL-6 expression but does not protect against muscle wasting in lewis lung cancer cachexia. Front Physiol 7:682

Auphan N, DiDonato JA et al (1995) Immunosuppression by glucocorticoids: inhibition of NF-kappa B activity through induction of I kappa B synthesis. Science 270(5234):286–290

Azevedo A, Torres T (2018) Tofacitinib: A New Oral Therapy for Psoriasis. Clin Drug Investig 38(2):101–112

Bagca BG, Avci CB (2020) The potential of JAK/STAT pathway inhibition by ruxolitinib in the treatment of COVID-19. Cytokine Growth Factor Rev 54:51–61

Banerjee S, Biehl A et al (2017) JAK-STAT signaling as a target for inflammatory and autoimmune diseases: current and future prospects. Drugs 77(5):521–546

Barbour AM, Sarov-Blat L et al (2013) Safety, tolerability, pharmacokinetics and pharmacodynamics of losmapimod following a single intravenous or oral dose in healthy volunteers. Br J Clin Pharmacol 76(1):99–106

Barnes PJ (2006) How corticosteroids control inflammation: Quintiles Prize Lecture 2005. Br J Pharmacol 148(3):245–254

Barnes PJ (2016) Inflammatory mechanisms in patients with chronic obstructive pulmonary disease. J Allergy Clin Immunol 138(1):16–27

Bhandari R, Khanna G et al (2021) Divulging the intricacies of crosstalk between NF-Kb and Nrf2-Keap1 pathway in neurological complications of COVID-19. Mol Neurobiol:1–15

Bhatti FUR, Hasty KA et al (2019) Anti-inflammatory role of TPCA-1 encapsulated nanosomes in porcine chondrocytes against TNF-alpha stimulation. Inflammopharmacology 27(5):1011–1019

Bian J, Wang K et al (2011) Caspase- and p38-MAPK-dependent induction of apoptosis in A549 lung cancer cells by Newcastle disease virus. Arch Virol 156(8):1335–1344

Blum S, Martins F et al (2016) Ruxolitinib in the treatment of polycythemia vera: patient selection and special considerations. J Blood Med 7:205–215

Bunim JJ, Black RL et al (1958) Studies on dexamethasone, a new synthetic steroid, in rheurheumatoid arthritis: a preliminary report; adrenal cortical, metabolic and early clinical effects. Arthritis Rheum 1(4):313–331

Burrack KS, Morrison TE (2014) The role of myeloid cell activation and arginine metabolism in the pathogenesis of virus-induced diseases. Front Immunol 5:428

Carty M, Guy C et al (2021) Detection of viral infections by innate immunity. Biochem Pharmacol 183:114316

Chen IY, Chang SC et al (2010) Upregulation of the chemokine (C-C motif) ligand 2 via a severe acute respiratory syndrome coronavirus spike-ACE2 signaling pathway. J Virol 84(15):7703–7712

Chen L, Deng H et al (2018) Inflammatory responses and inflammation-associated diseases in organs. Oncotarget 9(6):7204

Christie JD, Vaslef S et al (2015) A randomized dose-escalation study of the safety and anti-inflammatory activity of the p38 mitogen-activated protein kinase inhibitor dilmapimod in severe trauma subjects at risk for acute respiratory distress syndrome. Crit Care Med 43(9):1859–1869

Ciprandi G, Tosca MA et al (2013) High exhaled nitric oxide levels may predict bronchial reversibility in allergic children with asthma or rhinitis. J Asthma 50(1):33–38

Cohen B, Preuss CV (2021) Celecoxib. StatPearls, Treasure Island, FL

Coleman JW (2001) Nitric oxide in immunity and inflammation. Int Immunopharmacol 1(8):1397–1406

D'Amico F, Fiorino G et al (2018) Janus kinase inhibitors for the treatment of inflammatory bowel diseases: developments from phase I and phase II clinical trials. Expert Opin Investig Drugs 27(7):595–599

Davis KL, Martin E et al (2001) Novel effects of nitric oxide. Annu Rev Pharmacol Toxicol 41: 203–236

de Girolamo L, Peretti GM et al (2020) Covid-19-The real role of NSAIDs in Italy. J Orthop Surg Res 15(1):165

DeDiego ML, Nieto-Torres JL et al (2014) Inhibition of NF-kappaB-mediated inflammation in severe acute respiratory syndrome coronavirus-infected mice increases survival. J Virol 88(2):913–924

Dhillon S, Keam SJ (2020) Filgotinib: first approval. Drugs 80(18):1987–1997

DiDonato JA, Mercurio F et al (2012) NF-kappaB and the link between inflammation and cancer. Immunol Rev 246(1):379–400

Ding C (2006) Drug evaluation: VX-702, a MAP kinase inhibitor for rheumatoid arthritis and acute coronary syndrome. Curr Opin Investig Drugs 7(11):1020–1025

Dowty ME, Lin J et al (2014) The pharmacokinetics, metabolism, and clearance mechanisms of tofacitinib, a janus kinase inhibitor, in humans. Drug Metab Dispos 42(4):759–773

Enkhbaatar P, Murakami K et al (2003) The inducible nitric oxide synthase inhibitor BBS-2 prevents acute lung injury in sheep after burn and smoke inhalation injury. Am J Respir Crit Care Med 167(7):1021–1026

Fang L, Karakiulakis G, Roth M (2020) Are patients with hypertension and diabetes mellitus at increased risk for COVID-19 infection? Med, Lancet Respir

FitzGerald GA (2020) Misguided drug advice for COVID-19. Science 367(6485):1434

Flaherty KT, Robert C et al (2012) Improved survival with MEK inhibition in BRAF-mutated melanoma. N Engl J Med 367(2):107–114

Fridman JS, Scherle PA et al (2010) Selective inhibition of JAK1 and JAK2 is efficacious in rodent models of arthritis: preclinical characterization of INCB028050. J Immunol 184(9):5298–5307

Fu Y, Cheng Y et al (2020) Understanding SARS-CoV-2-mediated inflammatory responses: from mechanisms to potential therapeutic tools. Virologica Sinica 35(3):266–271

Gallagher PE, Ferrario CM et al (2008) MAP kinase/phosphatase pathway mediates the regulation of ACE2 by angiotensin peptides. Am J Physiol Cell Physiol 295(5):C1169–C1174

Gaspari V, Zengarini C et al (2020) Side effects of ruxolitinib in patients with SARS-CoV-2 infection: two case reports. Int J Antimicrob Agents 56(2):106023

Gaur P, Munjhal A et al (2011) Influenza virus and cell signaling pathways. Med Sci Monit 17(6): RA148–RA154

Genovese MC, Fleischmann R et al (2018) Safety and efficacy of upadacitinib in patients with active rheumatoid arthritis refractory to biologic disease-modifying anti-rheumatic drugs (SELECT-BEYOND): a double-blind, randomised controlled phase 3 trial. Lancet 391(10139):2513–2524

Gilmore TD, Herscovitch M (2006) Inhibitors of NF-kappaB signaling: 785 and counting. Oncogene 25(51):6887–6899

Gimeno A, Mestres-Truyol J et al (2020) Prediction of Novel inhibitors of the Main Protease (M-pro) of SARS-CoV-2 through consensus docking and drug reposition. Int J Mol Sci 21(11)

Giuliani C, Bucci I et al (2014) Resveratrol inhibits sodium/iodide symporter gene expression and function in rat thyroid cells. PLoS One 9(9):e107936
Gonzales AJ, Bowman JW et al (2014) Oclacitinib (APOQUEL((R))) is a novel Janus kinase inhibitor with activity against cytokines involved in allergy. J Vet Pharmacol Ther 37(4):317–324
Gonzales AJ, Fleck TJ et al (2016) IL-31-induced pruritus in dogs: a novel experimental model to evaluate anti-pruritic effects of canine therapeutics. Vet Dermatol 27(1):34–e10
Greaves J, Chamberlain LH (2007) Palmitoylation-dependent protein sorting. J Cell Biol 176(3):249–254
Greten FR, Arkan MC et al (2007) NF-kappaB is a negative regulator of IL-1beta secretion as revealed by genetic and pharmacological inhibition of IKKbeta. Cell 130(5):918–931
Grimes JM, Grimes KV (2020) p38 MAPK inhibition: a promising therapeutic approach for COVID-19. J Mol Cell Cardiol 144:63–65
Guimaraes LMF, Rossini CVT et al (2021) Implications of SARS-Cov-2 infection on eNOS and iNOS activity: consequences for the respiratory and vascular systems. Nitric Oxide 111–112: 64–71
Guo FH, De Raeve HR et al (1995) Continuous nitric oxide synthesis by inducible nitric oxide synthase in normal human airway epithelium in vivo. Proc Natl Acad Sci U S A 92(17):7809–7813
Harigai M (2019) Growing evidence of the safety of JAK inhibitors in patients with rheumatoid arthritis. Rheumatology (Oxford) 58(Suppl. 1):i34–i42
Hariharan A, Hakeem AR et al (2021) The role and therapeutic potential of NF-kappa-B pathway in severe COVID-19 patients. Inflammopharmacology 29(1):91–100
Harirforoosh S, Asghar W et al (2013) Adverse effects of nonsteroidal antiinflammatory drugs: an update of gastrointestinal, cardiovascular and renal complications. J Pharm Pharm Sci 16(5):821–847
Hirano T, Murakami M (2020) COVID-19: a new virus, but a familiar receptor and cytokine release syndrome. Immunity 52(5):731–733
Hoesel B, Schmid JA (2013) The complexity of NF-κB signaling in inflammation and cancer. Mol Cancer 12(1):1–15
Hong W, Chen Y et al (2020) Celebrex adjuvant therapy on coronavirus disease 2019: an experimental study. Front Pharmacol 11:561674
Hsu LC, Enzler T et al (2011) IL-1beta-driven neutrophilia preserves antibacterial defense in the absence of the kinase IKKbeta. Nat Immunol 12(2):144–150
Jeon KI, Xu X et al (2010) Vinpocetine inhibits NF-kappaB-dependent inflammation via an IKK-dependent but PDE-independent mechanism. Proc Natl Acad Sci U S A 107(21):9795–9800
Jorgensen SCJ, Tse CLY et al (2020) Baricitinib: a review of pharmacology, safety, and emerging clinical experience in COVID-19. Pharmacotherapy 40(8):843–856
Kaddoura M, AlIbrahim M et al (2020) COVID-19 Therapeutic options under investigation. Front Pharmacol 11:1196
Kahn N, Meister M et al (2012) Early detection of lung cancer by molecular markers in endobronchial epithelial-lining fluid. J Thorac Oncol 7(6):1001–1008
Kamiyama I, Kohno M et al (2014) A new technique of bronchial microsampling and proteomic analysis of epithelial lining fluid in a rat model of acute lung injury. Mol Immunol 59(2):217–225
Ke YY, Peng TT et al (2020) Artificial intelligence approach fighting COVID-19 with repurposing drugs. Biomed J 43(4):355–362
Kellner M, Noonepalle S et al (2017) ROS signaling in the pathogenesis of Acute Lung Injury (ALI) and Acute Respiratory Distress Syndrome (ARDS). Adv Exp Med Biol 967:105–137
Khanmohammadi S, Rezaei N (2021) Role of Toll-like receptors in the pathogenesis of COVID-19. J Med Virol 93(5):2735–2739

Kiang MV, Irizarry RA et al (2020) Every body counts: measuring mortality from the COVID-19 pandemic. Ann Intern Med 173(12):1004–1007
Klingstrom J, Akerstrom S et al (2006) Nitric oxide and peroxynitrite have different antiviral effects against hantavirus replication and free mature virions. Eur J Immunol 36(10):2649–2657
Klunder B, Mohamed MF et al (2018) Population pharmacokinetics of upadacitinib in healthy subjects and subjects with rheumatoid arthritis: analyses of Phase I and II clinical trials. Clin Pharmacokinet 57(8):977–988
Korhonen R, Lahti A et al (2005) Nitric oxide production and signaling in inflammation. Curr Drug Targets Inflamm Allergy 4(4):471–479
Kotyla PJ (2018) Are Janus kinase inhibitors superior over classic biologic agents in RA patients? Biomed Res Int 2018:7492904
Kumar V (2020) Toll-like receptors in sepsis-associated cytokines storm and their endogenous negative regulators as future immunomodulatory targets. Int immunopharmacol:107087
Kumar R, Khandelwal N et al (2018) Role of MAPK/MNK1 signaling in virus replication. Virus Res 253:48–61
Lancaster LH, de Andrade JA et al (2017) Pirfenidone safety and adverse event management in idiopathic pulmonary fibrosis. Eur Respir Rev 26(146)
Lawrence T (2009) The nuclear factor NF-kappaB pathway in inflammation. Cold Spring Harb Perspect Biol 1(6):a001651
Lee S, Channappanavar R et al (2020) Coronaviruses: innate immunity, inflammasome activation, inflammatory cell death, and cytokines. Trends Immunol
Li H, Liu SM et al (2020) Coronavirus disease 2019 (COVID-19): current status and future perspectives. Int J Antimicrob Agents 55(5):105951
Liao QJ, Ye LB et al (2005) Activation of NF-kappaB by the full-length nucleocapsid protein of the SARS coronavirus. Acta Biochim Biophys Sin (Shanghai) 37(9):607–612
Little P (2020) Non-steroidal anti-inflammatory drugs and covid-19. BMJ 368:m1185
Liu T, Zhang L et al (2017) NF-κB signaling in inflammation. Signal Transduction Target Ther 2(1):1–9
Luan J, Lu Y et al (2020) Spike protein recognition of mammalian ACE2 predicts the host range and an optimized ACE2 for SARS-CoV-2 infection. Biochem Biophys Res Commun 526(1):165–169
Lund LC, Kristensen KB et al (2020) Adverse outcomes and mortality in users of non-steroidal anti-inflammatory drugs who tested positive for SARS-CoV-2: a Danish nationwide cohort study. PLoS Med 17(9):e1003308
MacNee W, Allan RJ et al (2013) Efficacy and safety of the oral p38 inhibitor PH-797804 in chronic obstructive pulmonary disease: a randomised clinical trial. Thorax 68(8):738–745
Mahesh G, Kumar KA et al (2021) Overview on the discovery and development of anti-inflammatory drugs: Should the focus be on synthesis or degradation of PGE2? J Inflamm Res 14:253
Manes NP, Nita-Lazar A (2021) Molecular mechanisms of the Toll-like receptor, STING, MAVS, inflammasome, and interferon pathways. Msystems 6(3):e00336–e00321
Manik M, Singh RK (2021) Role of toll-like receptors in modulation of cytokine storm signaling in SARS-CoV-2-induced COVID-19. J Med Virol
Markham A, Keam SJ (2019) Peficitinib: first global approval. Drugs 79(8):887–891
Masiello, P., M. Novelli, et al. (2020). Can Hypericum perforatum (SJW) prevent cytokine storm in COVID-19 patients? Phytother Res.
Matsuo N (1999) The role of intrapulmonary nitric oxide generation in the development of adult respiratory distress syndrome. Surg Today 29(10):1068–1074
Mattson MP, Camandola S (2001) NF-kappaB in neuronal plasticity and neurodegenerative disorders. J Clin Invest 107(3):247–254
McIntosh, K. (2021). COVID-19: Epidemiology, virology, and prevention. UpToDate. Available online: https://www.uptodate.com/contents/covid-19-epidemiology-virology-and-prevention (accessed on 18 March 2021).

Mitchell JA, Kirkby NS (2019) Eicosanoids, prostacyclin and cyclooxygenase in the cardiovascular system. Br J Pharmacol 176(8):1038–1050
Mizutani T, Fukushi S et al (2004) Phosphorylation of p38 MAPK and its downstream targets in SARS coronavirus-infected cells. Biochem Biophys Res Commun 319(4):1228–1234
Mohamed MF, Camp HS et al (2016) Pharmacokinetics, safety and tolerability of ABT-494, a Novel selective JAK 1 inhibitor, in healthy volunteers and subjects with rheumatoid arthritis. Clin Pharmacokinet 55(12):1547–1558
Mohan S, Gupta D (2018) Crosstalk of toll-like receptors signaling and Nrf2 pathway for regulation of inflammation. Biomed Pharmacother 108:1866–1878
Mohanta TK, Sharma N et al (2020, 2020) Molecular insights into the MAPK cascade during viral infection: potential crosstalk between HCQ and HCQ analogues. BioMed Res Int
Montero P, Milara J et al (2021) Role of JAK/STAT in interstitial lung diseases: molecular and cellular mechanisms. Int J Mol Sci 22(12):6211
Nakayamada S, Kubo S et al (2016) Recent progress in JAK inhibitors for the treatment of rheumatoid arthritis. BioDrugs 30(5):407–419
Namour F, Diderichsen PM et al (2015) Pharmacokinetics and pharmacokinetic/pharmacodynamic modeling of Filgotinib (GLPG0634), a selective JAK1 inhibitor, in support of phase IIB dose selection. Clin Pharmacokinet 54(8):859–874
Namour F, Desrivot J et al (2016) Clinical confirmation that the selective JAK1 inhibitor filgotinib (GLPG0634) has a low liability for drug-drug interactions. Drug Metab Lett 10(1):38–48
Oeckinghaus A, Ghosh S (2009) The NF-kappaB family of transcription factors and its regulation. Cold Spring Harb Perspect Biol 1(4):a000034
Organization W H (2020). The use of non-steroidal anti-inflammatory drugs (NSAIDs) in patients with COVID-19.
O'Shea JJ, Schwartz DM et al (2015) The JAK-STAT pathway: impact on human disease and therapeutic intervention. Annu Rev Med 66:311–328
Pardanani A, Laborde RR et al (2013) Safety and efficacy of CYT387, a JAK1 and JAK2 inhibitor, in myelofibrosis. Leukemia 27(6):1322–1327
Pardanani A, Harrison C et al (2015) Safety and efficacy of Fedratinib in patients with primary or secondary myelofibrosis: a randomized clinical trial. JAMA Oncol 1(5):643–651
Platnich JM, Muruve DA (2019) NOD-like receptors and inflammasomes: a review of their canonical and non-canonical signaling pathways. Arch Biochem Biophys 670:4–14
Ramamoorthy S, Cidlowski JA (2016) Corticosteroids: mechanisms of action in health and disease. Rheum Dis Clin North Am 42(1):15–31. vii
Rehberg S, Maybauer MO et al (2010) The role of nitric oxide and reactive nitrogen species in experimental ARDS. Front Biosci (School Ed) 2:18–29
Ren Z, Wang L et al (2013) Resveratrol inhibits NF-κB signaling through suppression of p65 and IB kinase activities. Die Pharmazie-Int J Pharm Sci 68(8):689–694
Renouf DJ, Velazquez-Martin JP et al (2012) Ocular toxicity of targeted therapies. J Clin Oncol 30(26):3277–3286
Ripon MAR, Bhowmick DR et al (2021) Role of Arachidonic cascade in COVID-19 infection: a review. Prostagland Other Lipid Mediators 106539
Russell B, Moss C et al (2020) COVID-19 and treatment with NSAIDs and corticosteroids: should we be limiting their use in the clinical setting? Ecancermedical science 14:1023
Santoro MG, Rossi A et al (2003) NF-kappaB and virus infection: who controls whom. EMBO J 22(11):2552–2560
Sarawar SR, Doherty PC (1994) Concurrent production of interleukin-2, interleukin-10, and gamma interferon in the regional lymph nodes of mice with influenza pneumonia. J Virol 68(5):3112–3119
Satarker S, Tom AA et al (2021) JAK-STAT pathway inhibition and their implications in COVID-19 therapy. Postgraduate Med 133(5):489–507
Saxena SK, Mathur A et al (2001) Induction of nitric oxide synthase during Japanese encephalitis virus infection: evidence of protective role. Arch Biochem Biophys 391(1):1–7

Scheinman RI, Cogswell PC et al (1995) Role of transcriptional activation of I kappa B alpha in mediation of immunosuppression by glucocorticoids. Science 270(5234):283–286
Schumacher HR Jr, Boice JA et al (2002) Randomised double blind trial of etoricoxib and indometacin in treatment of acute gouty arthritis. BMJ 324(7352):1488–1492
Sestili P, Stocchi V (2020) Repositioning Chromones for Early Anti-inflammatory Treatment of COVID-19. Front Pharmacol 11:854
Sibbald B (2004) Rofecoxib (Vioxx) voluntarily withdrawn from market. CMAJ 171(9):1027–1028
Silverstein FE, Faich G et al (2000) Gastrointestinal toxicity with celecoxib vs nonsteroidal anti-inflammatory drugs for osteoarthritis and rheumatoid arthritis: the CLASS study: A randomized controlled trial. Celecoxib long-term arthritis safety study. JAMA 284(10):1247–1255
Singer JW, Al-Fayoumi S et al (2016) Comprehensive kinase profile of pacritinib, a nonmyelosuppressive Janus kinase 2 inhibitor. J Exp Pharmacol 8:11–19
Sittipunt C, Steinberg KP et al (2001) Nitric oxide and nitrotyrosine in the lungs of patients with acute respiratory distress syndrome. Am J Respir Crit Care Med 163(2):503–510
Smeitink, J., X. Jiang, et al. (2020). Hypothesis: mPGES-1-derived prostaglandin E2, a so far missing link in COVID-19 pathophysiology?.
Smith JA, Stallons LJ et al (2015) Suppression of mitochondrial biogenesis through toll-like receptor 4-dependent mitogen-activated protein kinase kinase/extracellular signal-regulated kinase signaling in endotoxin-induced acute kidney injury. J Pharmacol Exp Ther 352(2):346–357
Smith JA, Mayeux PR et al (2016) Delayed mitogen-activated protein kinase/extracellular signal-regulated kinase inhibition by trametinib attenuates systemic inflammatory responses and multiple organ injury in murine sepsis. Crit Care Med 44(8):e711–e720
Soares-Silva M, Diniz FF et al (2016) The mitogen-activated protein kinase (MAPK) pathway: role in immune evasion by trypanosomatids. Front Microbiol 7:183
Solt LA, May MJ (2008) The IkappaB kinase complex: master regulator of NF-kappaB signaling. Immunol Res 42(1-3):3–18
Stamler JS, Toone EJ et al (1997) (S) NO signals: translocation, regulation, and a consensus motif. Neuron 18(5):691–696
Takeuchi T, Tanaka Y et al (2016) Efficacy and safety of the oral Janus kinase inhibitor peficitinib (ASP015K) monotherapy in patients with moderate to severe rheumatoid arthritis in Japan: a 12-week, randomised, double-blind, placebo-controlled phase IIb study. Ann Rheum Dis 75(6):1057–1064
Talukdar J, Bhadra B et al (2020) Potential of natural astaxanthin in alleviating the risk of cytokine storm in COVID-19. Biomed Pharmacother 110886
Tang K, Shao X et al (2017) Correlation between nitric oxide content in exhaled breath condensate and the severity of acute respiratory distress syndrome. Int J Clin Exp Pathol 10(7):7350–7355
Taylor PC, Abdul Azeez M et al (2017) Filgotinib for the treatment of rheumatoid arthritis. Expert Opin Investig Drugs 26(10):1181–1187
Tefferi A (2016) Primary myelofibrosis: 2017 update on diagnosis, risk-stratification, and management. Am J Hematol 91(12):1262–1271
Tefferi A, Barraco D et al (2018) Momelotinib therapy for myelofibrosis: a 7-year follow-up. Blood Cancer J 8(3):29
Tomazini BM, Maia IS et al (2020) Effect of dexamethasone on days alive and ventilator-free in patients with moderate or severe acute respiratory distress syndrome and COVID-19: The CoDEX randomized clinical trial. JAMA 324(13):1307–1316
van der Vliet A, Eiserich JP et al (1999) Reactive nitrogen species and tyrosine nitration in the respiratory tract: epiphenomena or a pathobiologic mechanism of disease? Am J Respir Crit Care Med 160(1):1–9
Voiriot G, Philippot Q et al (2019) Risks related to the use of non-steroidal anti-inflammatory drugs in community-acquired pneumonia in adult and pediatric patients. J Clin Med 8(6):786

Wallet F, Delannoy B et al (2013) Evaluation of recruited lung volume at inspiratory plateau pressure with PEEP using bedside digital chest X-ray in patients with acute lung injury/ARDS. Respir Care 58(3):416–423

Westphal M, Enkhbaatar P et al (2008) Neuronal nitric oxide synthase inhibition attenuates cardiopulmonary dysfunctions after combined burn and smoke inhalation injury in sheep. Crit Care Med 36(4):1196–1204

Willsher K (2020) Anti-inflammatories may aggravate Covid-19, France advises. The Guardian 15

Yamamoto Y, Gaynor RB (2001) Therapeutic potential of inhibition of the NF-kappaB pathway in the treatment of inflammation and cancer. J Clin Invest 107(2):135–142

Yang J, Zhang E et al (2020) Longitudinal characteristics of T cell responses in asymptomatic SARS-CoV-2 infection. Virol Sinica 35(6):838–841

Zamora R, Vodovotz Y et al (2000) Inducible nitric oxide synthase and inflammatory diseases. Mol Med 6(5):347–373

Zhang X, Zhang Y et al (2020) Baricitinib, a drug with potential effect to prevent SARS-COV-2 from entering target cells and control cytokine storm induced by COVID-19. Int Immunopharmacol 86:106749

Zhao N, Di B et al (2021) The NLRP3 inflammasome and COVID-19: Activation, pathogenesis and therapeutic strategies. Cytokine Growth Factor Rev

Zhou T, Georgeon S et al (2014) Specificity and mechanism-of-action of the JAK2 tyrosine kinase inhibitors ruxolitinib and SAR302503 (TG101348). Leukemia 28(2):404–407

Zhou L, Huntington K et al (2020) MEK inhibitors reduce cellular expression of ACE2, pERK, pRb while stimulating NK-mediated cytotoxicity and attenuating inflammatory cytokines relevant to SARS-CoV-2 infection. Oncotarget 11(46):4201–4223

Chapter 18
Repurposing Drugs for Viruses and Cancer: A Novel Drug Repositioning Strategy for COVID-19

Ka Heng Wong, Chie-Min Lim, Ashley Jia Wen Yip, Isra Ahmad Farouk, Nur Zawanah Zabidi, Zheng Yao Low, and Sunil K. Lal

Abstract The high infection capacity and rapid mutations in coronavirus disease 2019 (COVID-19) has been no stranger to many. The etiological agent that contributed to this global health crisis is by no means the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). COVID-19 is characterized by an episode of immune fluctuations, followed by hyperactivation of inflammatory responses, known as the cytokine storm. The rapid progression of the COVID-19 pandemic calls for new and promising antiviral therapeutics. Repositioning anticancer drugs against the virus is very much explored due to the common similar pathways or targeting structures, opening new windows for many possibilities. As such, the repurposing of zidovudine for Friend leukemia virus and ouabain for Ebola virus are among the successful examples. Other potential FDA-approved anticancer drugs to be repositioned for COVID-19 include imatinib, saracatinib, and homoharringtonine, which have been studied for other coronaviruses in the past. Furthermore, current anticancer drugs like carmofur, carfilzomib, zotatifin, plitidepsin, and toremifene have gained interesting outcomes with respect to SARS-CoV-2. It is well recognized that to achieve viral replication, viruses antagonise or hijack host proteins and signaling pathways to gain productive infection, with SARS-CoV-2 indeed being no exception. This review aims to discuss the drug repositioning approaches concerning previously established anticancer drugs on viruses, especially on SARS-CoV-2. We accentuate this idea with specific examples of how potential anticancer inhibitors can effectively be used against SARS-CoV-2 as well as the limitations and future perspectives of drug repositioning.

K. H. Wong · C.-M. Lim · A. J. W. Yip · I. A. Farouk · N. Z. Zabidi · Z. Y. Low
School of Science, Monash University Malaysia, Subang Jaya, Selangor, Malaysia

S. K. Lal (✉)
School of Science, Monash University Malaysia, Subang Jaya, Selangor, Malaysia

Tropical Medicine and Biology Platform, Monash University Malaysia, Subang Jaya, Selangor, Malaysia
e-mail: sunil.lal@monash.edu

R. C. Sobti et al. (eds.), *Drug Repurposing for Emerging Infectious Diseases and Cancer*, https://doi.org/10.1007/978-981-19-5399-6_18

Keywords Drug repurposing · Drug repositioning · Anticancer · Antivirus · COVID-19 · SARS-CoV-2

18.1 Introduction

Affecting more than 490,000,000 individuals accompanied by 6,150,000 deaths worldwide, COVID-19 has been a significant threat to global health (Worldometer 2022). The etiological agent behind this disastrous episode is termed the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), well associated with the previous SARS-CoV and Middle East respiratory syndrome (MERS-CoV) (Low et al. 2021). Falling under the *Coronaviridae* family, coronaviruses are a group of positive-sense single-stranded RNA viruses with club-shaped spike projections on the virion surface (Shereen et al. 2020). Coronaviruses can be subdivided into four different genera: alpha, beta, gamma, and delta (Wu et al. 2020). Falling under the beta-coronavirus genus, SARS-CoV-2 closely resembles SARS-CoV with 94.4% similarity in amino acid sequence (Low et al. 2021). To date, the exact origin of SARS-CoV-2 remains undefined, though the evidence is pointing toward bats (Andersen et al. 2020). It is to note that patients infected with SARS-CoV-2 may experience severe symptoms such as acute respiratory distress syndrome (ARDS), multiple organ failure, septic shock, and pneumonia (Wehbe et al. 2020). Despite the lower estimated case fatality rate (3.4%), the enhanced infection capacity and rapid emergence of diverse SARS-CoV-2 variants have led to fear and anxiety among the public. To better monitor and facilitate research, the World Health Organization (WHO) has categorized existing variants into variants of concern (VOC), variants of interest (VOI), and variants under monitoring (VUM), in which the VOCs [Delta (B.1.617.2) and omicron (B.1.1.529)] are expressed with a higher degree of significance toward global public health in contrast to the VOIs, which entail the Lambda (C.37) and Mu (B.1.621) variants (World Health Organization 2022).

In such a dire need, drug repositioning is especially crucial to provide a swift and effective antiviral therapy against COVID-19. Drug repositioning, also termed drug repurposing or drug reprofiling, is an alternative route to provide new uses to an already established drug aside from its intended purposes. The advantages of drug repositioning include shorter and cheaper development stages accompanied by a well-established ADMET (absorption, distribution, metabolism, excretion, and toxicity) data of the existing drugs (Low et al. 2020). For instance, tenofovir, a nucleotide analog used in HIV treatment, can hinder the binding of SARS-CoV-2 spike (S) protein to the host ACE2 receptor via the interaction with papain-like protease (PL^{pro}) and M^{pro}, resulting in the reduction of nonstructural protein (NSP) production required for viral replication. Tenofovir also acts on the SARS-CoV-2 RNA-dependent RNA polymerase (RdRp), a replicase essential for viral replication, to reduce the viral load (Zanella et al. 2021). Another exciting example would be ivermectin, an antiparasitic drug that showed antiviral activity against SARS-CoV-2. Recently, ivermectin was revealed to bind with $3CL^{pro}$ and S protein, disrupting viral attachment and replication. Moreover, ivermectin hinders the importin heterodimer

complex, attenuating the cytoplasmic-nuclear shuttling of viral proteins, resulting in a decreased cytokine storm (Low et al. 2022).

Several clinically approved drugs are now being considered in the conquest for an effective antiviral treatment against SARS-CoV-2. Interestingly, it has been suggested that several anticancer drugs may possess such properties. In light of that, this chapter presents classic examples of anticancer drugs repurposed for viruses and investigates their potential inhibitory mechanism against SARS-CoV-2 replication; thereby, allowing us to repurpose anticancer drugs for SARS-CoV-2 antiviral therapy, especially in such urgent needs.

18.2 Classic Examples of Anticancer Drug Repositioning

Drug repositioning has opened many possibilities for disease treatment, particularly in providing rapid responses to highly mutated and novel disease outbreaks. Anticancer drugs are now gaining traction for drug repositioning against viruses due to the similar pathways or structures targeted. For instance, the close structural resemblance between viral RdRp and the cancer-contributing human telomerase reverse transcriptase (hTERT) suggests that anticancer drugs can be a potential antiviral drug (Machitani et al. 2020). Given those above, discussed below are some examples of potential anticancer drugs that have demonstrated antiviral properties in the past, shedding some light on an effective antiviral drug for COVID-19.

18.2.1 Zidovudine

Zidovudine, also known as azidothymidine (AZT), is a thymidine analog originally developed to thwart cancer. It was later disregarded due to its lack of anticancer activity and high concentration toxicity (Chow et al. 2009). The anticancer properties of zidovudine were revisited in 1989 when it was discovered to restore cisplatin sensitivity in resistant human colon carcinoma cells by incorporating itself into tumor cell DNA (Scanlon et al. 1989). Yet, the lack of favorable outcomes on such mechanisms in humans under normal circumstances means the need for co-administration with other anticancer agents. Additionally, zidovudine has successfully re-sensitized gemcitabine-resistant pancreatic cancer cells by inhibiting Akt-GSK3β-Snail1 pathway and restoring downregulated human equilibrative nucleoside transporter 1 (hENT1), all of which contribute to gemcitabine resistance (Namba et al. 2015). The antiviral properties of zidovudine were firstly discovered in 1960 in the Friend leukemia virus (Ostertag et al. 1974). Since then, it is now repositioned as an antiretroviral drug for HIV treatment, which selectively inhibits the reverse transcriptase of HIV and subsequent production of cDNA from viral RNA. The combination of zidovudine and other agents, such as lamivudine, has also exhibited a synergistic effect on HIV compared to either agent alone (Eron et al.

1995). The repurposing of zidovudine is especially important due to the emergence of HIV drug resistance. This tends to occur across the same class of drugs due to the same mode of inhibition, limiting the efficacy of existing drugs (Clouser et al. 2010).

18.2.2 Cardiac Glycosides

Plant-derived compounds contribute to 20–30% of existing drugs and more than 60% of clinically approved cancer drugs. One of the many is cardiac glycosides, a group of organic compounds that are often used as an antiarrhythmic agent via the inhibition of sodium–potassium pump activity (Na^+/K^+ ATPase). Some well-known cardiac glycosides include ouabain, oleandrin, digoxin, and digitoxin. Since human cancer cells express certain isoforms of subunits that make the Na^+/K^+ ATPase, it was then postulated that cancer cells are susceptible to cardiac glycosides (Kepp et al. 2012). Indeed, this is prominent in lung cancer where cardiac glycosides exhibit anticancer activity against the growth and proliferation of lung cancer cells that overexpress the α-subunit of Na^+/K^+ATPase (Kim et al. 2016; Kaushik et al. 2017). Apart from anticancer activities, some cardiac glycosides possess antiviral properties. Digitoxin, g-strophanthin, and ouabain were demonstrated to inhibit the viral gene expression and protein synthesis of the herpes simplex virus (Dodson et al. 2007; Amarelle and Lecuona 2018). These cardiac glycosides and a few others can also inhibit HIV-1 gene expression via the inhibition of MEK1/2-ERK1/2 signaling and alteration of viral pre-RNA splicing (Wong et al. 2013, 2018; Laird et al. 2014). Additionally, ouabain, digoxin, and lanatoside C can inhibit the viral protein translation of the influenza A virus (Amarelle et al. 2019). In particular, ouabain attenuates the viral entry of coronaviruses (feline, murine, and MERS-CoV) and Ebola virus via the inhibition of ATPA1-mediated Src signaling and interruption of cellular interacting proteins, respectively (García-Dorival et al. 2014; Burkard et al. 2015; Dowall et al. 2016).

18.3 Anticancer Drug Candidates for Previous SARS-CoV and MERS-CoV

Prior to the SARS-CoV-2 pandemic outbreak, coronaviruses, specifically SARS-CoV, were not considered significant human pathogens as they were mainly reported to cause mild respiratory infections or simply low numbers of cases (Dyall et al. 2014). Nevertheless, many lives were lost due to the SARS-CoV and MERS-CoV epidemics, which urgently called for treatment options. Drug repositioning became a viable option as US Food and Drug Administration (FDA)-approved drugs required less time to be permitted for repositioning as compared to the development of novel drugs (Aherfi et al. 2021). Among many FDA-approved drugs, anticancer drugs

were considered and studied for repositioning potential. Anticancer drugs target an array of cellular pathways, including those manipulated during viral infection, making them ideal candidates to reposition for SARS and MERS (Dyall et al. 2014). Drugs that target protein synthesis, for example, were hypothesized to be potentially valuable candidates. In this section, we will explore approved anticancer drugs identified for their potential in inhibiting SARS-CoV or MERS-CoV infections and considered for repositioning (Table 18.1).

18.3.1 Imatinib

Imatinib is a tyrosine kinase inhibitor commonly used to treat several diverse cancers as an apoptosis inducer and antineoplastic agent (PubChem 2022a). Imatinib specifically inhibits Abelson kinase receptors and is classified as a benzamide drug (PubChem 2022a). Many cellular processes are regulated independently of transcription/translation and are instead regulated through kinase-mediated signaling pathways. Kinase inhibitors like imatinib were granted FDA approval in 2012–2015 and are now considered among the most common classes of target gene therapy in cancer (Dyall et al. 2017). It was identified that imatinib and another kinase inhibitor, dasatinib inhibit MERS-CoV and SARS-CoV through in vitro assays. Imatinib interferes with SARS and MERS-CoV infection after viral internalization and endosomal trafficking by not allowing the fusion of virions to the cellular endosomal membrane (Coleman et al. 2016). Research revealed that SARS and MERS-CoV specifically require the Abelson tyrosine-protein kinase 2 (Abl2) for efficient replication; thus, imatinib's action on this target significantly inhibits viral replication (Coleman et al. 2016).

18.3.2 Saracatinib

Saracatinib is a drug that AstraZeneca originally developed as an anticancer agent. In 2019, saracatinib was granted orphan drug designation by the FDA for the treatment of idiopathic pulmonary fibrosis (IPF). Saracatinib is classified under quinazolines, which potently inhibit the Src-family of tyrosine kinases (SFK) and is used to manage cancer as an antineoplastic agent, apoptosis inducer, radio-sensitizing agent and autophagy inducer (PubChem 2022b). In the search for inhibitors of the highly infectious MERS-CoV, one study in 2018 screened 2334 approved drugs and identified saracatinib (AZD0530) (Shin et al. 2018). This was achieved using a high-throughput screening (HTS) method, and the drug was then evaluated for antiviral effects through in vitro experiments. Saracatinib was identified through a cell-based antiviral screening assay that contains data based on the protection of cells from viral cytopathic effects (CPE); thus, exhibiting significant antiviral activity against MERS-CoV. The drug was tested against several MERS-CoV strains by infection

Table 18.1 Studied FDA-approved anticancer drug candidates to reposition against MERS and SARS-CoV diseases

Drug name	Original purpose	Mechanism of action	Target coronavirus	Research finding	Reference
Imatinib	Treatment of wide range of cancers (i.e., acute lymphocytic leukemia, chronic myeloid leukemia, gastrointestinal stromal tumors)	Kinase inhibitor (Abelson kinase receptors)	MERS-CoV, SARS-CoV	Interferes with SARS- and MERS-CoV infection after viral internalization and endosomal trafficking by halting the fusion of virions to the cellular endosomal membrane	Coleman et al. (2016)
Saracatinib	Treatment of wide range of cancers (i.e., colorectal, ovary, prostate, breast)	Inhibitor of Src-family of tyrosine kinases	MERS-CoV	In vitro antiviral activity observed using virus plaque assay Combination assay of saracatinib-gemcitabine reported lower cytotoxicity with complete abolishment of progeny virus	Shin et al. (2018)
Homoharringtonine	Treatment of wide range of cancers (i.e., CML/AML, myelodysplastic syndrome, lymphoblastic leukemia)	Protein synthesis inhibitor - inhibits the translation elongation process	MERS-CoV	Significant reduction in virus titer in vitro was observed after 12 and 16 h under drug treatment Significant reduction of viral N protein expression in cell lysates	Cao et al. (2015)

of human hepatoma (Huh-7) cells. Viral progeny titer was measured via plaque assay from the supernatant of saracatinib-treated Huh-7 cells followed by infection with MERS-CoV. As expected, a marginal reduction of progeny virus titer was observed (Shin et al. 2018). In addition, saracatinib was found to have synergistic and enhanced antiviral activity in combination with gemcitabine, a deoxycytidine analog anticancer drug. Gemcitabine was first evaluated for its anti-MERS-CoV potential using the same cell-based assay at a noncytotoxic concentration for the combination study. In vitro plaque assay, post-treatment with gemcitabine resulted in a complete absence of MERS-CoV virus particle production. The combination of saracatinib-gemcitabine also reduced cellular toxicity compared to gemcitabine alone, suggesting the suitability of use in low-dose combination therapy (Shin et al. 2018).

18.3.3 Homoharringtonine

Homoharringtonine (HHT) is a plant benzazepine alkaloid and a cyclic acetal with known anticancer properties (PubChem 2022c). HHT is a protein synthesis inhibitor that prevents the initial elongation step of protein synthesis in eukaryotic cells. Protein synthesis inhibitors function as anticancer agents by inducing differentiation and apoptosis of some cancer cell types. HHT has been widely used in many countries to treat chronic and acute myeloid leukemia (CML and AML) (Lü and Wang 2014). Omacetaxine mepesuccinate is a semi-synthetic form of HHT with enhanced bioavailability and was approved for anticancer therapy usage by the FDA in 2012 for CML (Lü and Wang 2014). In a screening study of small molecules that have potential anticoronavirus activity, it was found that HTT inhibits MERS-CoV (Cao et al. 2015; Dyall et al. 2017). However, the drug was not reported to have any antiviral activity against SARS-CoV.

18.4 The General Life Cycle and Pathogenesis

18.4.1 Viral Entry and Membrane Fusion

Viruses are transmitted between individuals via respiratory droplets and aerosols (El Bairi et al. 2020; Parasher 2021). It enters exposed individuals' bodies and binds to the host receptors located on cell membranes, such as the angiotensin-converting enzyme 2 (ACE2) (Parasher 2021). Viral entry and membrane fusion are mediated by the viral surface glycoproteins, the S protein. Within the host, virus infection is initiated by the recognition and binding of S to host ACE2. The S protein consists of two subunits—spike protein subunit 1 (S1) and spike protein subunit 2 (S2) (Parasher 2021). The receptor-binding domain (RBD) located on the S1 subunit binds to the host cell receptor, while the S2 subunit is responsible for viral and host membrane fusion (Wang et al. 2020). Upon host entry, the S protein is

cleaved by host serine protease at the site between S1 and S2 to undergo an irreversible conformational change that activates the S protein for membrane fusion (Huang et al. 2020; Parasher 2021). Among serine proteases such as furin and cathepsin L, the transmembrane protease serine protease 2 (TMPRSS2), a co-expressed protease by ACE2 on nasal epithelial cells, lungs and bronchial branches, may also be involved in the cleavage of S protein (Queirós-Reis et al. 2021). Given that the host cell receptors are highly expressed on human pulmonary epithelial cells, it is no stranger that we are highly susceptible to SARS-CoV-2 infection (El Bairi et al. 2020). Considering that virus–host cell membrane fusion marks the success of virus–host cell invasion, several studies have shown that it may be inhibited by targeting host serine proteases like TMPRSS2 (El Bairi et al. 2020). For example, the drug camostat which inhibits prostate cancer metastasis in vivo has been associated with suppressing TMPRSS2, conferring potential as a druggable target (El Bairi et al. 2020).

18.4.2 Replication and Virus Assembly

Upon membrane fusion, the virus disassembles, releasing genetic material and viral nucleocapsid (N) into the host cytoplasm via caveolae and clathrin-mediated endocytosis (El Bairi et al. 2020). The genomic RNA released produces a replicase transcriptase complex that synthesizes various NSPs, such as the RdRp and helicase, which are vital for viral transcription, translation, and replication (El Bairi et al. 2020). Given the miniature and limited genetic material in viruses, SARS-CoV-2 also utilizes host-derived components to translate its proteins (Montero et al. 2019). Following that, the N protein will bind to the newly synthesized viral genome and assemble with other viral proteins to form new virions. Then, the Golgi vesicles containing new virions will migrate towards the host membrane and be released by exocytosis. Newly released virions will then be capable of infecting surrounding healthy cells and exit the body to infect new hosts (Parasher 2021). This allows the rapid transmission of COVID-19, causing a widespread pandemic.

18.5 Pathophysiology

COVID-19 has varying degrees of disease severity (Li et al. 2021). Individuals may experience mild, moderate, severe symptoms, or asymptomatic (Parasher 2021). To complicate things, there are also preasymptomatic (infectious before exhibiting symptoms) and true asymptomatic (infectious without exhibiting symptoms) in COVID-19 (Johansson et al. 2021). During the initial viral invasion, individuals may remain asymptomatic for several days as the viruses undergo replication and propagation, but are nonetheless infectious (Parasher 2021). Additionally, a short immune response against the viruses may be elicited; albeit, temporarily during the

asymptomatic phase (El Bairi et al. 2020; Parasher 2021). As the virus migrates from the nasal epithelium to the upper respiratory tract (URT) via the conduction airways, symptoms like fever, malaise, and dry cough may start to manifest. In this phase, a more robust immune response will be elicited as infected cells release various immunomodulatory cytokines such as C-X-C motif chemokine ligand 10 (CXCL-10) and interferons (IFNs) like IFN-β and IFN-λ to combat the virus and contain the spread of infection. In contrast, virus infection spreading into the lower respiratory tract (LRT) contributes to severe COVID-19 symptoms. This occurs in one-fifth of COVID-19 infected patients (Parasher 2021). The virus replicates within the ACE2-expressing type-2 alveolar epithelial cells upon LRT invasion, triggering the host immune-inflammatory response against infected pneumocytes, mast cells, and macrophages that release various pro-inflammatory cytokines such as IFN-β, interleukins (IL-1, IL-6, IL-8, IL-12, IL-120), tumor necrosis factor-α (TNF-α), CXCL-10, monocyte chemoattractant protein-1 (MCP-1), and inflammatory protein-1α (MIP-1α). This sudden influx of pro-inflammatory cytokines is known as the "cytokine storm." Cytokine storm attracts various circulating immune cells such as neutrophils, macrophages, CD^{4+} helper T-cells, and CD^{8+} cytotoxic T-cells to the site of infection, leading to massive tissue damage due to capillary injury and diffuse alveolar damage. Eventually, it may progress to acute lung injury, resulting in ARDS and multiple organ failure, the key to mortality. In addition, leukopenia and lymphopenia with lowered natural killer (NK) cell levels and abnormally elevated C-reactive protein (CRP) levels are also observed in individuals with severe COVID-19 (Cao 2020). Many studies have suggested the use of immunomodulatory drugs to adequately suppress and control elevated cytokine levels in severe COVID-19 patients, reducing the overexpressed immune-associated mortality rate (Mani et al. 2019; Ciliberto et al. 2020; El Bairi et al. 2020; Aldea et al. 2021).

18.6 Anticancer Drugs with Potential Antiviral Properties

Anticancer drugs have been suggested as an antiviral against SARS-CoV-2 via several in silico or in vitro studies. Several anticancer drugs have now undergone clinical trials against COVID-19. These drugs inhibit virus replication by targeting viral protease (carmofur; carfilzomib), targeting transcription complex proteins (zotatifin, plitidepsin), and inhibiting viral entry (toremifene) (Table 18.2).

18.6.1 Inhibition of Virus Replication by Targeting the Main Protease (M^{pro})

Protease enzymes play an essential role in the maturation of viral proteins by cleaving the proproteins after translation into the host cytosol (Mengist et al.

Table 18.2 Summary of anticancer drugs with potential antiviral properties

Anticancer drug	The original purpose of the drug	Implicated mechanism of antiviral activity	Supporting evidence	References
Carmofur	Treatment for colorectal cancer. Also investigated for gastric, bladder, and breast cancer therapy	Inhibition of M^{pro} to prevent virus replication	In silico analysis implicated high inhibitory potential of carmofur in targeting M^{pro} of SARS CoV-2 based on high throughput screening In vitro analysis demonstrated potent SARS-CoV-2 inhibition in Vero E6 cells	Jin et al. (2020a, b), Ma et al. (2020)
Carfilzomib	Treatment for relapsed or refractory multiple myeloma that one or more lines have previously treated with therapy	Inhibition of M^{pro} to prevent virus replication	In silico analysis showed high potential of carfilzomib in targeting M^{pro} based on docking-screening results	Waxman et al. (2018); Wang (2020)
Zotatifin	Clinical trial drug candidate for the treatment of advanced solid tumor malignancies that are refractory or intolerant to standard cancer treatments	Prevents viral protein synthesis by inhibiting the transcription complex protein eIF4A	Protein interaction analysis of SARS-CoV-2 showed zotatifin as an attractive antiviral candidate with lower cytotoxicity than other rocaglates against SARS-CoV-2 by inhibiting the transcription complex protein (eIF4A) Currently, zotatifin has advanced to clinical trial studies of patients with mild and moderate COVID-19	Obermann et al. (2022)
Plitidepsin	Clinical trial drug candidate investigated for multiple myeloma treatment, leukemia, and lymphomas	Prevents viral protein synthesis by inhibiting the transcription complex protein eEF1A2	In vitro assays demonstrated reduction in subgenomic RNA of SARS-CoV-2 N protein in Vero E6 and hACE2-293 T cells treated with	White et al. (2021); Service (2021)

(continued)

Table 18.2 (continued)

Anticancer drug	The original purpose of the drug	Implicated mechanism of antiviral activity	Supporting evidence	References
			plitidepsin In vivo mouse models showed reduced SARS-CoV-2 infection and lung inflammation after plitidepsin treatment, comparable to remdesivir. Plitidepsin has advanced to phase 2/3 clinical trials	
Toremifene	Nonsteroidal selective estrogen receptor modulator (SERM) to treat metastatic breast cancer	Prevent viral entry by inhibiting membrane fusion through interactions with S protein or NSP14	In silico analysis found that toremifene may inhibit entry and replication of SARS-CoV-2 through unfamiliar pathways	Dyall et al. (2014); Jeon et al. (2020); Martin and Cheng (2020); Zhou et al. (2020a)

2021). This allows the uncleaved and unassembled polyproteins to be converted into mature proteins for new virions (Babé and Craik 1997). Moreover, it was found that inhibition of the virus proteases reduces the assembly of viral particles; therefore, conferring a great potential for pharmaceutical intervention. Currently, multiple FDA-approved drugs targeting proteases have been developed and used for viral infections (Mengist et al. 2021). For instance, saquinavir, atazanavir, lopinavir, darunavir, amprenavir, tipranavir, and ritonavir are several drugs used in treating HIV-1 infection (Wang et al. 2015).

In SARS-CoV-2, the main protease (M^{pro}) is responsible for mediating the maturation cleavage of important viral polyproteins during virus replication (Mengist et al. 2021). To recap, the RNA genome is ~29.9 kb, with two-thirds containing the main open reading frame 1a and 1b (ORF1ab) replicase from the 5′-end, encoding for NSPs 1 to 16. The other one-third encodes the different structural proteins, such as S, envelope (E), membrane (M) and N proteins. In addition, variable ORFs alternate at the 3′-end of the genome, such as the ORF3a, ORF3d, ORF6, ORF7a, ORF7b, ORF8, ORF9b, ORF14, and ORF10 to produce accessory proteins. Here, M^{pro} is encoded by ORF1ab. During the initial phase of infection, M^{pro} (nsp5) cleaves two functional polyproteins known as polyprotein 1a and 1b (pp1a and pp1b, respectively) in ORF1ab into the above mentioned 16 NSPs, including M^{pro} and papain-like protease. Due to the highly conserved M^{pro} among coronaviruses and its significance in the viral life cycle, M^{pro} inhibitors are deemed

to work against other coronaviruses, making them a potential druggable target for repositioning anticancer drugs, particularly those targeting proteases (Jin et al. 2020b; Mengist et al. 2021).

18.6.1.1 Carmofur

A 5-fluorouracil (5-FU) derivative, carmofur, is an FDA-approved antineoplastic drug used in colorectal cancer treatments and has been investigated for gastric, bladder, and breast cancer therapy (Jin et al. 2020a; Cui et al. 2020). The anticancer activity of carmofur involves the inhibition of thymidylate synthase, a catalytic enzyme in thymidylate production (Dementiev et al. 2019; Ma et al. 2020). Thus, the inhibition mentioned above can halt DNA biosynthesis as thymidylate is essential for DNA biosynthesis (Mengist et al. 2021). However, it was later found that carmofur prevents the activity of human acid ceramidase by modifying the catalytic C143 residue in the active site (Dementiev et al. 2019). As the upregulation of acid ceramidase is often associated with cancer cell growth and survival, carmofur was used to achieve anticancer therapeutic outcomes (El Bairi et al. 2020).

Recent studies have suggested many antiviral prospects of carmofur against SARS-CoV-2 through the targeting of M^{pro} in an in silico analysis, potentially inhibiting viral replication and transcription (Ma et al. 2017; Jin et al. 2020b, a; Cui et al. 2020). Additionally, mass spectrometry data have demonstrated covalent binding of carmofur to the catalytic component of SARS-CoV-2 M^{pro}, with an IC_{50} of 1.82 ± 0.06 μM. Similarly, another in silico study revealed that the carbonyl reactive group of carmofur binds covalently to the catalytic Cys145 residue of SARS-CoV-2 M^{pro} via X-ray crystallography (Jin et al. 2020b). Meanwhile, the same study showed that carmofur inhibited SARS-CoV-2 replication (MOI = 0.05) at various concentrations (0.4–100 μM) in Vero E6 cells based on the reduced SARS-CoV-2 N levels post-treatment. Furthermore, carmofur was shown to have a CC_{50} of 133.4 μM in cytotoxicity assays and an EC_{50} of 24.30 μM; hence, adding great potential against SARS-CoV-2 (Jin et al. 2020b). Interestingly, Ma et al. (2020) showed reduced viral inhibition by carmofur in the presence of 1,4-dithiothreitol (DTT) in contrast to without DTT. In common practice, DTT is a reducing agent added to maintain the active form of enzymes and prevent non-specific binding between thiol-reactive compounds and cysteine. Consequently, this encounter suggested that carmofur may not bind specifically to M^{pro}, but also toward other cysteine proteases, which may cause devastating effects. Therefore, a further clinical evaluation must be done to evaluate the efficacy and safety of carmofur, especially since it has been associated with drug-related leukoencephalopathy (El Bairi et al. 2020).

18.6.1.2 Carfilzomib

Carfilzomib is a modified epoxyketone, which is a selective and irreversible proteasome inhibitor used in treating relapsed or refractory multiple myeloma (MM) patients that had previously undergone ≤3 lines of therapy (Vij et al. 2009; El Bairi et al. 2020). The associated mechanism of action for anticancer activity involves the selective targeting of intracellular proteasome enzymes by carfilzomib, in which it binds irreversibly to the active sites located on 20S proteasomes, eventually inhibiting cell proliferation and subsequently inducing cell death in malignant cells (Coux et al. 1996; Kortuem and Stewart 2013). Moreover, proteasomes play essential roles in cellular mechanisms by maintaining optimal intracellular proteins involved in cell cycle progression, cell apoptosis, and normal cellular processes via ubiquitin-dependent or ubiquitin-independent protein degradation (Jayaweera et al. 2021). Thus, disruption of proteasomal activity leads to cell cycle arrest and apoptosis of malignant plasma cells. Apart from that, it may be used as a monotherapy or combined with dexamethasone or lenalidomide to achieve a more potent effect (Jayaweera et al. 2021).

According to virtual docking screening results, carfilzomib has recently shown repurposed potential for SARS-CoV-2 antiviral. Like carmofur, a structure-based virtual screening for approved and clinical trial drug candidates for M^{pro} at key binding sites or domains has emerged with carfilzomib as the top candidate (Wang 2020). Besides that, other promising anticancer candidates, such as eravacycline, valrubicin, lopinavir, and elbasvir, now find enormous antiviral potential against SARS-CoV-2 (Wang 2020). Certainly, further in vitro and in vivo studies of carfilzomib against SARS-CoV-2 must be done to validate this interaction. Nonetheless, carfilzomib has been associated with adverse cardiovascular events, such as severe hypertension, cardiac arrhythmia, and cardiac arrest (Waxman et al. 2018). A meta-analysis involving 24 clinical trials has shown that 2594 patients experienced all-grade (18.1%) and high-grade (8.2%) cardiovascular adverse events (CVAE) (Waxman et al. 2018). Therefore, safety precautions and strict dosage should remain in sight.

18.6.2 Inhibition of Viral Protein Synthesis by Targeting Transcription-Complex Proteins

As viral proliferation is dependent on viral protein synthesis, disruption of the latter will subsequently interrupt the viral life cycle, thereby preventing replication (Müller et al. 2021; Taroncher-Oldenburg et al. 2021). Notably, eukaryotic factors that initiate translation have received significant interest as an antiviral target (Gordon et al. 2020). For instance, zotatifin and plitidepsin, two significant anticancer drugs with potential antiviral activity against SARS-CoV-2, will be further discussed.

18.6.2.1 Zotatifin

Zotatifin is a potent and selective synthetic rocaglate that inhibits eukaryotic initiation factor 4A (eIF4A) (El Bairi et al. 2020). The eIF4A protein is a DEAD-box RNA helicase central to the activity of the eukaryotic translation initiation complex eIF4F (Müller et al. 2021). The DEAD-box helicases are ATP-dependent eukaryotic RNA helicases involved in cellular RNA metabolism (ranging from biogenesis to decay). Apart from its well-established role, it has also been postulated to modulate viral infection by recognizing viral RNA and contributing to antiviral immune signaling pathways (Taschuk and Cherry 2020; Taroncher-Oldenburg et al. 2021). Altogether, this makes rocaglate a drug candidate for preclinical cancer studies (Müller et al. 2021). To date, zotatifin has already progressed to clinical trials to treat advanced solid tumor malignancies that are refractory or intolerant to standard cancer treatments (in Phases 1/2) (Obermann et al. 2022).

The associated mechanism of action involved forming "stacking interactions" between the compounds and polypurine sequences within 5′-untranslated regions (UTR) of capped mRNAs. Zotatifin disrupts the unwinding of mRNA, leading to the dissociation of the mRNA-eIF4A complex from other eukaryotic translation factors; thus, disrupting the eIF4A-mediated translation in many oncogenes (eIF4E and eIF4G) (Müller et al. 2021). Notably, RNA viruses were also found to utilize the helicase activity of eIF4A to form the 43S-preinitiation complex that translates their viral mRNA. For instance, the Ebola virus, hepatitis E, Zika virus, SARS-CoV, MERS-CoV, and SARS-CoV-2 have utilized host eIF4A to translate its viral proteins (Montero et al. 2019; Nebigil et al. 2020; Taroncher-Oldenburg et al. 2021). Hence, rocaglates may be repurposed as a broad-spectrum antiviral candidate.

Multiple studies have demonstrated promising anticancer effects of zotatifin in preclinical settings (Neveu et al. 2012; Chan et al. 2019; Thompson et al. 2021; Gerson-Gurwitz et al. 2021). Furthermore, a study by Gordon et al. (2020) has also suggested repurposing zotatifin for COVID-19 treatment in a protein interaction map study for SARS-CoV-2. In that study, affinity-purification mass spectrometry identified 332 protein–protein interactions between hosts and SARS-CoV-2 with 69 compounds, including 29 FDA-approved drugs that target 66 different human proteins, entailing those that demonstrated antiviral activity against mRNA translation. Hence, the proposal of zotatifin as a compelling candidate for disrupting protein biogenesis through the inhibition of eIF4A presents an attractive antiviral strategy against SARS-CoV-2 during the early infection stages (Gordon et al. 2020). Besides that, an in vitro study conducted by Obermann et al. (2022) has demonstrated the efficacy of zotatifin, natural rocaglate silvesterol, and other synthetic rocaglate, the CR-1-31-B for RNA-clamping and antiviral activity against human coronavirus 229E (HCoV-229E) in Medical Research Council cell strain 5 (MRC-5 cells). It was found that silvesterol and CR-1-31-B demonstrated more potent results than zotatifin; however, the latter was less cytotoxic toward host immune cells. Currently, zotatifin is being investigated in a dose-escalating study involving COVID-19 patients (mild and moderate disease severity) (Obermann et al. 2022).

18.6.2.2 Plitidepsin

Plitidepsin (dehydrodidemnin) is a marine cyclic depsipeptide found in a Caribbean marine organism known as *Aplidum albicans* (El Bairi et al. 2020; Papapanou et al. 2021). It was initially investigated for its antitumor properties; however, recent drug screening studies found its potential antiviral use against SARS-CoV-2.

Plitidepsin exhibits antineoplastic effects through antiproliferation and antiangiogenesis; hence, contributing to its antitumor properties (Papapanou et al. 2021). It was found to induce cell cycle arrest or apoptosis and inhibit vascular endothelial growth factors (Alonso-Álvarez et al. 2017; Papapanou et al. 2021). The primary target for plitidepsin is the eukaryotic translation elongation factor 1 alpha 2 (eEF1A2) which is responsible for the enzymatic delivery of aminoacyl tRNA to ribosomes (White et al. 2021; Martinez 2021; Papapanou et al. 2021). As eEF1A2 facilitates noncanonical pro-oncogenic activity, inhibition of the protein by plitidepsin alters cell activity, including controlling proteasomal degradation of unfolded proteins, heat shock response, oxidative stress regulation, as well as actin building and cytoskeleton reorganization (Papapanou et al. 2021). In addition, the drug has an established safety profile in humans (White et al. 2021). Plitidepsin has been investigated for multiple myeloma, leukemia, and lymphomas either as a monotherapy or in combination with dexamethasone (El Bairi et al. 2020; Papapanou et al. 2021). However, due to the arrival of competitive targeted agents and poor enrolment of plitidepsin in clinical trials, the development of plitidepsin has been discontinued (El Bairi et al. 2020).

On the contrary, plitidepsin has shown antiviral properties in several SARS-CoV-2 in vitro and in vivo models. A recent study conducted by White et al. (2021) showed that plitidepsin inhibited eEF1A in human cells and has demonstrated solid antiviral activity against SARS-CoV-2 in Vero E6 and human ACE2 293 T (hACE2-293 T) cell lines. The N protein subgenomic RNA was reduced as early as 4 h post-SARS-CoV-2 infection, and the impact of plitidepsin on viral RNA and protein production continued throughout the time course study. Furthermore, plitidepsin significantly reduced SARS-CoV-2 infection and inflammation of lung tissues in mouse models. The reduction of SARS-CoV-2 was comparable to the positive control involving remdesivir-treated mice, which has been used to improve the disease outcomes of COVID-19 patients. Therefore, plitidepsin is now being investigated in a Phase 2/3 trial by a Spanish pharmaceutical company, PharmaMar (Service 2021).

18.6.3 Inhibition of Viral Entry into Hostspiepr146 Cells

18.6.3.1 Toremifene

Toremifene is an FDA-approved, first-generation nonsteroidal selective estrogen receptor modulator (SERM) used to treat metastatic breast cancer (Zhou et al.

2020a). It has similar efficacy to tamoxifen in advanced breast cancer (Mao et al. 2012). In previous studies, toremifene has successfully inhibited virus infections such as MERS CoV, SARS-CoV, and the Ebola virus (Johansen et al. 2013; Dyall et al. 2014). A study of toremifene on the Ebola virus revealed toremifene prevented membrane fusion of virus and host endosome through the interaction and destabilization of the membrane glycoprotein (Zhao et al. 2016; Zhou et al. 2020a). This led to the disruption of viral entry into host cells. Notably, it was determined that toremifene inhibited virus entry and internalization of Ebola, regardless of the estrogen-modulating activity in the following in vivo studies (Johansen et al. 2013).

In many drug screening and molecular docking studies, toremifene has been selected as a potential antiviral drug against SARS-CoV-2 (Jeon et al. 2020; Martin and Cheng 2020). Although the exact antiviral mechanism of toremifene has yet to be elucidated, it was suggested that toremifene likely interacts with the S glycoprotein or NSP14, which subsequently inhibits viral entry and replication, disrupting the life cycle of SARS-CoV-2 (Martin and Cheng 2020). Furthermore, several network-based analyses have suggested that using toremifene in combination with emodin, a naturally occurring compound in Chinese traditional medicine used to prevent and treat viral influenza, may enhance clinical efficacy compared to toremifene alone (Dong et al. 2016; Zhou et al. 2020a, b). Moreover, toremifene may also interact with other proteins such as ribosomal proteins RPL19, NPM1, EIF3E, HNRNPA1, and EIR3F (Zhou et al. 2020a). Despite that, the addition of toremifene to Vero E6 cells prior to SARS-CoV-2 virus infection in a drug screening study has demonstrated poor (little to none) virus inhibition according to immunofluorescence analyses (Jeon et al. 2020). Apart from that, the IC_{50} and CC_{50} of toremifene were 3.58 μM and 10.11 μM, respectively, suggesting potential adverse effects for the user (Jeon et al. 2020). Therefore, further preclinical investigation of toremifene is still warranted.

18.7 Targeting Cellular Pathway Mechanisms as a Strategy for Drug Repositioning

Due to the nature of the diverse functions of proteins in the human body, many signaling pathways inevitably partake in many different biological processes. Therefore, a specific signaling pathway may play a role in several diseases or conditions. Among the many, the Janus kinase signal transducer and activator of transcription (JAK-STAT) pathway is notably involved in antiviral responses, cancer development, autoimmune, and inflammatory responses (Fleming 2016; Banerjee et al. 2017; Owen et al. 2019). As such, repositioning drugs based on overlapping host factors in different diseases or conditions is a legitimate strategy that has been granted with great success, aspirin being one of the best examples (Jourdan et al. 2020). In addition, targeting host factors has an inherent advantage of circumventing the development of drug resistance (Prudencio and Mota 2012). Thus, targeting host

factors presents itself as a desirable strategy. Here, we describe three common host factors and signaling pathways that play a significant role in both antiviral responses and cancer development.

18.7.1 PI3K/AKT/mTOR

The phosphatidylinositol-3-kinase/Akt (PI3K/AKT) and mammalian target of rapamycin (mTOR) signaling pathways are two crucial pathways in regulating cellular proliferation and differentiation, apoptosis, and cellular metabolism. Despite being two distinct pathways, both PI3K/AKT and mTOR pathways share many cellular processes, and therefore is termed the PI3K/AKT/mTOR pathway (Porta et al. 2014). As mentioned above, the PI3K/AKT/mTOR pathway is strongly associated with cellular growth, which is no stranger that it has been studied extensively in cancer therapeutics such as gain-of-function mutations that lead to disrupted and dysregulated cell growth. This results in several classical hallmarks of cancer, such as unlimited proliferation and angiogenesis (Porta et al. 2014). The FDA has approved several PI3K/AKT/mTOR inhibitors, namely copanlisib, duvelisib, and idelalisib, in addition to many others still in clinical trials (Yang et al. 2019). SARS-CoV-2 can hijack and overexpress the PI3K/AKT/mTOR pathway to facilitate its viral replication and growth (Fattahi et al. 2022). As PI3K/AKT/mTOR pathway is also closely associated with the activation of pro-inflammatory cytokines, an over-activation of the pathway by the virus leads to an overexpression of such cytokines, resulting in cytokine storm and severe cellular damage (Khezri et al. 2022). As such, this pathway gained attraction and was proposed as a potential target for anti-SARS-CoV-2 therapy (Abu-Eid and Ward 2021). Several studies have already suggested the antiviral effects of dactolisib, a PI3K inhibitor (Khezri et al. 2022). Currently, numerous clinical trials are ongoing in evaluating the potential of mTOR inhibitors like rapamycin, as antiviral agents against SARS-CoV-2 (Patocka et al. 2021).

18.7.2 STAT-3

The signal transducer and activator of transcription-3 (STAT-3) is an essential key player in mediating multiple cellular processes such as cellular proliferation, immune responses, inflammation, and apoptosis (Yu et al. 2014). As such, STAT-3 has been studied extensively. Dysregulation of STAT-3 is associated with many diseases and conditions such as type 1 diabetes, arthritis, autoimmunity, and microbial infections (Vogel et al. 2015). Among other pro-inflammatory cytokines, the hyper-activation of STAT-3 is strongly associated with the cytokine storm during SARS-CoV-2 infection (Jafarzadeh et al. 2021). The hyper-elevation of STAT-3 leads to downstream activation of multiple inflammatory pathways, including

JAK-STAT and NF-κB, resulting in an uncontrolled infiltration of inflammatory cells such as monocytes and macrophages into the lung alveoli, causing ALI or ARDS (Jafarzadeh et al. 2021). In contrast, the hyper-production of STAT-3 in cancer is attributed to a gain-of-function mutation of the STAT-3 gene, akin to that demonstrated during SARS-CoV-2 viral infection (Faletti et al. 2021). Recent developments in cancer immunotherapy have resulted in several FDA-approved drugs targeting STAT-3, such as BBI608 and celecoxib (Zou et al. 2020). Since STAT-3 is associated with a wide range of inflammatory cytokines such as IL-6 and JAK, STAT-3 inhibitors can act directly or indirectly. As the name suggests, the direct inhibitors target STAT-3 specifically, while indirect inhibitors target STAT-3-associated cytokines (Zou et al. 2020). Therefore, STAT-3 is a potential druggable target for anticancer and antiviral development; thus, warranting more attention in repurposing STAT-3 inhibitors from cancer therapeutics to SARS-CoV-2 therapies.

18.7.3 VEGF

Vascular endothelial growth factor (VEGF) is a glycoprotein initially identified as a permeability factor, and was later shown to possess mitogenic and angiogenic properties (Barratt et al. 2014). Due to its angiogenic properties, VEGF became a popular target in cancer therapeutics as it can promote endothelial cell growth and survival. Multiple VEGF inhibitors have been approved against various cancers, namely colorectal and breast cancer. These VEGF inhibitors include, but are not limited to bevacizumab, sorafenib, sunitinib, and nilotinib (Escalante and Zalpour 2011). Furthermore, its role as a prime regulator in vascular permeability is often associated with ALI, which is characterized by pulmonary edema, hypoxia, and diffuse pulmonary infiltrates (Tomita et al. 2020). In the context of SARS-CoV-2, a clinical study published in 2020 noted that COVID-19 patients possess elevated levels of VEGF (Kong et al. 2020); therefore, presenting itself as a potential biomarker for clinical diagnostics. As mentioned earlier, VEGF was identified by its properties to increase pulmonary vascular permeability; hence, was strongly linked to playing a pivotal role in the pathogenesis of ALI (Medford 2006). Combining these literature data, targeting VEGF should consequently lead to clinical improvements in COVID-19 patients. A single-armed clinical trial published in early 2021 successfully improved the outcome of COVID-19 patients using bevacizumab, a VEGF inhibitor plus standard care compared to just standard care (Pang et al. 2021). The authors had a similar concept of inhibiting VEGF as hypoxia induces VEGF expression, leading to increased vascular leakiness. Another clinical trial in Bangladesh treated COVID-19 patients with bevacizumab and reported that 23 out of the 25 patients had a favorable clinical outcome (Al-Mahtab et al. 2021). Conclusively, this shows the potential of VEGF inhibitors to be repositioned against SARS-CoV-2.

18.8 Limitations and Future Perspectives of Drug Repositioning

As discussed above, drug repositioning confers a plethora of benefits and undiscovered potential for using readily available drugs for other indications. Particularly during a pandemic like COVID-19, there presents an urgent need for antiviral drugs to reduce the mortality and morbidity rates within the global population. Nonetheless, there remain a number of challenges that need to be addressed.

18.8.1 Accessibility to Data and Compound

Pharmaceutical industries' shelved drugs hold various opportunities to discover a medical miracle with indications that are even outside the industries' core disease area. Despite this, some are reluctant to share their chemical libraries, including a record of their failed drugs. Should they choose to collaborate with other firms or academic groups, administrative procedures pertaining to the compound distribution and material transfer agreement are vital to be made flexible and clearly outlined. Compounds that are derived from generic active pharmaceutical ingredients may cause some trouble if they are suddenly removed from the international market. Therefore, finding a reliable source for the compound of interest is imperative (Talevi and Bellera 2020).

Open-source platforms are becoming an increasingly popular approach to drug discovery and repositioning (Allarakhia 2013). Nevertheless, there is still limited public access to valuable data such as clinical trial outcomes. Additionally, data mining and manipulation are less feasible with some other types of data, more so for those presented in a nonstandardized manner (Pushpakom et al. 2019). To integrate them together would then require specialized experts and appropriate computational resources to increase the power of analysis (Ritchie et al. 2015).

18.8.2 Drug Safety and Toxicity

Drugs that are readily available and have been approved by regulatory agencies have undergone multiple phases of evaluation to determine the maintenance of their safety and efficacy. Aspects that are looked into include whether the dosage being given is within the well-defined safety margins, the ability of the drug to be delivered to the target region and if the activity of the drug can be achieved for a minimal length of time (Oprea et al. 2011). Particularly in anticancer research, the presenting challenge is directing the drug action to specific cancer cells instead of nonspecific targeting to normal, healthy cells (Senapati et al. 2018). Developing novel formulations or delivery mechanisms are viable strategies for improvement. Unfortunately, it has

been observed that discovery teams often lack collaboration with pharmaceutical and toxicological scientists to do so. Safety margins of novel drug–target interactions as well as older drugs that have not been comprehensively profiled are often disclosed in literature. Based on what is reported thus far, the potency is usually at the micromolar level. Consequently, this creates a therapeutic window that poses constraints on prospective indications. Researchers are pressurized with the burden of proof to demonstrate that clinical benefits may still be obtained for the treatment of the disease of interest within the pre-established dose ranges. If the drug potency falls outside those ranges, researchers will need to kickstart Phase 1 clinical trials. In this case, the efforts are defeating the process of drug repositioning, rather, it is more to de novo drug discovery (Oprea et al. 2011).

18.8.3 Exhaustion of Conventional Drug Repositioning Strategies

For any given disease, conventional drug repositioning strategies may rapidly exhaust the number of potential candidates considering that the pool is limited and only slowly expanding due to the financial burden of drug discovery. This raises the question of whether drug repositioning itself may gradually cease upon full screening of already known drugs (Talevi and Bellera 2020). An answer to this question may be to readjust the approach and look into other target disorders. For instance, instead of the typical monotherapy, drug combinations can be evaluated to amplify the possibility of drug repositioning (Sun et al. 2016). Another alternative which can widen the range of drug candidates is precision and system medicine, whereby they are personalized to patients for better delivery and advanced outcomes while potentially covering lower healthcare costs. The disease signatures and accurate stratification of patients based on their genomic component will need to be well understood (Taylor et al. 2019). During drug development, pharmaceutical industries often generate hundreds of other compounds sharing the same active scaffold as the lead compound. They are intended to build structure–activity relationships in hit-to-lead and lead-optimization programs. Upon identification of a suitable candidate with successful activity against the target of interest, this whole set of compounds may be retrieved to be further studied for other indications (Talevi and Bellera 2020).

18.8.4 Intellectual Property Protection

There are a number of reasons hampering the efforts of applying for intellectual property (IP) protection over repositioned drugs. Although a majority of the pharmaceutical industry allows for their protection, some national legislations are against patenting the same drug for additional indications. Even if the latter carries on,

different countries have varying rulings (Talevi and Bellera 2020). For instance, the European Union grants 8 years of data protection in addition to 2 years of market exclusivity. Should another indication be discovered during the 8-year period, an extra year of protection will be given (Sonia Ribeiro 2018). On the other hand, the United States permits an initial period of 5 years, followed by another 3 years for a new indication. In spite of that, as these additional years do not necessitate a profitable return of investment, further financial incentives are required to ensure the efforts are cost-effective (Talevi and Bellera 2020). Another concern is that many potential repositioning uses have already been published or better yet, already implemented as a clinical practice, albeit, are regarded as off-label, nonregistered prescription. Only if the correlation between drug, target, and disease is truly unique can protected marketing rights be favored. Considering that drug repositioning remains to be a newly growing field, experts specializing in its legal issues are still lacking; thus, various issues may arise, potentially resulting in the discovery team not wanting to seek patent protection at all (Oprea et al. 2011).

18.8.5 Challenges of Bioinformatics Approaches

Classical statistical approaches confer a number of limitations, including the ineffectiveness in identifying the molecular target of a drug against the abundant number of genes. Furthermore, the other hundreds and thousands of gene products that indirectly respond to the changes in the activity of the target are yet to be fully considered. These approaches rely on small datasets and biological networks that originate from experiments carried out on varying platforms. Resultantly, research outcomes may be inconsistent or only provide partial knowledge of the studied area. Hence, derived conclusions may not entirely be replicable (Jarada et al. 2020). In response to that, multiple strategies like semantic technologies involving machine learning, deep learning, and network analysis may be combined to attain a higher success rate (Zeng et al. 2019; Rodriguez et al. 2021; Siminea et al. 2022). Their advantages and disadvantages must certainly be weighed to achieve the most fruitful data. For example, the use of deep neural network models through deep learning requires adjustments to fit the data, causing the efforts to be time-consuming. Additionally, decision-making over which machine learning technique or similarity measure to opt for is not straightforward and dependent on the dataset (Jarada et al. 2020). Regardless, integrating data on drug candidates and how they target different diseases and the host can provide a vast amount of enriching knowledge for the modeling of computational drug repositioning.

18.9 Conclusion

In such a dire need for an effective therapeutic approach for COVID-19, the potential of drug repositioning cannot be overlooked. Given the many successes and studies done in the context of anticancer for antivirals such as zidovudine for Friend leukemia virus and ouabain for Ebola virus, to name a few, it is no doubt anticancer may be the new paradigm for the treatment of the virus, saving cost, time, and resources. Cancer and viruses share many cellular pathways and host proteins for activation and propagation, making anticancer drugs a vital candidate to be evaluated and considered for SARS-CoV-2. Notably, previously FDA-approved anticancer drugs have been studied for other coronaviruses, such as imatinib, saracatinib, and HTT for SARS-CoV and MERS-CoV. In addition, the exciting outcomes of SARS-CoV-2 inhibition contributed by current anticancer drugs like carmofur, carfilzomib, zotatifin, plitidepsin, and toremifene, further affirm the importance to study potential anticancer inhibitors. Apart from that, numerous cancers associated with cellular pathways, such as the PI3K/AKT/mTOR pathway, JAK-STAT pathway, and VEGF pathway, cross path with virus infection mechanisms, making anticancer agents associated with these pathways a potential candidate for SARS-CoV-2 viral inhibitors. While there are certain limitations to the drug repositioning approach like data and compound accessibility, drug safety and toxicity for repositioning purposes, narrow pools of potential candidates, IP protection, and challenges arising from preliminary bioinformatics studies, the potential of anticancer agents for antiviral therapy, especially for SARS-CoV-2, remains of great interest.

Author Contributions

KHW, CML, AJWP, IAF, NZZ, and ZYL contributed equally to the preparation and drafting of the manuscript. Critical revisions, supervision and conceptualization of the article were done by SKL.
Conflict of Interest All authors have read and agreed to the published version of the manuscript. All authors have declared that there is no conflict of interest.

References

Abu-Eid R, Ward FJ (2021) Targeting the PI3K/Akt/mTOR pathway: a therapeutic strategy in COVID-19 patients. Immunol Lett 240:1–8. https://doi.org/10.1016/j.imlet.2021.09.005

Aherfi S, Pradines B, Devaux C et al (2021) Drug repurposing against SARS-CoV-1, SARS-CoV-2 and MERS-CoV. Future Microbiol 16:1341–1370. https://doi.org/10.2217/fmb-2021-0019

Aldea M, Michot J-M, Danlos F-X et al (2021) Repurposing of anticancer drugs expands possibilities for antiviral and anti-inflammatory discovery in COVID-19. Cancer Discov 11:1336–1344. https://doi.org/10.1158/2159-8290.CD-21-0144

Allarakhia M (2013) Open-source approaches for the repurposing of existing or failed candidate drugs: learning from and applying the lessons across diseases. DDDT 753. https://doi.org/10.2147/DDDT.S46289

Al-Mahtab M, Akbar SM, Huq AF et al (2021) Extraordinary survival benefits of severe and critical patients with COVID-19 by immune modulators: the outcome of a clinical trial in Bangladesh. Euroasian J Hepato-Gastroenterol 10:68–75. https://doi.org/10.5005/jp-journals-10018-1327

Alonso-Álvarez S, Pardal E, Sánchez-Nieto D et al (2017) Plitidepsin: design, development, and potential place in therapy. DDDT 11:253–264. https://doi.org/10.2147/DDDT.S94165

Amarelle L, Lecuona E (2018) The antiviral effects of Na, K-ATPase inhibition: a min ireview. Int J Mol Sci 19:2154. https://doi.org/10.3390/ijms19082154

Amarelle L, Katzen J, Shigemura M et al (2019) Cardiac glycosides decrease influenza virus replication by inhibiting cell protein translational machinery. Am J Phys Lung Cell Mol Phys 316:L1094–L1106. https://doi.org/10.1152/ajplung.00173.2018

Andersen KG, Rambaut A, Lipkin WI et al (2020) The proximal origin of SARS-CoV-2. Nat Med 26:450–452. https://doi.org/10.1038/s41591-020-0820-9

Babé LM, Craik CS (1997) Viral proteases: evolution of diverse structural motifs to optimize function. Cell 91:427–430. https://doi.org/10.1016/S0092-8674(00)80426-2

Banerjee S, Biehl A, Gadina M et al (2017) JAK–STAT signaling as a target for inflammatory and autoimmune diseases: current and future prospects. Drugs 77:521–546. https://doi.org/10.1007/s40265-017-0701-9

Barratt S, Medford AR, Millar AB (2014) Vascular endothelial growth factor in acute lung injury and acute respiratory distress syndrome. Respiration 87:329–342. https://doi.org/10.1159/000356034

Burkard C, Verheije MH, Haagmans BL et al (2015) ATP1A1-mediated Src signaling inhibits coronavirus entry into host cells. J Virol 89:4434–4448. https://doi.org/10.1128/JVI.03274-14

Cao X (2020) COVID-19: immunopathology and its implications for therapy. Nat Rev Immunol 20:269–270. https://doi.org/10.1038/s41577-020-0308-3

Cao J, Forrest JC, Zhang X (2015) A screen of the NIH clinical collection small molecule library identifies potential anti-coronavirus drugs. Antivir Res 114:1–10. https://doi.org/10.1016/j.antiviral.2014.11.010

Chan K, Robert F, Oertlin C et al (2019) eIF4A supports an oncogenic translation program in pancreatic ductal adenocarcinoma. Nat Commun 10:5151. https://doi.org/10.1038/s41467-019-13086-5

Chow WA, Jiang C, Guan M (2009) Anti-HIV drugs for cancer therapeutics: back to the future? Lancet Oncol 10:61–71. https://doi.org/10.1016/S1470-2045(08)70334-6

Ciliberto G, Mancini R, Paggi MG (2020) Drug repurposing against COVID-19: focus on anticancer agents. J Exp Clin Cancer Res 39:86. https://doi.org/10.1186/s13046-020-01590-2

Clouser CL, Patterson SE, Mansky LM (2010) Exploiting drug repositioning for discovery of a novel HIV combination therapy. J Virol 84:9301–9309. https://doi.org/10.1128/JVI.01006-10

Coleman CM, Sisk JM, Mingo RM et al (2016) Abelson kinase inhibitors are potent inhibitors of severe acute respiratory syndrome coronavirus and Middle East respiratory syndrome coronavirus fusion. J Virol 90:8924–8933. https://doi.org/10.1128/JVI.01429-16

Coux O, Tanaka K, Goldberg AL (1996) Structure and functions of the 20S and 26S proteasomes. Annu Rev Biochem 65:801–847. https://doi.org/10.1146/annurev.bi.65.070196.004101

Cui W, Yang K, Yang H (2020) Recent Progress in the drug development targeting SARS-CoV-2 Main protease as treatment for COVID-19. Front Mol Biosci 7:616341. https://doi.org/10.3389/fmolb.2020.616341

Dementiev A, Joachimiak A, Nguyen H et al (2019) Molecular mechanism of inhibition of acid ceramidase by Carmofur. J Med Chem 62:987–992. https://doi.org/10.1021/acs.jmedchem.8b01723

Dodson AW, Taylor TJ, Knipe DM, Coen DM (2007) Inhibitors of the sodium potassium ATPase that impair herpes simplex virus replication identified via a chemical screening approach. Virology 366:340–348. https://doi.org/10.1016/j.virol.2007.05.001

Dong X, Fu J, Yin X et al (2016) Emodin: a review of its pharmacology, toxicity and pharmacokinetics: Emodin: pharmacology, toxicity and pharmacokinetics. Phytother Res 30:1207–1218. https://doi.org/10.1002/ptr.5631

Dowall SD, Bewley K, Watson RJ et al (2016) Antiviral screening of multiple compounds against Ebola virus. Viruses 8:277. https://doi.org/10.3390/v8110277

Dyall J, Coleman CM, Hart BJ et al (2014) Repurposing of clinically developed drugs for treatment of Middle East respiratory syndrome coronavirus infection. Antimicrob Agents Chemother 58: 4885–4893. https://doi.org/10.1128/AAC.03036-14

Dyall J, Gross R, Kindrachuk J et al (2017) Middle East respiratory syndrome and severe acute respiratory syndrome: current therapeutic options and potential targets for novel therapies. Drugs 77:1935–1966. https://doi.org/10.1007/s40265-017-0830-1

El Bairi K, Trapani D, Petrillo A et al (2020) Repurposing anticancer drugs for the management of COVID-19. Eur J Cancer 141:40–61. https://doi.org/10.1016/j.ejca.2020.09.014

Eron JJ, Benoit SL, Jemsek J et al (1995) Treatment with lamivudine, zidovudine, or both in HIV-positive patients with 200 to 500 CD^{4+} cells per cubic millimeter. N Engl J Med 333:1662–1669. https://doi.org/10.1056/NEJM199512213332502

Escalante CP, Zalpour A (2011) Vascular endothelial growth factor inhibitor-induced hypertension: basics for primary care providers. Cardiol Res Pract 2011:1–8. https://doi.org/10.4061/2011/816897

Faletti L, Ehl S, Heeg M (2021) Germline STAT3 gain-of-function mutations in primary immunodeficiency: impact on the cellular and clinical phenotype. Biom J 44:412–421. https://doi.org/10.1016/j.bj.2021.03.003

Fattahi S, Khalifehzadeh-Esfahani Z, Mohammad-Rezaei M et al (2022) PI3K/Akt/mTOR pathway: a potential target for anti-SARS-CoV-2 therapy. Immunol Res 70:269. https://doi.org/10.1007/s12026-022-09268-x

Fleming S (2016) Viral inhibition of the IFN-induced JAK/STAT signaling pathway: development of live attenuated vaccines by mutation of viral-encoded IFN-antagonists. Vaccine 4:23. https://doi.org/10.3390/vaccines4030023

García-Dorival I, Wu W, Dowall S et al (2014) Elucidation of the Ebola virus VP24 cellular Interactome and disruption of virus biology through targeted inhibition of host-cell protein function. J Proteome Res 13:5120–5135. https://doi.org/10.1021/pr500556d

Gerson-Gurwitz A, Young NP, Goel VK et al (2021) Zotatifin, an eIF4A-selective inhibitor, blocks tumor growth in receptor tyrosine kinase driven tumors. Front Oncol 11:766298. https://doi.org/10.3389/fonc.2021.766298

Gordon DE, Jang GM, Bouhaddou M et al (2020) A SARS-CoV-2 protein interaction map reveals targets for drug repurposing. Nature 583:459–468. https://doi.org/10.1038/s41586-020-2286-9

Huang Y, Yang C, Xu X et al (2020) Structural and functional properties of SARS-CoV-2 spike protein: potential antivirus drug development for COVID-19. Acta Pharmacol Sin 41:1141–1149. https://doi.org/10.1038/s41401-020-0485-4

Jafarzadeh A, Nemati M, Jafarzadeh S (2021) Contribution of STAT3 to the pathogenesis of COVID-19. Microb Pathog 154:104836. https://doi.org/10.1016/j.micpath.2021.104836

Jarada TN, Rokne JG, Alhajj R (2020) A review of computational drug repositioning: strategies, approaches, opportunities, challenges, and directions. J Cheminform 12:46. https://doi.org/10.1186/s13321-020-00450-7

Jayaweera SPE, Wanigasinghe Kanakanamge SP, Rajalingam D, Silva GN (2021) Carfilzomib: a promising proteasome inhibitor for the treatment of relapsed and refractory multiple myeloma. Front Oncol 11:740796. https://doi.org/10.3389/fonc.2021.740796

Jeon S, Ko M, Lee J et al (2020) Identification of antiviral drug candidates against SARS-CoV-2 from FDA-approved drugs. Antimicrob Agents Chemother 64:e00819–e00820. https://doi.org/10.1128/AAC.00819-20

Jin Z, Du X, Xu Y et al (2020a) Structure of Mpro from SARS-CoV-2 and discovery of its inhibitors. Nature 582:289–293. https://doi.org/10.1038/s41586-020-2223-y

Jin Z, Zhao Y, Sun Y et al (2020b) Structural basis for the inhibition of SARS-CoV-2 main protease by antineoplastic drug carmofur. Nat Struct Mol Biol 27:529–532. https://doi.org/10.1038/s41594-020-0440-6

Johansen LM, Brannan JM, Delos SE et al (2013) FDA-approved selective estrogen receptor modulators inhibit Ebola virus infection. Sci Transl Med 5:190ra79. https://doi.org/10.1126/scitranslmed.3005471

Johansson MA, Quandelacy TM, Kada S et al (2021) SARS-CoV-2 transmission from people without COVID-19 symptoms. JAMA Netw Open 4:e2035057. https://doi.org/10.1001/jamanetworkopen.2020.35057

Jourdan J-P, Bureau R, Rochais C, Dallemagne P (2020) Drug repositioning: a brief overview. J Pharm Pharmacol 72:1145–1151. https://doi.org/10.1111/jphp.13273

Kaushik V, Azad N, Yakisich JS, Iyer AKV (2017) Antitumor effects of naturally occurring cardiac glycosides convallatoxin and peruvoside on human ER+ and triple-negative breast cancers. Cell Death Discov 3:1–9. https://doi.org/10.1038/cddiscovery.2017.9

Kepp O, Menger L, Vacchelli E et al (2012) Anticancer activity of cardiac glycosides. Oncoimmunology 1:1640–1642. https://doi.org/10.4161/onci.21684

Khezri MR, Varzandeh R, Ghasemnejad-Berenji M (2022) The probable role and therapeutic potential of the PI3K/AKT signaling pathway in SARS-CoV-2 induced coagulopathy. Cell Mol Biol Lett 27:6. https://doi.org/10.1186/s11658-022-00308-w

Kim N, Yim HY, He N et al (2016) Cardiac glycosides display selective efficacy for STK11 mutant lung cancer. Sci Rep 6:29721. https://doi.org/10.1038/srep29721

Kong Y, Han J, Wu X et al (2020) VEGF-D: a novel biomarker for detection of COVID-19 progression. Crit Care 24:373. https://doi.org/10.1186/s13054-020-03079-y

Kortuem KM, Stewart AK (2013) Carfilzomib. Blood 121:893–897. https://doi.org/10.1182/blood-2012-10-459883

Laird GM, Eisele EE, Rabi SA et al (2014) A novel cell-based high-throughput screen for inhibitors of HIV-1 gene expression and budding identifies the cardiac glycosides. J Antimicrob Chemother 69:988–994. https://doi.org/10.1093/jac/dkt471

Li X, Zhong X, Wang Y et al (2021) Clinical determinants of the severity of COVID-19: a systematic review and meta-analysis. PLoS One 16:e0250602. https://doi.org/10.1371/journal.pone.0250602

Low ZY, Yip AJW, Lal SK (2022, 1868) Repositioning Ivermectin for Covid-19 treatment: molecular mechanisms of action against SARS-CoV-2 replication. Biochim Biophys Acta (BBA): Mol Basis Dis:166294. https://doi.org/10.1016/j.bbadis.2021.166294

Low ZY, Farouk IA, Lal SK (2020) Drug repositioning: new approaches and future prospects for life-debilitating diseases and the COVID-19 pandemic outbreak. Viruses 12:1058. https://doi.org/10.3390/v12091058

Low ZY, Yip AJW, Sharma A, Lal SK (2021) SARS coronavirus outbreaks past and present—a comparative analysis of SARS-CoV-2 and its predecessors. Virus Genes 57:307–317. https://doi.org/10.1007/s11262-021-01846-9

Lü S, Wang J (2014) Homoharringtonine and omacetaxine for myeloid hematological malignancies. J Hematol Oncol 7:2. https://doi.org/10.1186/1756-8722-7-2

Ma X, Xie L, Wartchow C et al (2017) Structural basis for therapeutic inhibition of influenza a polymerase PB2 subunit. Sci Rep 7:9385. https://doi.org/10.1038/s41598-017-09538-x

Ma C, Hu Y, Townsend JA et al (2020) Ebselen, disulfiram, Carmofur, PX-12, Tideglusib, and Shikonin are nonspecific promiscuous SARS-CoV-2 Main protease inhibitors. ACS Pharmacol Transl Sci 3:1265–1277. https://doi.org/10.1021/acsptsci.0c00130

Machitani M, Yasukawa M, Nakashima J et al (2020) RNA-dependent RNA polymerase, RdRP, a promising therapeutic target for cancer and potentially COVID-19. Cancer Sci 111:3976–3984. https://doi.org/10.1111/cas.14618

Mani D, Wadhwani A, Krishnamurthy PT (2019) Drug repurposing in antiviral research: a current scenario. JYP 11:117–121. https://doi.org/10.5530/jyp.2019.11.26

Mao C, Yang Z-Y, He B-F et al (2012) Toremifene versus tamoxifen for advanced breast cancer. Cochrane Database Syst Rev 2021:CD008926. https://doi.org/10.1002/14651858.CD008926.pub2

Martin WR, Cheng F (2020) Repurposing of FDA-approved Toremifene to treat COVID-19 by blocking the spike glycoprotein and NSP14 of SARS-CoV-2. J Proteome Res 19:4670–4677. https://doi.org/10.1021/acs.jproteome.0c00397

Martinez MA (2021) Plitidepsin: a repurposed drug for the treatment of COVID-19. Antimicrob Agents Chemother 65:e00200–e00221. https://doi.org/10.1128/AAC.00200-21

Medford ARL (2006) Vascular endothelial growth factor (VEGF) in acute lung injury (ALI) and acute respiratory distress syndrome (ARDS): paradox or paradigm? Thorax 61:621–626. https://doi.org/10.1136/thx.2005.040204

Mengist HM, Dilnessa T, Jin T (2021) Structural basis of potential inhibitors targeting SARS-CoV-2 Main protease. Front Chem 9:622898. https://doi.org/10.3389/fchem.2021.622898

Montero H, Pérez-Gil G, Sampieri CL (2019) Eukaryotic initiation factor 4A (eIF4A) during viral infections. Virus Genes 55:267–273. https://doi.org/10.1007/s11262-019-01641-7

Müller C, Obermann W, Karl N et al (2021) The rocaglate CR-31-B (−) inhibits SARS-CoV-2 replication at non-cytotoxic, low nanomolar concentrations in vitro and ex vivo. Antivir Res 186:105012. https://doi.org/10.1016/j.antiviral.2021.105012

Namba T, Kodama R, Moritomo S et al (2015) Zidovudine, an anti-viral drug, resensitizes gemcitabine-resistant pancreatic cancer cells to gemcitabine by inhibition of the Akt-GSK3-β-snail pathway. Cell Death Dis 6:e1795–e1795. https://doi.org/10.1038/cddis.2015.172

Nebigil CG, Moog C, Vagner S et al (2020) Flavaglines as natural products targeting eIF4A and prohibitins: from traditional Chinese medicine to antiviral activity against coronaviruses. Eur J Med Chem 203:112653. https://doi.org/10.1016/j.ejmech.2020.112653

Neveu G, Barouch-Bentov R, Ziv-Av A et al (2012) Identification and targeting of an interaction between a tyrosine motif within hepatitis C virus Core protein and AP2M1 essential for viral assembly. PLoS Pathog 8:e1002845. https://doi.org/10.1371/journal.ppat.1002845

Obermann W, Friedrich A, Madhugiri R et al (2022) Rocaglates as antivirals: comparing the effects on viral resistance, anti-Coronaviral activity, RNA-clamping on eIF4A and immune cell toxicity. Viruses 14:519. https://doi.org/10.3390/v14030519

Oprea TI, Bauman JE, Bologa CG et al (2011) Drug repurposing from an academic perspective. Drug Discovery Today: Therapeutic Strategies 8:61–69. https://doi.org/10.1016/j.ddstr.2011.10.002

Ostertag W, Roesler G, Krieg CJ et al (1974) Induction of endogenous virus and of thymidine kinase by Bromodeoxyuridine in cell cultures transformed by friend virus. Proc Natl Acad Sci 71:4980–4985. https://doi.org/10.1073/pnas.71.12.4980

Owen K, Brockwell N, Parker B (2019) JAK-STAT signaling: a double-edged sword of immune regulation and cancer progression. Cancers 11:2002. https://doi.org/10.3390/cancers11122002

Pang J, Xu F, Aondio G et al (2021) Efficacy and tolerability of bevacizumab in patients with severe Covid-19. Nat Commun 12:814. https://doi.org/10.1038/s41467-021-21085-8

Papapanou M, Papoutsi E, Giannakas T, Katsaounou P (2021) Plitidepsin: mechanisms and clinical profile of a promising antiviral agent against COVID-19. JPM 11:668. https://doi.org/10.3390/jpm11070668

Parasher A (2021) COVID-19: current understanding of its pathophysiology, clinical presentation and treatment. Postgrad Med J 97:312–320. https://doi.org/10.1136/postgradmedj-2020-138577

Patocka J, Kuca K, Oleksak P et al (2021) Rapamycin: drug repurposing in SARS-CoV-2 infection. Pharmaceuticals 14:217. https://doi.org/10.3390/ph14030217

Porta C, Paglino C, Mosca A (2014) Targeting PI3K/Akt/mTOR signaling in cancer. Front Oncol 4. https://doi.org/10.3389/fonc.2014.00064

Prudencio M, Mota M (2012) Targeting host factors to circumvent anti-malarial drug resistance. CPD 19:290–299. https://doi.org/10.2174/1381612811306020290

PubChem (2022a) Imatinib. https://pubchem.ncbi.nlm.nih.gov/compound/5291. Accessed 3 Apr 2022

PubChem (2022b) Saracatinib. https://pubchem.ncbi.nlm.nih.gov/compound/10302451. Accessed 3 Apr 2022

PubChem (2022c) Homoharringtonine,(S). https://pubchem.ncbi.nlm.nih.gov/compound/16219462. Accessed 3 Apr 2022
Pushpakom S, Iorio F, Eyers PA et al (2019) Drug repurposing: progress, challenges and recommendations. Nat Rev Drug Discov 18:41–58. https://doi.org/10.1038/nrd.2018.168
Queirós-Reis L, Gomes da Silva P, Gonçalves J et al (2021) SARS-CoV-2 virus−host interaction: currently available structures and implications of variant emergence on infectivity and immune response. IJMS 22:10836. https://doi.org/10.3390/ijms221910836
Sonia Ribeiro (2018) Data exclusivity, market protection, orphan and paediatric rewards
Ritchie MD, Holzinger ER, Li R et al (2015) Methods of integrating data to uncover genotype–phenotype interactions. Nat Rev Genet 16:85–97. https://doi.org/10.1038/nrg3868
Rodriguez S, Hug C, Todorov P et al (2021) Machine learning identifies candidates for drug repurposing in Alzheimer's disease. Nat Commun 12:1033. https://doi.org/10.1038/s41467-021-21330-0
Scanlon KJ, Kashani-Sabet M, Sowers LC (1989) Overexpression of DNA replication and repair enzymes in cisplatin-resistant human colon carcinoma HCT8 cells and circumvention by azidothymidine. Cancer Commun 1:269–275
Senapati S, Mahanta AK, Kumar S, Maiti P (2018) Controlled drug delivery vehicles for cancer treatment and their performance. Sig Transduct Target Ther 3:7. https://doi.org/10.1038/s41392-017-0004-3
Service RF (2021) A call to arms. Science 371:1092–1095. https://doi.org/10.1126/science.371.6534.1092
Shereen MA, Khan S, Kazmi A et al (2020) COVID-19 infection: emergence, transmission, and characteristics of human coronaviruses. J Adv Res 24:91–98. https://doi.org/10.1016/j.jare.2020.03.005
Shin J, Jung E, Kim M et al (2018) Saracatinib inhibits Middle East respiratory syndrome-coronavirus replication in vitro. Viruses 10:283. https://doi.org/10.3390/v10060283
Siminea N, Popescu V, Sanchez Martin JA et al (2022) Network analytics for drug repurposing in COVID-19. Brief Bioinform 23:bbab490. https://doi.org/10.1093/bib/bbab490
Sun W, Sanderson PE, Zheng W (2016) Drug combination therapy increases successful drug repositioning. Drug Discov Today 21:1189–1195. https://doi.org/10.1016/j.drudis.2016.05.015
Talevi A, Bellera CL (2020) Challenges and opportunities with drug repurposing: finding strategies to find alternative uses of therapeutics. Expert Opin Drug Discovery 15:397–401. https://doi.org/10.1080/17460441.2020.1704729
Taroncher-Oldenburg G, Müller C, Obermann W et al (2021) Targeting the DEAD-box RNA helicase eIF4A with Rocaglates—a pan-antiviral strategy for minimizing the impact of future RNA virus pandemics. Microorganisms 9:540. https://doi.org/10.3390/microorganisms9030540
Taschuk F, Cherry S (2020) DEAD-box helicases: sensors, regulators, and effectors for antiviral defense. Viruses 12:181. https://doi.org/10.3390/v12020181
Taylor K, Das S, Pearson M et al (2019) Systematic drug repurposing to enable precision medicine: a case study in breast cancer. Digit Med 5:180. https://doi.org/10.4103/digm.digm_28_19
Thompson PA, Eam B, Young NP et al (2021) Targeting oncogene mRNA translation in B-cell malignancies with eFT226, a potent and selective inhibitor of eIF4A. Mol Cancer Ther 20:26–36. https://doi.org/10.1158/1535-7163.MCT-19-0973
Tomita K, Saito Y, Suzuki T et al (2020) Vascular endothelial growth factor contributes to lung vascular hyperpermeability in sepsis-associated acute lung injury. Naunyn Schmiedeberg's Arch Pharmacol 393:2365–2374. https://doi.org/10.1007/s00210-020-01947-6
Vij R, Wang L, Orlowski RZ et al (2009) Carfilzomib (CFZ), a novel proteasome inhibitor for relapsed or refractory multiple myeloma, is associated with minimal peripheral neuropathic effects. Blood 114:430–430. https://doi.org/10.1182/blood.V114.22.430.430
Vogel TP, Milner JD, Cooper MA (2015) The Ying and Yang of STAT3 in human disease. J Clin Immunol 35:615–623. https://doi.org/10.1007/s10875-015-0187-8

Wang J (2020) Fast identification of possible drug treatment of coronavirus Disease-19 (COVID-19) through computational drug repurposing study. J Chem Inf Model 60:3277–3286. https://doi.org/10.1021/acs.jcim.0c00179

Wang Y, Lv Z, Chu Y (2015) HIV protease inhibitors: a review of molecular selectivity and toxicity. HIV 95. https://doi.org/10.2147/HIV.S79956

Wang M-Y, Zhao R, Gao L-J et al (2020) SARS-CoV-2: structure, biology, and structure-based therapeutics development. Front Cell Infect Microbiol 10:587269. https://doi.org/10.3389/fcimb.2020.587269

Waxman AJ, Clasen S, Hwang W-T et al (2018) Carfilzomib-associated cardiovascular adverse events: a systematic review and meta-analysis. JAMA Oncol 4:e174519. https://doi.org/10.1001/jamaoncol.2017.4519

Wehbe Z, Hammoud S, Soudani N et al (2020) Molecular insights into SARS COV-2 interaction with cardiovascular disease: role of RAAS and MAPK signaling. Front Pharmacol 11

White KM, Rosales R, Yildiz S et al (2021) Plitidepsin has potent preclinical efficacy against SARS-CoV-2 by targeting the host protein eEF1A. Science 371:926–931. https://doi.org/10.1126/science.abf4058

Wong RW, Balachandran A, Ostrowski MA, Cochrane A (2013) Digoxin suppresses HIV-1 replication by altering viral RNA processing. PLoS Pathog 9:e1003241. https://doi.org/10.1371/journal.ppat.1003241

Wong RW, Lingwood CA, Ostrowski MA et al (2018) Cardiac glycoside/aglycones inhibit HIV-1 gene expression by a mechanism requiring MEK1/2-ERK1/2 signaling. Sci Rep 8:850. https://doi.org/10.1038/s41598-018-19298-x

World Health Organization (2022) Tracking SARS-CoV-2 variants. World Health Organization, In. https://www.who.int/health-topics/typhoid/tracking-SARS-CoV-2-variants. Accessed 29 Mar 2022

Worldometer (2022) COVID live—coronavirus statistics—Worldometer. In: Worldometer. https://www.worldometers.info/coronavirus/. Accessed 30 Mar 2022

Wu D, Wu T, Liu Q, Yang Z (2020) The SARS-CoV-2 outbreak: what we know. Int J Infect Dis 94:44–48. https://doi.org/10.1016/j.ijid.2020.03.004

Yang J, Nie J, Ma X et al (2019) Targeting PI3K in cancer: mechanisms and advances in clinical trials. Mol Cancer 18:26. https://doi.org/10.1186/s12943-019-0954-x

Yu H, Lee H, Herrmann A et al (2014) Revisiting STAT3 signaling in cancer: new and unexpected biological functions. Nat Rev Cancer 14:736–746. https://doi.org/10.1038/nrc3818

Zanella I, Zizioli D, Castelli F, Quiros-Roldan E (2021) Tenofovir, another inexpensive, well-known and widely available old drug repurposed for SARS-COV-2 infection. Pharmaceuticals 14:454. https://doi.org/10.3390/ph14050454

Zeng X, Zhu S, Liu X et al (2019) deepDR: a network-based deep learning approach to in silico drug repositioning. Bioinformatics 35:5191–5198. https://doi.org/10.1093/bioinformatics/btz418

Zhao Y, Ren J, Harlos K et al (2016) Toremifene interacts with and destabilizes the Ebola virus glycoprotein. Nature 535:169–172. https://doi.org/10.1038/nature18615

Zhou Y, Hou Y, Shen J et al (2020a) Network-based drug repurposing for novel coronavirus 2019-nCoV/SARS-CoV-2. Cell Discov 6:14. https://doi.org/10.1038/s41421-020-0153-3

Zhou Y, Wang F, Tang J et al (2020b) Artificial intelligence in COVID-19 drug repurposing. The Lancet Digital Health 2:e667–e676. https://doi.org/10.1016/S2589-7500(20)30192-8

Zou S, Tong Q, Liu B et al (2020) Targeting STAT3 in cancer immunotherapy. Mol Cancer 19:145. https://doi.org/10.1186/s12943-020-01258-7

Chapter 19
Drug Repurposing for COVID-19 Therapy: Pipeline, Current Status and Challenges

Rohit Verma, Shivani Raj, Umang Berry, C. T. Ranjith-Kumar, and Milan Surjit

Abstract Repurposed drugs such as Remdesivir, Fabipiravir and Molnupiravir became life saver drugs during the peak of the COVID-19 pandemic, attesting the efficacy of the repurposing approach. By definition, drug repurposing is the process of identification of new therapeutic use of an existing drug or drug candidate that has already passed the safety, toxicity and pharmacology tests for human use. Although drug repurposing approach involves a significant level of challenge, affordability and faster discovery pipeline outweighs the risks in the event of emergency situations like the current COVID-19 pandemic. In this chapter, we provide a brief summary of the advantages of the drug repurposing approach, followed by an overview of the drug repurposing pipeline and finally end with an update on the status of drug repurposing in developing effective anti-viral therapeutics against COVID-19.

Keywords COVID-19 · SARS-CoV-2 · Antivirals · Drug repurposing · Remdesivir · Fabipiravir · Molnupiravi

19.1 Introduction

Within 5 months of discovery of the severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2), the causative agent of coronavirus-induced disease-2019 (COVID-19), a nucleotide analogue prodrug called GS-5734 or remdesivir, was granted the emergency use authorization by the United States Food and Drug Administration for the treatment of COVID-19 patients. Remdesivir was originally identified as an antiviral against the Ebola virus infection (Pardo et al. 2020). This

R. Verma · U. Berry · M. Surjit (✉)
Virology Laboratory, Vaccine and Infectious Disease Research Centre, Translational Health Science and Technology Institute, NCR Biotech Science Cluster, Faridabad, Haryana, India
e-mail: milan@thsti.res.in

S. Raj · C. T. Ranjith-Kumar
University School of Biotechnology, Guru Gobind Singh Indraprastha University, New Delhi, India

R. C. Sobti et al. (eds.), *Drug Repurposing for Emerging Infectious Diseases and Cancer*, https://doi.org/10.1007/978-981-19-5399-6_19

illustrates the power of the repurposing approach in drug discovery. Drug repurposing is defined as the process of identification of new therapeutic use of an existing drug or drug candidate that has already passed the safety, toxicity and pharmacology tests for human use. As a matter of fact, most of the drugs approved so far for standard care in COVID-19 patients belong to the repurposed category, further emphasizing the advantages of the repurposing approach. Although drug repurposing approach involves a significant level of challenge, affordability and faster discovery pipeline outweighs the risks in the event of emergency situations like the current COVID-19 pandemic. This chapter begins with a brief summary of the advantages of the drug repurposing pipeline followed by an update on the status of drug repurposing in developing effective anti-viral therapeutics against COVID-19.

19.2 Advantages of Drug Repurposing

The emphasis of conventional drug discovery is to treat new and chronic diseases, while repurposing or repositioning of drugs is an approach to discover methods for already available and approved drugs that can be useful for the development of rapidly emerging and re-emerging infectious diseases and many other neglected diseases (Pushpakom et al. 2019). In spite of immense knowledge of the disease and advancements in technology, translating this into welfare of drug development is slower than expected. New drugs, for the treatment of any disease, need to abide stringent regulations before entering the market. De novo drug approval process takes up to 10–15 years for the development and this process is extremely expensive and time consuming (Pushpakom et al. 2019). Drug repurposing is one solution which holds the promise of immediate therapeutic impact at much lower expense than *de novo* drug for diseases (Parvathaneni et al. 2019; Corsello et al. 2017). The present expensive and cumbersome process of drug discovery is not well equipped to combat rapid evolving infections such as avian flu, West Nile virus, Zika virus, SARS-CoV-2, etc. Also, various auto-immune diseases, bacterial and rare disorders are not inherited and because of their idiopathic nature their treatment becomes more difficult.

Nobel laureate Sir James Black remarkably stated that ‘The most fruitful basis for the discovery of a new drug is to start with an old drug’ (Raju 1999). Repurposing of the existing available drugs is attractive and pragmatic and has various advantages over development of new drug molecule (Fig. 19.1) (Jourdan et al. 2020). The cost of development for the de novo drugs is reduced in drug repurposing as they have already passed phases such as clinical trials and toxicity tests, which provides a major advantage for drug reprofiling over conventional drug development method (Pushpakom et al. 2019). Repurposed drugs are at much lower risks as they have been found to be harmless in preclinical and human trials so the probability of failing in the safety point of view in subsequent efficacy trials is less (Pushpakom et al. 2019). The development of de novo drug through conventional method will cost

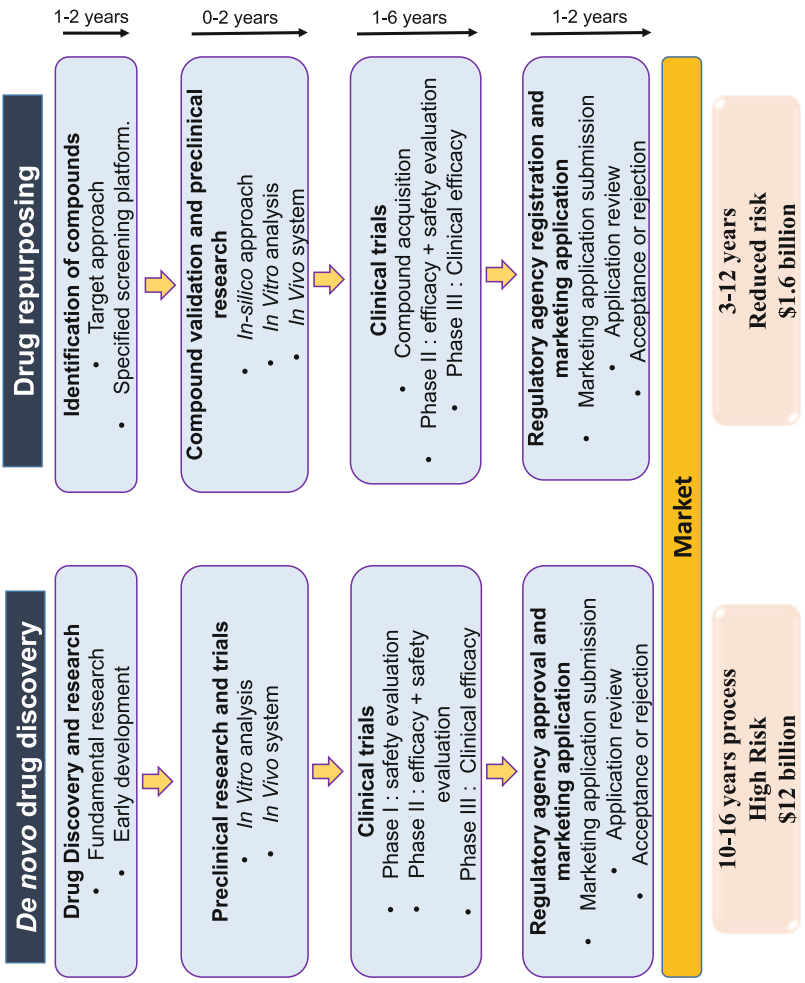


Fig. 19.1 Schematic representation of advantages of drug repurposing over conventional drug discovery approach

around $12 billion, while the costing of new drug using drug repurposing method will reduce to around $1.6 billion (Badria 2020). For the drug repurposing, the information of the drug, their safety profile and clinical efficacy are readily available from their initial trials of drug development. This will reduce the risk of failure associated with the new drugs in their earlier stage of development, which usually occur in the conventional approach. Furthermore, repurposed drug can be available in market within 3–12 years, which is less than a new drug. There are more than 3000 drugs approved and available in various countries, which signifies the availability of vast unexploited resource and that can be widely used for the treatment of various rare diseases (Mele et al. 2015).

Now governments worldwide have started to invest in drug repurposing like National Centre for Advancing Translational Sciences (NCATS) of USA has introduced Discovering New Therapeutic Uses for Existing Molecules Programme to enhance and develop cures and treatments for the disease by investigating already available drugs (Langedijk et al. 2015). NCATS has also collaborated with C-Path and U.S. FDA (Food and Drug Administration) to initiate CURE Drug Repurposing Collaboratory (CDRC), a public–private partnership, for the assortment of available world clinical data to accelerate repositioning of drugs (Yong and D'Cruz 2008). Similarly, the OPEN act (Orphan Product Extension Now Accelerating Cures and Treatment Act of 2015) was re-established for use of repurposed drugs for rare diseases (Volberding et al. 1990). European Medicines Agency (EMA) and the Heads of Medicine Agencies (HMA) of Europe have also launched pilot project to support drug repositioning. British Medical Research Centre (MRC) and consortium pharmaceutical companies have made accessible around 70 drugs for repositioning, which were deprioritized earlier due to their sub-optimal efficacy in clinical trials (Elder and Tindall 2020). These initiatives indicate the importance of drug repurposing in modern world of medicine and treatment.

Many drugs are now approved for indications other than what it was initially intended for. Some examples of successful drug repurposing are azidothymidine which was unsuccessful for the chemotherapy purpose but later on it was used in the treatment of human immunodeficiency virus (Volberding et al. 1990); mycophenolate mofetil was originally used for the organ transplant, later it has been used for the treatment of lupus nephritis (Yong and D'Cruz 2008). Successful repurposed drugs have paved path to emergence of more repurposing of drugs. Another examples of repurposed drugs are sildenafil, a phosphodiesterase-5 inhibitor (PDE5) initially developed for the treatment of coronary artery disease which was then repurposed for the treatment of erectile dysfunction. Canakinumab is a drug previously used for the rheumatoid arthritis and has been repurposed for the treatment of Muckle–Wells syndrome (Roessler et al. 2021). Some more examples of the repurposed drugs are listed in Table 19.1. Such repurposed drugs are becoming increasingly available in the market and perhaps will help in finding a treatment in a time efficient manner.

Table 19.1 Notable examples of repurposed drugs

Drug name	Original indication	Repurposed indication	Year of approval for repurposed use
Bupropion	Depression	Smoking cessation	1997 (US) and 2000 (UK)
Thalidomide	Nausea	Leprosy	1964 (US)
Sildenafil	Angina	Erectile dysfunction Pulmonary arterial hypertension	1998 (US) 2005 (US)
Zidovudine	Cancer	HIV/AIDS	1987 (US)
Bromocriptine	Parkinson	Type-2 diabetes	2009 (US)
Rituximab	Cancer	Rheumatoid arthritis	2006 (US)
Finasteride	Benign prostatic hyperplasia	Androgenetic alopecia	1997 (US)
Galantamine	Polio and paralysis	Alzheimer	2004 (US)
Imatinib	Chronic myelogenous leukaemia	Gastrointestinal tumours	2001 (US)
Raloxifene	Osteoporosis	Breast cancer	2007 (US)
Duloxetine	Depression	Chronic musculoskeletal pain	2010 (US)
Sunitinib	Gastrointestinal stromal tumours	Pancreatic cancer Recurrent renal cell carcinoma	2011 (US) 2017 (US)
Cycloserine	Urinary tract infection	Tuberculosis	2011 (US)
Crizotinib	Lymphoma	NSCLC	2016 (US)
Atomoxetine	Parkinson	ADHD	2008 (US)

19.3 Drug Repurposing for COVID-19 Treatment

COVID-19 originated in China in December 2019 and rapidly spread across the globe, resulting in a pandemic that caused high burden on hospitals and failure of the health care system. The disease took millions of lives and caused unprecedented damage to the global socio-economic dynamics (W.H.O. 2022). Scientists and clinicians across the globe have been working relentlessly to develop potent vaccines and therapeutics against the disease and significant success have been achieved in a very short time span. Nevertheless, SARS-CoV-2, the virus responsible for COVID-19, continues to evolve and still pose a prominent threat for human health. A clear understanding of the viral RNA structure, proteins encoded by the virus, the life cycle of the virus and its pathogenesis mechanism are vital for identifying suitable targets for antiviral development.

SARS-CoV-2 is a positive strand RNA virus. After entry into the cells by a receptor-dependent process, viral genomic RNA is translated by the cellular translation machinery to produce the viral nonstructural polyprotein, which is essential for its replication. The polyprotein is processed into individual functional proteins by its proteolytic cleavage by the virus-encoded proteases. SARS-CoV-2 encodes two proteases, namely, papain-like protease (PLpro) and the chymotrypsin-like main

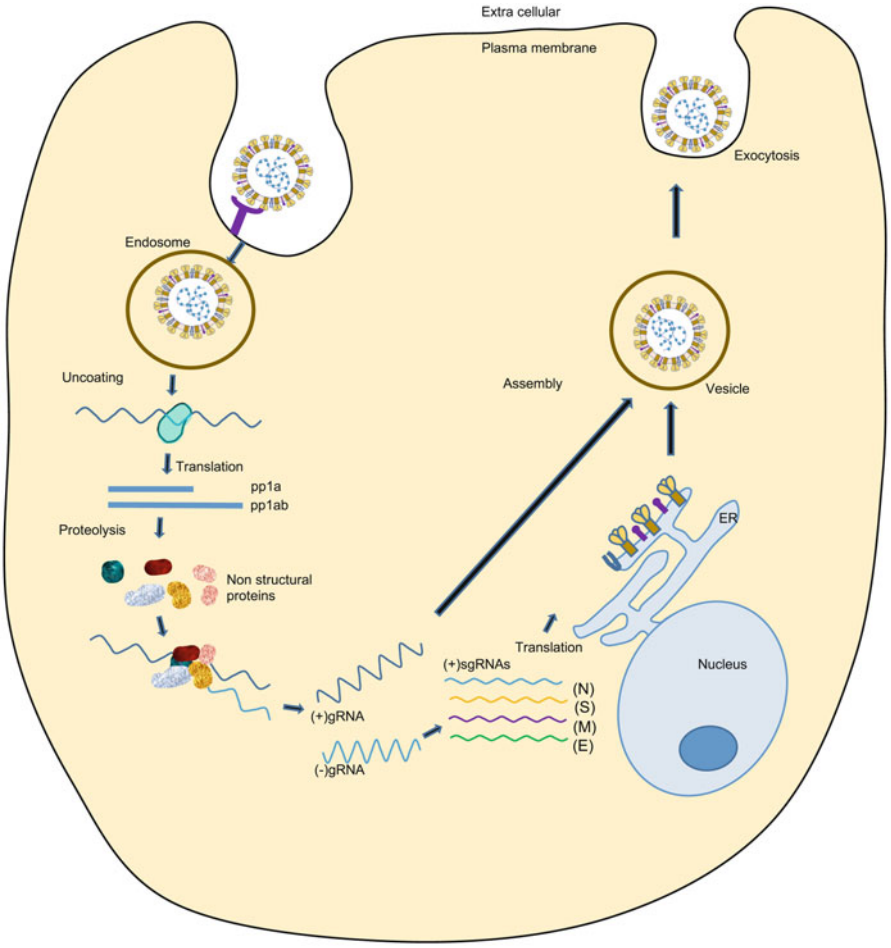


Fig. 19.2 Proposed life cycle of the SARS-CoV-2

protease (Mpro). Mpro plays a major role in polyprotein processing. The nonstructural proteins include the viral RNA-dependent RNA polymerase (RdRp), which initiates the assembly of a multi-protein complex consisting of both viral and cellular proteins that mediate replication of the viral genome. Subsequently, viral sub-genomic RNAs are synthesized through a discontinuous transcription process. These sub-genomic RNAs are translated to produce the structural proteins, which mediate assembly of progeny viruses. Progeny viruses exit the infected cells to spread the infection (V'kovski et al. 2021). A summary of SARS-CoV-2 life cycle is illustrated in Fig. 19.2.

COVID-19 disease symptoms are generated by a combination of cytopathic effects of the virus-encoded factors and defence response of the host to the viral

infection. Successful treatment of COVID-19 needs to involve strategies to reduce the viral infection and spread as well as minimize hyper reactivation of the host defence pathways. Broadly speaking, following three categories of drugs are being investigated as therapeutic options against the COVID-19.

(a) First category of drugs is directed at the viral-encoded proteins. Since each stage in the viral life cycle involves multiple steps and one or more viral proteins are central players of that particular stage, they are important targets for development of virus-specific antivirals, also called as direct acting antivirals (DAA). Antivirals targeting the viral factors are the first category of drugs for COVID-19 therapy. Though DAAs are considered safe due to their specificity towards the viral targets and non-interference with the host processes, a key limitation of DAAs is evolution of drug-resistant variants of the virus (Pawlotsky 2011).
(b) Second category of antivirals targets the cross-talk between the virus and its host. Since viruses entirely depend upon the host machinery for their survival and spread, host–virus interactions are crucial determinants of success of the virus. Antivirals that block essential virus–host interactions are potential therapeutic options (Gordon et al. 2020; Baggen et al. 2021). However, they may have safety and toxicity issue or cause undesired side effects that need to be evaluated by rigorous testing and trials (Lin and Gallay 2013).
(c) Third category of drugs plays roles in alleviating the COVID-19 disease symptoms by directly/indirectly targeting the host factors/processes/pathways. These drugs include immune modulators, tissue remodellers, anti-inflammatory, pain relievers, etc. These are not antivirals *per se*, but they help in improvement of health of the patient. Hence, they are also preferred choice for patient management (Samuel et al. 2021; Recpvery Collaborative Group 2021).

19.4 Drug Repurposing Pipeline for COVID-19 Therapy

Both wet and dry lab research have been followed to identify repurposed drugs for COVID-19 therapy that acts by targeting either the virus-encoded factors or by blocking the virus–host cross-talk. As illustrated in Fig. 19.3 and summarized below, the drug repurposing pipeline involves multiple modern biotechnology and computational and mathematical biology tools.

19.4.1 Wet Lab-Based Research

As mentioned in previous sections, a thorough knowledge of life cycle of the virus and its pathogenesis mechanism is key to antiviral development. Wet lab studies involving cell-based models of the virus infection, small animal models showing the virus-induced disease phenotypes and direct analysis of virus-infected patient samples provide valuable crucial information regarding different stages of the viral life

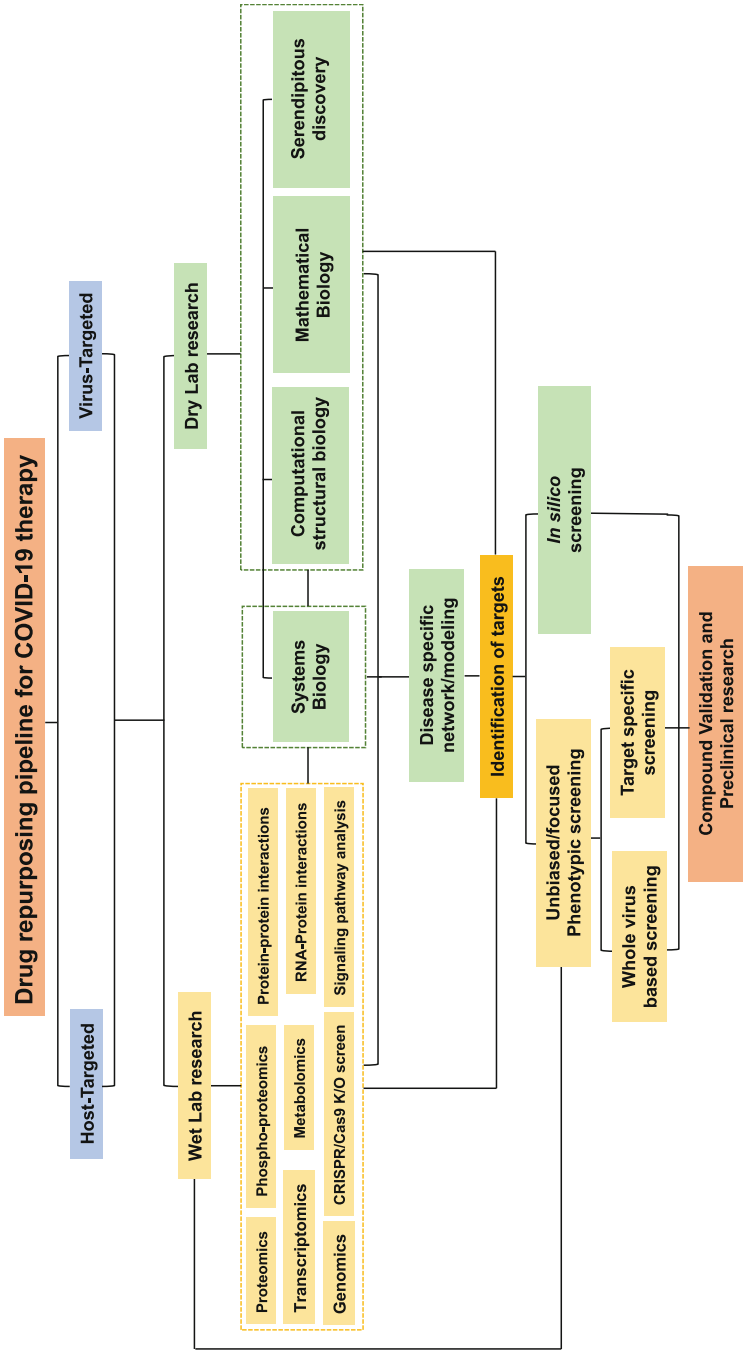


Fig. 19.3 Drug repurposing pipeline for COVID-19 therapy

cycle and pathogenesis mechanism (Blanco-Melo et al. 2020; Shen et al. 2020; Xiong et al. 2020). Emergence of powerful proteomics and genomics technologies has allowed researchers to perform time course kinetics studies of virus infection-induced alteration in global proteome, phospho-proteome, metabolome, transcriptome, etc. (Bouhaddou et al. 2020; Migaud et al. 2020; Liu et al. 2021). Further, as SARS-CoV-2 is a positive strand RNA virus, omics technologies have been widely used to identify the intra-viral as well as virus–host protein–protein interactome and RNA–protein interactomes (Gordon et al. 2020; Schmidt et al. 2021; Verma et al. 2021). Additionally, genome-wide CPRISR/CAS9 K/O screens have been carried out to identify key host factors required for infection and replication of the virus (Hoffmann et al. 2021a). Several independent studies have also been performed to independently measure the perturbations in major cellular signalling pathways upon SARS-CoV-2 infections (Perfetto et al. 2021). All these studies have directly contributed to identification of new targets for drug repurposing studies. Moreover, the datasets identified through omics technologies have been used by systems biologists as startup resources for performing integrative omics-based drug repurposing studies.

19.4.2 Dry Lab-Based Research

With the availability of powerful computational and informatics tools and establishment of public repositories that allow uninterrupted access to multiple data sets, there is increased use of dry lab approaches for proposing a hypothesis and initial hit screening for drug repurposing studies. As illustrated in Fig. 19.3, drug repurposing by dry lab research can be classified into four major areas: (a) Systems biology, (b) Computational structural biology, (c) Mathematical biology and (d) Serendipitous discovery.

19.4.2.1 Systems Biology

Systems biology refers to analysis and modelling of complex biological systems using in silico or wet lab experiment-derived data sets. By integrating multiple independent observations related to the biological systems in question using mathematical algorithms built into the computational programmes, systems biology is capable of generating novel insights about the system, which is not possible by applying the tools of pure biology. For example protein–protein interactions or RNA–protein interactions of SARS-CoV-2-infected samples will demonstrate the profile of interaction among those proteins or RNA. Similarly, transcriptome analysis of same samples will only represent the change in gene expression, and metabolome analysis will represent the profile of metabolites in infected samples. It will not be possible to estimate gene expression profile from interactome/metabolome data or link these three independent observations using the

conventional techniques used in biological research. Systems biology tools allow scientists to integratively analyse these data sets so that a correlation between interactome, transcriptome and metabolome can be established. This not only improves the confidence of the output data and provides mechanistic insight but also identifies key targets for designing potent therapeutic intervention strategies.

For drug repurposing studies, available information related to drugs such as pharmacological, chemical, biomedical and genomics information is taken into consideration. Genomic information refers to global gene expression profile in response to a disease and/or treatment with a drug. A widely used systems biology approach in drug repurposing studies employs connectivity map (cMap) and its extended library of Integrated Network-based Cellular Signatures (LINCS) (Lotfi Shahreza et al. 2018). cMap represents a combination of genome-wide transcriptome data that helps in decoding the connections between genes, drugs and diseases (Lotfi Shahreza et al. 2018). In addition, genome-wide association studies (GWAS), chemical structure-focused weighted ensemble similarity (WES) algorithm and drug molecule-proteome signature-based 'computational analysis of novel drug opportunities' (CANDO) platform have been used in drug repurposing studies (Jarada et al. 2020). Automated tools developed for data mining and text mining, combined with machine learning protocols, have further advanced the unique benefits of systems biology approaches in drug repurposing studies (Tayara et al. 2021; Beck et al. 2020). An elegant study by Tomazou *et al.* utilized integrative omics approach to identity repurposed drugs against COVID-19 (Tomazou et al. 2021). In addition to identifying remdesivir and dexamethasone, which are already used for COVID-19 therapy, the authors identified several new repurposed drug candidates such as src-Tyrosine kinase inhibitors (bosutinib, dasatinib, cytarabine and saracatinib), anti-inflammatory drugs (dactolisib and methotrexate) and histone deactylase inhibitors (hydroquinone and varinostat). Some of these drugs are in clinical trials for COVID-19 therapy (Table 19.2).

19.4.2.2 Computational Structural Biology

Computational structural biology (CSB) refers to use of computational biology tools to decipher the three-dimensional (3D) structure of biological molecules in their functionally active state (Fetrow and Babbitt 2018; Nussinov et al. 2019). Since biological molecules usually do not function as static 3D structure, an important aspect of computational structural studies involves generation and analysis of multiple interconverting states of the molecule to evaluate its function. Molecular docking studies are performed to unravel the molecular details of protein–protein interactions (PPIs), RNA–protein interactions (RPIs), protein–drug interaction and RNA–drug interaction. Free energy landscape and conformational stability are important parameters while performing docking studies to measure PPIs, RPIs and drug screening. The advantage of CSB in drug repurposing is attributed to simultaneous analysis and optimization of 3D structure of the drug molecule, which significantly expedites the optimization process. However, CSB approach has

Table 19.2 Repurposed drugs undergoing clinical trials for COVID-19 therapy

Lead molecule/ drug name	Original use	Target/observed therapeutic benefits in COVID-19	No. of phase III clinical trials	Reference
(A) Drugs that show antiviral activity by targeting viral proteins				
Amantadine	Influenza A virus	Inhibits ion channel activity of envelope and ORF10 proteins of SARS-CoV-2	3	Toft-Bertelsen et al. (2021)
Andrographolide	Cold and flu symptoms, immune booster	Viral main protease (Mpro) inhibitor	1	Enmozhi et al. (2021)
AT-527	Flaviviruses	Viral RNA-dependent RNA polymerase (RdRp) inhibitor	1	Manfredonia et al. (2020)
Atazanavir	Human immunodeficiency virus (HIV)	Viral main protease (Mpro) inhibitor	2	Chaves et al. (2021)
Ciclesonide	Asthma, allergic rhinitis	Viral replication inhibitor	4	Matsuyama et al. (2020)
Daclatasvir	Hepatitis C virus	Viral papain lke protease (PLpro) inhibitor	2	Sacramento et al. (2021)
Enisamium iodide	Influenza A virus	Viral RNA-dependent RNA polymerase (RdRp) inhibitor	1	Walker et al. (2020)
Ingavirin	Ion channel modulator	Viral RNA-dependent RNA polymerase (RdRp) inhibitor	1	Kouznetsov (2020)
Isavuconazonium	Anti-fungal	Viral main protease (Mpro) inhibitor	1	Achilonu et al. (2020)
Ledipasvir	Hepatitis C virus	Viral RNA-dependent RNA polymerase (RdRp) inhibitor	1	Pirzada (2021)
Leflunomide	Dihydroorotate dehydrogenase (DHODH) inhibitor	Viral replication inhibitor, inhibitor of cytokine release and inflammation	1	Hu et al. (2020)
Lopinavir/ ritonavir	Human immunodeficiency virus (HIV)	Viral papain lke protease (PLpro) inhibitor	34	Yeh et al. (2006)
Sitagliptin	Type-2 Diabetes	Viral papain lke protease (PLpro) inhibitor	1	Narayanan et al. (2022)
Sofosbuvir	Hepatitis C virus	Viral RNA-dependent RNA polymerase (RdRp) inhibitor	6	Sacramento et al. (2021)

(continued)

Table 19.2 (continued)

Lead molecule/ drug name	Original use	Target/observed therapeutic benefits in COVID-19	No. of phase III clinical trials	Reference
Tenofovir/ emtricitabine	Human immunodeficiency virus (HIV)	Viral RNA-dependent RNA polymerase (RdRp) inhibitor	6	Parienti et al. (2021)
Triazavirin	Broad spectrum antiviral	Viral papain lke protease (PLpro) inhibitor	1	Hudecova (2021)
(b) Drugs that show antiviral activity by targeting the host				
Artemisinin	Malaria	Antiviral activity against SARS-CoV-2	2	Cao et al. (2020)
Camostat mesylate	Chronic pancreatitis	Antiviral activity against SARS-CoV-2	7	Hoffmann et al. (2021b)
Captopril	Hypertension and heart failure	Antiviral activity against SARS-CoV-2	2	Chatterjee and Thakur (2020)
Carrimycin	Acute tracheal-bronchitis	Antiviral activity against SARS-CoV-2	1	Yan et al. (2021)
Chloropromazine	Antipsychotic	Antiviral activity against SARS-CoV-2	2	Sohn et al. (2021), Stip et al. (2020)
Cyproheptadine	Antiallergic	Antiviral activity against SARS-CoV-2	4	Qu et al. (2021)
IMU-838	Autoimmune diseases	Antiviral activity against SARS-CoV-2	1	Hahn et al. (2020)
Interferon beta-1a	Antiviral signaling activator	Antiviral activity against SARS-CoV-2	6	Bosi et al. (2020)
Isotretinoin	13-cis-retinoic acid	Antiviral activity against SARS-CoV-2	2	Baggen et al. (2021)
Ketotifen	Atopic asthma and allergic conjunctivitis	Antiviral activity against SARS-CoV-2	1	Kiani et al. (2021)
Lactoferrin	Immune modulator and maintain iron home	Antiviral activity against SARS-CoV-2	4	Salaris et al. (2021)
Loratadine	Atopic asthma and allergic conjunctivitis	Antiviral activity against SARS-CoV-2	2	Hou et al. (2021)
Lumefantrine	Malaria	Antiviral activity against SARS-CoV-2	1	Gendrot et al. (2020)
Mefloquine	Malaria	Antiviral activity against SARS-CoV-2	3	Xiao et al. (2020)
Nafamostat	TMPRSS2 inhibitor	Antiviral activity against SARS-CoV-2	4	Baggen et al. (2021)
Niclosamide	Inhibition of endocytosis	Antiviral activity against SARS-CoV-2	3	Weiss et al. (2021)

Novaferon	Interferon a2b, chronic heptitis B treatment	Antiviral activity against SARS-CoV-2	4	Zheng et al. (2020)
Panduratin A	Anti-oxidant	Antiviral activity against SARS-CoV-2	1	Kanjanasirirat et al. (2020)
Peginterferon lambda-1A	Antiviral signaling activator	Antiviral activity against SARS-CoV-2	2	Jagannathan et al. (2021)
Proxalutamide	Androgen receptor antagonist	Antiviral activity against SARS-CoV-2	5	McCoy et al. (2021)
Pyronaridine artesunate	Malaria	Antiviral activity against SARS-CoV-2	1	Smail et al. (2021)
Raloxifene	Selective estrogen receptor modulator (SERAMnt)	Antiviral activity against SARS-CoV-2	1	Xiao et al. (2020)
Rapamycin/ sirolimus	mTOR Inhibitor	Antiviral activity against SARS-CoV-2	1	Bouhaddou et al. (2020)
Ribavirin	Hepatitis C virus	Antiviral activity against SARS-CoV-3	4	Unal et al. (2021)
Ruxolitinib	JAK/STAT inhibitor	Antiviral activity against SARS-CoV-2	2	Kouznetsov (2020)
Silymarin	Antioxidant, anti-inflammatory	Antiviral activity against SARS-CoV-2	1	Palit et al. (2021)
Spironolactone	Aldosterone receptor antagonist	Antiviral activity against SARS-CoV-2	2	Cadegiani (2020)
Verapamil/ amiodarone	Antiarrhythmic, ebola disease	Antiviral activity against SARS-CoV-2	1	Aherfi et al. (2021)
(C) Drugs that act indirectly and reduce disease severity				
Acalabrutinib	Non-Hodgkin lymphoma	Improvement in disease symptoms	1	Bianconi et al. (2020)
Acetylsalicylic acid	Pain, and inflammation	Improvement in disease symptoms	6	Panossian and Brendler (2020)
ADAPT-232	Respiratory infections and convalescence	Improvement in disease symptoms	1	Karosanidze et al. (2022), Sanchis-Gomar et al. (2020)
Amiodarone	Anti-arrhythmic	Improvement in disease symptoms	1	Barnes et al. (2020)
Anakinra	Recombinant human interleukin 1 receptor antagonist	Improvement in disease symptoms	5	Schoot et al. (2020), Bridgewater (2020)
Atorvastatin	Anti-cholesesteremic	Improvement in disease symptoms	7	Tomazou et al. (2021)
Brensocatib	Chronic obstructive pulmonary disease	Improvement in disease symptoms	1	Khanna et al. (2020)
Bucillamine	Anti-rheumatic	Improvement in disease symptoms	1	Tornling et al. (2021)

(continued)

Table 19.2 (continued)

Lead molecule/ drug name	Original use	Target/observed therapeutic benefits in COVID-19	No. of phase III clinical trials	Reference
C21	Idiopathic pulmonary fibrosis	Improvement in disease symptoms	1	Song et al. (2022)
CD24Fc	Anti-inflammatory	Improvement in disease symptoms	1	Yousefi et al. (2021)
Cromolyn sodium	Anti-allergic	Improvement in disease symptoms	1	Cure and Cure (2020)
Dapagliflozin	Type-2 diabetes	Improvement in disease symptoms	2	Kanwar et al. (2021)
Dapsone	Immune thrombocytopenia	Improvement in disease symptoms	1	Liu et al. (2020a)
Darunavir	HIV protease inhibitor	Improvement in disease symptoms	3	Yeh et al. (2006)
Dipyridamole	Reduces the concentration of D-dimers	Improvement in disease symptoms	1	Earhart (2020)
Dornase alfa	Reduces neutrophil extracellular traps (NETs)	Improvement in disease symptoms	2	Cadegiani (2020)
Dutasteride	Anti-androgen	Improvement in disease symptoms	1	Olivera et al. (2020)
Endoxaban	Anti-coagulant (Factor Xa inhibitor)	Improvement in disease symptoms	2	Drago et al. (2020)
Enoxaparin	Anti-thromboembolic	Improvement in disease symptoms	17	Pharm J (n.d.)
ESCIN	Anti-edematous, anti-inflammatory, venotonic	Improvement in disease symptoms	1	Alimova et al. (2020)
Fostamatinib	Chronic immune thrombocytopenia (tyrosine kinase inhibitor)	Improvement in disease symptoms	2	Hippensteel et al. (2020)
Heparin	Anti-coagulant	Improvement in disease symptoms	28	Berger et al. (2020)
Icosapent ethyl	Anti-lipemic	Improvement in disease symptoms	1	Sun et al. (2021)
Imatinib	Anti-leukemic (tyrosine kinase inhibitor)	Improvement in disease symptoms	2	Aigner (2020)
Leukotriene receptor antagonist	Anti-inflammatory	Improvement in disease symptoms	3	Al-Kuraishy et al. (2021)
Levamisole	Anti-helminthic	Improvement in disease symptoms	3	Abuhasira et al. (2021)
Linagliptin	Type-2 diabetes	Improvement in disease symptoms	1	Samuel et al. (2021)
Metformin	Type-2 diabetes	Improvement in disease symptoms	2	Poe and Corn (2020)

N-acetylcysteine	Anti-oxidant	Improvement in disease symptoms	4	Costanzo et al. (2020)
Oseltamivir	Influenza treatment	Improvement in disease symptoms	12	Sen (2020)
Plitidepsin	Anti-cancer, EEF1A1 inhibitor	Improvement in disease symptoms	1	Kiani et al. (2021)
Prolectin-M	Anti-emetic, galectin antagonist	Improvement in disease symptoms	1	Milan, Italy and San Mateo (2021)
Reparixin	Anti-cancer, CXC chemokine receptor types 1 (CXCR1) and 2 (CXCR2) inhibitor	Improvement in disease symptoms	2	Szolnoky (2020)
Sulodexide	Peripheral arterial thrombosis and venous thrombosis	Improvement in disease symptoms	1	Liu et al. (2020b)
Tacrolimus	Immunosupressant, calcineurin inhibitor	Improvement in disease symptoms	1	Bridgewater (2020)
Thymosin-a-1	Hepatitis B and C treatment, immuno modulator	Improvement in disease symptoms	1	Favalli (2020)
Tofacitinib	Rheumatoid arthritis, psoriatic arthritis, and ulcerative colitis	Improvement in disease symptoms	1	Materiamedica (2020)
Ergoferon	Antiviral complex drug containing released-active forms of antibodies to interferon gamma, CD4, and histamine	Improvement in disease symptoms	1	Nakazono et al. (2021)
Fluticasone propionate	Corticosteroid	Improvement in disease symptoms	2	Shekhar et al. (2022)
Indomethacin	Nonsteroidal anti-inflammatory drug, used in inflammation, pain	Improvement in disease symptoms	1	Zheng et al. (2020)

inherent limitations such as known 3D structure of biological molecules and drugs are not readily available, computational resources might be limiting to run all analyses and molecular docking have high false-positive rates (Macip et al. 2022).

19.4.2.3 Mathematical Biology

Mathematical modelling has been used to interrogate biological systems to answer specific questions in healthy or diseased state. Mathematical modelling relies on a combination of different types of differential equations, Petrinet agent-based models, enrichment analysis and centrality analysis to decipher an output. For example, ordinary differential equations (ODE) and delay differential equations (DDE) are commonly used in mathematical biology to represent biological phenomena in steady state or upon infection by a pathogen, respectively, over a period of time, with different rate constants and with or without time delays (Sarmah et al. 2021). Note that disease modelling in mathematical biology does not depend on high-throughput omics data. It only requires information of the disease dynamics to establish and analyse the model. A major limitation of the mathematical biology approach concerns its ability to analyse only a part of the system, whereas systems biology is capable of analysing the entire system.

19.4.2.4 Serendipitous Discovery

Drug repurposing studies have been conducted on the basis of serendipitous observations of the hallmarks of the new disease, either in a clinical setup or by proposing a hypothesis of the disease dynamics and expected therapeutic benefit of the repurposed drug on the basis of prior knowledge. These repurposed drugs may be evaluated in vitro, followed by clinical trial or undergo direct clinical trial in the context of the new disease. Many of the repurposed drugs used in COVID-19 therapy such as paracetamols, antibiotics, zinc, corticosteroids, etc. may be assigned to this category.

19.4.2.5 Screening of Repurposed Drugs

Apart from in silico screening, unbiased/focused phenotypic screening has been widely used for identifying antivirals against the SARS-CoV-2 (Jeon et al. 2020; Chen et al. 2021). Target-specific screening has been conducted using FDA-approved repurposed drug libraries to identify inhibitors of viral proteins such as the RdRp and the proteases (PL-pro, M-pro) and inhibitors of host proteins such as TMPRSS2. These strategies have identified several candidate molecules, which are under investigation. Several reports have also been published where drug repurposing screening was performed using the cell-based infection model of the SARS-CoV-2. Collectively, these studies have identified several lead molecules, which are being evaluated in different stages of pre-clinical and clinical trials.

19.5 Current Status of Drug Repurposing for COVID-19 Therapy

19.5.1 Drugs That Show Antiviral Activity by Targeting Viral Proteins

Based on the knowledge generated by years of research on other RNA viruses such as the hepatitis C virus, Ebola virus, influenza virus, severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS) CoVs, it was possible to predict the importance of viral proteases (Papain-like protease and main protease) and RNA-dependent RNA polymerase (RdRp) in the life cycle of SARS-CoV-2. Subsequently, drug repurposing studies were undertaken to identify the inhibitors of these enzymes and their therapeutic protentional was evaluated (Beck et al. 2020). Drugs used in COVID-19 treatment such as remdesivir, favipiravir and molnupiravir belong to the category of RdRp inhibitors (Table 19.3). Note that remdesivir was originally identified as an inhibitor of RdRp of the Ebola virus, and molnupiravir and favipiravir were originally identified as inhibitors of RdRp of the influenza virus (Agrawal et al. 2020; Kokic et al. 2021; Jayk Bernal et al. 2022). Additionally, 6 more RdRp-inhibitor drug candidates are being evaluated in phase 3 clinical trials (Table 19.2). Some of these drugs were

Table 19.3 Drugs approved for use in COVID-19 treatment

Drugs	Original use	Observed therapeutic benefits in COVID-19	Reference
Amlodipine	Hypertension	Improvement in disease symptoms	Zhang et al. (2020)
Baricitinib	Rheumatoid arthritis	Improvement in disease symptoms	Ely et al. (2022)
Famotidine	Acidity and gastro-esophageal reflux	Improvement in disease symptoms	Freedberg and Famotidine Research Group (2020)
Favipiravir	Influenza	Inhibitor of SARS-CoV-2 replication	Agrawal et al. (2020)
Fluvoxamine	Obsessive-compulsive disorder (OCD)	Improvement in disease symptoms	Sukhatme et al. (2021)
Losartan	Hypertension	Improvement in disease symptoms	Yan et al. (2020)
Merimepodib	Broad spectrum antiviral	Inhibitor of SARS-CoV-2 replication	Evans and Liu (2021)
Molnupiravir	Influenza	Inhibitor of SARS-CoV-2 replication	Jayk Bernal et al. (2022)
Nirmatrelvir/ Ritonavir[a]	Human immunodeficiency virus (HIV)	Inhibitor of SARS-CoV-2 main protease (Mpro)	Hammond et al. (2022)
Remdesivir	Ebola	Inhibitor of SARS-CoV-2 replication	Kokic et al. (2021)

[a]Ritonavir has been shown to inhibit HIV protease. Nirmatrelvir is not repurposed

already known to inhibit the RdRp of other RNA viruses. For example, sofosbuvir, a direct acting antiviral originally used against the HCV is being evaluated in phase 3 clinical trial for COVID-19 treatment (Sayad et al. 2020). AT-527 and enisamium iodide inhibit flavivirus and influenza A virus RdRp, respectively (Manfredonia et al. 2020; Walker et al. 2020). Among the viral protease inhibitors, nirmatrelvir/ritonavir combination has been approved for COVID-19 treatment (Table 19.3). Ritonavir was originally identified as an inhibitor of HIV protease. It also binds to and inhibits CYP3A4 enzyme in the liver, which metabolizes other protease inhibitors. Therefore, its combination with other protease inhibitors (such as nirmatrelvir) improves the overall antiviral activity of the drug (Yeh et al. 2006; Hammond et al. 2022). Apart from that, seven more SARS-CoV-2 protease inhibitors are being evaluated in phase 3 clinical trials for COVID-19 therapy (Table 19.2). Another drug under phase 3 clinical trial, named amantadine, shows antiviral activity against the SARS-CoV-2 by inhibiting the ion channel activity (viroporin function) of the viral envelope (E) and ORF 10 proteins (Table 19.2; Toft-Bertelsen et al. 2021). Note that viroporins are important players in the life cycle of many viruses, including the SARS-CoV-2. Hence, inhibition of viroporin is supposed to be an effective therapeutic strategy. Drugs specifically targeting the viral proteins are generally considered safer as they do not interfere with the host cellular processes. However, these drugs also cause stringent selection pressure on the virus, leading to emergence of new variants of virus that is resistant to such drugs. Recent studies have also demonstrated the feasibility of targeting viral RNA structures for antiviral development. Notably, amilorides were found to inhibit the enterovirus-71 replication by targeting its internal ribosome entry site (IRES) (Davila-Calderon et al. 2020). Elegant studies reported by Zafferani et al. and Manfredria et al. have mapped the RNA structures in SARS-CoV2 genome and identified therapeutically relevant elements (Zafferani et al. 2021; Manfredonia et al. 2020). Further, Zafferani et al. demonstrated the ability of amiloride derivatives in inhibiting SARS-CoV-2 replication (Zafferani et al. 2021).

19.5.2 Drugs That Show Antiviral Activity by Targeting the Host

Angiotensin converting enzyme 2 (ACE2) and transmembrane protease serine 2 precursor (TMPRSS2) are the major determinants of cellular entry of SARS-CoV-2 (Evans and Liu 2021). Therefore, drugs targeting these proteins are supposed to be potent antiviral therapeutics. However, there have been concerns of side effects of ACE2 inhibitory drugs for COVID-19 therapy in individuals having hypertension and other cardiovascular indications. More importantly, ACE2 is not inhibited by conventional ACE inhibitors. On the other hand, several TMPRSS2 inhibitors like camostat and nafamostat are being evaluated in phase 3 clinical trial for COVID-19 therapy (Table 19.2).

Host signalling/process-dependent functional activation of essential viral factors or host-dependent direct or indirect interactions among viral factors as well as host–virus interactions are essential for entry and survival of the SARS-Cov-2 inside the host. The activity/interactions *per se* may not be easy to target using the existing drugs. However, it is possible to achieve therapeutic benefit by using drugs that target the function of key intermediates upstream or downstream of that particular pathway/process. Such intervention strategies have been a major focus of drug repurposing approach. Using a combination of omics technologies and systems biology tools, several drug candidates have been identified for possible use in COVID-19 therapy. For example, merimepodib, an inhibitor of the enzyme inosine monophosphate dehydrogenase, is approved for use in COVID-19 therapy (Table 19.3). Inosine monophosphate dehydrogenase (IMPDH) is an essential enzyme in the 'Guanine' biosynthetic pathway and several IMPDH inhibitors such as merimepodib and ribavirin show both antiviral and immunosuppressive effects (Unal et al. 2021). Note that ribavirin is also being evaluated for COVID-19 therapy in phase 3 clinical trial (Table 19.2). Additionally, in vitro studies have identified anti-SARS-CoV-2 activity of many anti-androgens such as proxalutamide and spironolactone (McCoy et al. 2021; Cadegiani 2020). Many studies have also identified in vitro anti-SARS-CoV-2 activity of anti-malaria drugs such as artemisin, mefloquine and lumefantrine, which are being evaluated in phase 3 clinical trials (Cao et al. 2020; Gendrot et al. 2020; Xiao et al. 2020). Table 19.2 summarizes the list of repurposed drugs currently evaluated by phase 3 clinical trials.

19.5.3 Drugs That Act Indirectly by Reducing the Disease Severity

Effective management of COVID-19 disease requires potent inhibition of viral spread as well as control of clinical symptoms associated with the disease. Clinical observation, understanding of the disease biology as well as systems biology-based disease modelling studies have identified several repurposed drug candidates for COVID-19 therapy. Some of the drugs such as baricitinib (Janus kinase 1/2 inhibitor), famotidine (histamine H_2 receptor antagonist), fluvoxamine (serotonin reuptake inhibitor), amlodipine and losartan (anti-hypertension drugs) have been approved for therapeutic use in COVID-19 patients (Table 19.3). Many other drugs are being evaluated in phase 3 clinical trials (Table 19.2). Notably, these drugs include diverse range of formulations such as host immunomodulators, anti-inflammatory molecules, anti-coagulants, pain relievers, antibody/antibody cocktail of host immune modulators and anti-arrhythmic compounds (Table 19.2). It is hoped that completion of these trials should add additional therapeutic drugs for COVID-19 treatment.

19.6 Challenges in Drug Repurposing Against COVID-19

Although drug repurposing has resulted in the availability of multiple therapeutics for COVID-19 treatment, nonetheless, there have been many failures as well. Well-known example includes the anti-malarial drug hydroxychloroquine and the anthelmintic drug ivermectin. Although initial studies reported beneficial effect of both the drugs against SARS-CoV2, lack of noticeable benefit in patients has led to the discontinuation of these drugs in COVID-19 treatment (Devaux et al. 2020; Wang et al. 2020; Saag 2020; Lim et al. 2022). Therefore, it is important to factor in the potential challenges associated with repurposing of any future drug candidate for COVID-19 therapy.

Major challenges in drug repurposing against COVID-19 include sub-optimal in vivo activity of the drug compound, and limited access to compounds and related data.

19.6.1 Sub-optimal In Vivo Activity of the Drug Compounds

Many repurposed drugs show potent antiviral activity in vitro and in pre-clinical studies; however, they fail to deliver the desired therapeutic benefit in patients. Therefore, even though a repurposed drug is safe and approved for clinical use, it needs in-depth experimental validation as well as rigorous clinical trials to ensure its therapeutic benefit against the new disease. Hydroxychloroquine and ivermectin studies illustrate how bypassing the rigorous validation criteria defeats the expected benefits of drug repurposing approach. Failure of a repurposed drug may be attributed to inefficient delivery of the drug compound to the target site, inefficient binding of the compound towards the repurposed targets, inadequate doses of the compound against the new disease or inefficient functional activity of the repurposed drug in vivo. Owing to the extraordinary threat posed by the SARS-CoV-2, a number of laboratories across the globe worked on a war footing step to discover antiviral therapeutics against the virus. This led to identification of a number of potential antiviral candidates, of which more than 80 compounds are being tested in phase 3 clinical trials. Given the ideal conditions employed in the screening of the drug compounds in vitro, it is not surprising that these compounds display potent antiviral activity against SARS-CoV-2 in a controlled experimental setup. However, the real test of their antiviral potential is determined in a randomized clinical trial involving a significant number of participants. Therefore, success in the phase 3 clinical trial is the actual proof of antiviral potency of a repurposed drug. Thus, due emphasis must be given to design and data interpretation of the phase 3 clinical trial to ensure the expected therapeutic benefit of the drug candidate.

At the same time, it is important to note that complete lack of therapeutic benefit or sub-optimal therapeutic effect of a repurposed drug candidate in the clinical trial

should not be treated as the end point of the evaluation of antiviral potential of the drug candidates. Careful analysis of the properties and composition of the drug formulation based on the pharmaceutical and clinical knowledge in the context of its original application vis-a-vis the repurposed application may help in developing a modified drug formulation with the desired therapeutic benefit. However, such modifications need to be evaluated from the pre-clinical study onwards, thereby significantly escalating the time cost of the entire study. It is even more challenging to perform such studies for COVID-19 therapy because of the urgency of the situation and global race for developing a potent antiviral to control the pandemic.

19.6.2 Limited Access to Compounds and Related Data

COVID-19 pandemic has reshaped the conventional research environment across the globe. Scientists across the globe from all disciplines and specializations are committed to and contributing to decode the different areas of SARS-CoV-2 biology and COVID-19 disease. This has resulted in identification of many potential targets for drug development. However, very few laboratories have resources to access large number of compounds for screening against the targets. Vast libraries available in big pharma industries are usually inaccessible to researchers outside the company. Moreover, all data related to the compounds are not available in public domain. Repurposing of a particular target may not be preferred in a pharma company if the repurposed indication is not within their areas of expertise. Therefore, there is a need of providing open access of the compounds and related data for efficient drug repurposing. Recently, collaborations between external researchers and pharma companies have been initiated to promote drug repurposing projects. Notably, pharma majors AstraZeneca, GlaxoSmithKline, Pfizer and Bayer have constituted platforms to allow drug repurposing projects by external researchers.

Another major roadblock in drug repurposing project relates to the intellectual property right of the repurposed molecules (Breckenridge and Jacob 2019). Even though an existing drug may show promising antiviral effect against COVID-19, off-label use of the drug is generally not encouraged by regulatory agencies in the absence of extensive research and clinical trial data. Existing drugs are usually covered by stringent patent clause, which prevents commercial production and use of the drug by companies other than the patent holders or its licensee. Therefore, many repurposed drug candidates do not reach the stage of clinical trial due to lack of funding. Considering the seriousness of COVID-19 pandemic, government and non-profit foundations should sponsor research and development of such promising candidates.

19.7 Conclusion and Future Prospects

As summarized in this chapter and many other reviews, drug repurposing approaches have certainly proven to be the preferred option for developing a potent antiviral against the COVID-19. Repurposed drugs became life saver drugs during the peak of the COVID-19 pandemic, testifying the efficacy of the repurposing approach. It was also learnt that thorough evaluation of the repurposed drugs through large clinical trials is essential to rule out any ambiguity regarding their therapeutic benefits. On-going clinical trials should add more drugs to the list of already existing repurposed drugs approved for therapeutic use in the near future. Further, fast-paced research across the globe has identified a vast resource of potential drug targets against the COVID-19, which should be used in drug repurposing screenings to identify new antiviral candidates. It is important to determine the optimal dose and formulation of these antiviral candidates through rigorous research and clinical trials. Considering the high mutation rate of RNA viruses, it is unlikely that a single therapeutic strategy will be effective for treatment in a long run. A combination of different therapeutic formulations is required to reduce the selection pressure as well as to arrest the disease at different stages. Finally, better policy regarding patenting of repurposed drugs, broader exchange of resources between academic research establishment and industries, better funding opportunities for drug repurposing projects are the need of the hour to expedite the development of potent antivirals against the COVID-19.

References

Abuhasira R et al (2021) A randomized clinical trial of linagliptin vs. standard of care in patients hospitalized with diabetes and COVID-19. Front Endocrinol 12

Achilonu I et al (2020) Targeting the SARS-CoV-2 main protease using FDA-approved Isavuconazonium, a P2-P3 α-ketoamide derivative and Pentagastrin: an in-silico drug discovery approach. J Mol Graph Model 101:107730

Agrawal U et al (2020) Favipiravir: a new and emerging antiviral option in COVID-19. Med J Armed Forces India 76(4):370–376

Aherfi S et al (2021) Drug repurposing against SARS-CoV-1, SARS-CoV-2 and MERS-CoV. Future Microbiol 16(17):1341–1370

Aigner L (2020) The leukotriene receptor antagonist montelukast as a potential COVID-19 therapeutic. Front Mol Biosci 400

Alimova M et al (2020) A high content screen for mucin-1-reducing compounds identifies fostamatinib as a candidate for rapid repurposing for acute lung injury during the COVID-19 pandemic. bioRxiv

Al-Kuraishy et al (2021) Levamisole therapy in COVID-19. Viral Immunol 34(10):722–725

Badria FA (ed) (2020) Drug repurposing: hypothesis, molecular aspects and therapeutic applications. BoD–Books on Demand

Baggen J et al (2021) Cellular host factors for SARS-CoV-2 infection. Nat Microbiol 6(10): 1219–1232

Barnes BJ et al (2020) Targeting potential drivers of COVID-19: neutrophil extracellular traps. J Exp Med 217(6)

Beck BR et al (2020) Predicting commercially available antiviral drugs that may act on the novel coronavirus (SARS-CoV-2) through a drug-target interaction deep learning model. Comput Struct Biotechnol J 18:784–790
Berger AA et al (2020) Icosapent ethyl–A successful treatment for symptomatic COVID-19 infection. Cureus 12(9)
Bianconi V et al (2020) Is acetylsalicylic acid a safe and potentially useful choice for adult patients with COVID-19? Drugs 80(14):1383–1396
Blanco-Melo D et al (2020) Imbalanced host response to SARS-CoV-2 drives development of COVID-19. Cell 181(5):1036–1045
Bosi E et al (2020) Interferon β-1a (IFNβ-1a) in COVID-19 patients (INTERCOP): study protocol for a randomized controlled trial. Trials 21(1):1–11
Bouhaddou M et al (2020) The global phosphorylation landscape of SARS-CoV-2 infection. Cell 182(3):685–712
Breckenridge A, Jacob R (2019) Overcoming the legal and regulatory barriers to drug repurposing. Nat Rev Drug Discov 18:1–2
Bridgewater, N.J., April 23, 2020 /PRNewswire. https://investor.insmed.com/2020-04-23-Brensocatib-Formerly-INS1007-to-be-Studied-in-Patients-with-Severe-COVID-19-in-Investigator-Initiated-Trial
Cadegiani FA (2020) Repurposing existing drugs for COVID-19: an endocrinology perspective. BMC Endocrine Disord 20(1):1–19
Cao R et al (2020) Anti-SARS-CoV-2 potential of artemisinins in vitro. ACS Infect Dis 6(9): 2524–2531
Chatterjee B, Thakur SS (2020) ACE2 as a potential therapeutic target for pandemic COVID-19. RSC Adv 10(65):39808–39813
Chaves OA et al (2021) Atazanavir is a competitive inhibitor of SARS-CoV-2 Mpro, impairing variants replication in vitro and in vivo. Pharmaceuticals 15(1):21
Chen CEZ et al (2021) Drug repurposing screen for compounds inhibiting the cytopathic effect of SARS-CoV-2. Front Pharmacol 11:592737
Corsello SM et al (2017) The drug repurposing hub: a next-generation drug library and information resource. Nat Med 23(4):405–408
Costanzo M et al (2020) SARS-CoV-2: recent reports on antiviral therapies based on lopinavir/ritonavir, darunavir/umifenovir, hydroxychloroquine, remdesivir, favipiravir and other drugs for the treatment of the new coronavirus. Curr Med Chem 27(27):4536–4541
Cure E, Cure MC (2020) Can dapagliflozin have a protective effect against COVID-19 infection? A hypothesis. Diab Metab Syndrome: Clin Res Rev 14(4):405–406
Davila-Calderon J et al (2020) IRES-targeting small molecule inhibits enterovirus 71 replication via allosteric stabilization of a ternary complex. Nat Commun 11(1):4775
Devaux CA et al (2020) New insights on the antiviral effects of chloroquine against coronavirus: what to expect for COVID-19? Int J Antimicrobial Agents 55(5):105938
Drago F et al (2020) Use of enoxaparin to counteract COVID-19 infection and reduce thromboembolic venous complications: a review of the current evidence. Front Pharmacol 1469
Earhart AP (2020) Consideration of dornase alfa for the treatment of severe COVID-19 acute respiratory distress syndrome. New Microbes New Infect 35:100689
Elder D, Tindall S (2020) The many advantages of repurposing existing drugs. Eur Pharm Rev 25(3):34–37
Ely EW et al (2022) Efficacy and safety of baricitinib plus standard of care for the treatment of critically ill hospitalised adults with COVID-19 on invasive mechanical ventilation or extracorporeal membrane oxygenation: an exploratory, randomised, placebo-controlled trial. Lancet Respir Med
Enmozhi SK et al (2021) Andrographolide as a potential inhibitor of SARS-CoV-2 main protease: an in silico approach. J Biomol Struct Dynam 39(9):3092–3098
Evans JP, Liu SL (2021) Role of host factors in SARS-CoV-2 entry. J Biol Chem 297(1):100847

Favalli EG (2020) COVID-19 infection and rheumatoid arthritis: Faraway, so close! Autoimmunity Rev 19(5):102523
Fetrow JS, Babbitt PC (2018) New computational approaches to understanding molecular protein function. PLoS Comput Biol 14(4):e1005756
Freedberg DE, Famotidine Research Group et al (2020) Famotidine use is associated with improved clinical outcomes in hospitalized COVID-19 patients: a propensity score matched retrospective cohort study. Gastroenterology 159(3):1129–1131. e3
Gendrot M et al (2020) Antimalarial artemisinin-based combination therapies (ACT) and COVID-19 in Africa: in vitro inhibition of SARS-CoV-2 replication by mefloquine-artesunate. Int J Infect Dis 99:437–440
Gordon DE et al (2020) A SARS-CoV-2 protein interaction map reveals targets for drug repurposing. Nature 583:459–468
Hahn F et al (2020) IMU-838, a developmental DHODH inhibitor in phase II for autoimmune disease, shows anti-SARS-CoV-2 and broad-spectrum antiviral efficacy in vitro. Viruses 12(12):1394
Hammond J et al (2022) Oral nirmatrelvir for high-risk, nonhospitalized adults with Covid-19. N Engl J Med. https://doi.org/10.1056/NEJMoa2118542
Hippensteel JA et al (2020) Heparin as a therapy for COVID-19: current evidence and future possibilities. Am J Physiol-Lung Cell Mol Physiol 319(2):L211–L217
Hoffmann HH et al (2021a) Functional interrogation of a SARS-CoV-2 host protein interactome identifies unique and shared coronavirus host factors. Cell Host Microbe 29(2):267–280
Hoffmann M et al (2021b) Camostat mesylate inhibits SARS-CoV-2 activation by TMPRSS2-related proteases and its metabolite GBPA exerts antiviral activity. EBioMedicine 65:103255
Hou Y et al (2021) Testing of the inhibitory effects of loratadine and desloratadine on SARS-CoV-2 spike pseudotyped virus viropexis. Chemico-Biol Interact 338:109420
Hu K et al (2020) A small-scale medication of leflunomide as a treatment of COVID-19 in an open-label blank-controlled clinical trial. Virol Sin 35(6):725–733
Hudecova I (2021) Triazavirin might be the new hope to fight Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Triazavirín by mohol byť novovu nádejou v súboji s koronavírusom 2 vyvolávajúcim ťažký akútny respiračný syndróm (SARS-CoV-2). Ceska a Slovenska farmacie: casopis Ceske farmaceuticke spolecnosti a Slovenske farmaceuticke spolecnosti 70(1):18–25
Jagannathan P et al (2021) Peginterferon Lambda-1a for treatment of outpatients with uncomplicated COVID-19: a randomized placebo-controlled trial. Nat Commun 12:1967
Jarada TN et al (2020) A review of computational drug repositioning: strategies, approaches, opportunities, challenges, and directions. J Cheminform 12(1):1–23
Jayk Bernal A et al (2022) Molnupiravir for oral treatment of Covid-19 in nonhospitalized patients. N Engl J Med 386(6):509–520
Jeon S et al (2020) Identification of antiviral drug candidates against SARS-CoV-2 from FDA-approved drugs. Antimicrob Agents Chemother 64(7):e00819–e00820
Jourdan JP et al (2020) Drug repositioning: a brief overview. J Pharm Pharmacol 72(9):1145–1151
Kanjanasirirat P et al (2020) High-content screening of Thai medicinal plants reveals Boesenbergia rotunda extract and its component Panduratin A as anti-SARS-CoV-2 agents. Sci Rep 10(1): 1–12
Kanwar B et al (2021) Specific treatment exists for SARS-CoV-2 ARDS. Vaccine 9(6):635
Karosanidze I et al (2022) Efficacy of adaptogens in patients with long COVID-19: a randomized, quadruple-blind, placebo-controlled trial. Pharmaceuticals 15(3):345
Khanna K et al (2020) Thiol drugs decrease SARS-CoV-2 lung injury in vivo and disrupt SARS-CoV-2 spike complex binding to ACE2 in vitro. bioRxiv
Kiani P et al (2021) In vitro assessment of the antiviral activity of ketotifen, indomethacin and naproxen, alone and in combination, against SARS-CoV-2. Viruses 13(4):558
Kokic G et al (2021) Mechanism of SARS-CoV-2 polymerase stalling by remdesivir. Nat Commun 12:279

Kouznetsov VV (2020) COVID-19 treatment: Much research and testing, but far, few magic bullets against SARS-CoV-2 coronavirus. Eur J Med Chem 203:112647
Langedijk J et al (2015) Drug repositioning and repurposing: terminology and definitions in literature. Drug Discov Today 20(8):1027–1034
Lim SCL et al (2022) Efficacy of ivermectin treatment on disease progression among adults with mild to moderate COVID-19 and comorbidities: the I-TECH randomized clinical trial. JAMA Internal Med 10:1001
Lin K, Gallay P (2013) Curing a viral infection by targeting the host: the example of cyclophilin inhibitors. Antivir Res 99(1):68–77
Liu DY et al (2021) Drug repurposing for COVID-19 treatment by integrating network pharmacology and transcriptomics. Pharmaceutics 13(4):545
Liu X et al (2020a) Potential therapeutic effects of dipyridamole in the severely ill patients with COVID-19. Acta Pharm Sin B 10(7):1205–1215
Liu Y et al (2020b) Thymosin alpha 1 (Tα1) reduces the mortality of severe COVID-19 by restoration of lymphocytopenia and reversion of exhausted T cells. Clin Infect Dis
Lotfi Shahreza M et al (2018) A review of network-based approaches to drug repositioning. Brief Bioinform 19(5):878–892
Macip G et al (2022) Haste makes waste: a critical review of docking-based virtual screening in drug repurposing for SARS-CoV-2 main protease (M-pro) inhibition. Med Res Rev 42(2): 744–769
Manfredonia I et al (2020) Genome-wide mapping of SARS-CoV-2 RNA structures identifies therapeutically-relevant elements. Nucleic Acids Res 48(22):12436–12452
Materiamedica, 30/01/2020., https://materiamedica.ru/en/news/coronavirus/
Matsuyama S et al (2020) The inhaled steroid ciclesonide blocks SARS-CoV-2 RNA replication by targeting the viral replication-transcription complex in cultured cells. J Virol 95(1):e01648–e01620
McCoy J et al (2021) Proxalutamide reduces the rate of hospitalization for COVID-19 male outpatients: a randomized double-blinded placebo-controlled trial. Front Med (Lausanne) 8: 668698
Mele BH et al (2015) Drug repositioning can accelerate discovery of pharmacological chaperones. Orphanet J Rare Dis 10(1):1–3
Migaud M et al (2020) Metabolomics to predict antiviral drug efficacy in COVID-19. Am J Respir Cell Mol Biol 63(3):396–398
Milan, Italy and San Mateo, Calif., June 4, 2021. https://www.dompe.com/en/media/press-releases/domp%C3%A9-announces-activation-of-u-s-clinical-trial-sites-for-phase-3-study-of-reparixin-an-oral-investigational-cxcr1-cxcr2-inhibitor-in-hospitalized-patients-with-severe-covid-19-pneumonia
Nakazono A et al (2021) Fluticasone propionate suppresses poly (I: C)-induced ACE2 in primary human nasal epithelial cells. Front Cell Infect Microbiol 11:347
Narayanan A et al (2022) Identification of SARS-CoV-2 inhibitors targeting Mpro and PLpro using in-cell-protease assay. Commun Biol 5(1):1–17
Nussinov R et al (2019) Computational structural biology: successes, future directions, and challenges. Molecules 24(3):637
Olivera PE et al (2020) Edoxaban Vs. low molecular weight heparin as anticoagulant therapy in hospitalized patients with atrial fibrillation and COVID-19 infection. Blood 136:22–23
Palit P et al (2021) Phyto-pharmacological perspective of Silymarin: a potential prophylactic or therapeutic agent for COVID-19, based on its promising immunomodulatory, anti-coagulant and anti-viral property. Phytother Res 35(8):4246–4257
Panossian A, Brendler T (2020) The role of adaptogens in prophylaxis and treatment of viral respiratory infections. Pharmaceuticals 13(9):236
Pardo J et al (2020) The journey of remdesivir: from Ebola to COVID-19. Drugs Context 9:4–14

Parienti JJ et al (2021) Effect of tenofovir disoproxil fumarate and emtricitabine on nasopharyngeal SARS-CoV-2 viral load burden amongst outpatients with COVID-19: a pilot, randomized, open-label phase 2 trial. EClinicalMedicine 38:100993
Parvathaneni V et al (2019) Drug repurposing: a promising tool to accelerate the drug discovery process. Drug Discov Today 24(10):2076–2085
Pawlotsky JM (2011) Treatment failure and resistance with direct-acting antiviral drugs against hepatitis C virus. Hepatology 53(5):1742–1751
Perfetto L et al (2021) A resource for the network representation of cell perturbations caused by SARS-CoV-2 infection. Genes 12(3):450
Heparin and zilucoplan added to COVID-19 ACCORD trials platform. Pharm J. https://doi.org/10.1211/PJ.2020.20208023
Pirzada RH (2021) Remdesivir and ledipasvir among the FDA-approved antiviral drugs have potential to inhibit SARS-CoV-2 replication. Cell 10(5):1052
Poe FL, Corn J (2020) N-Acetylcysteine: a potential therapeutic agent for SARS-CoV-2. Med Hypotheses 143:109862
Pushpakom S et al (2019) Drug repurposing: progress, challenges and recommendations. Nat Rev Drug Discov 18(1):41–58
Qu C et al (2021) Could histamine h1 receptor antagonists be used for treating covid-19? Int J Mol Sci 22(11):5672
Raju TN (1999) The nobel chronicles. Lancet 354(9175):347
Recpvery Collaborative Group (2021) Dexamethasone in hospitalized patients with Covid-19. N Engl J Med 384(8):693–704
Roessler HI et al (2021) Drug repurposing for rare diseases. Trends Pharmacol Sci
Saag MS (2020) Misguided use of hydroxychloroquine for COVID-19: the infusion of politics into science. JAMA 324(21):2161–2162
Sacramento CQ et al (2021) In vitro antiviral activity of the anti-HCV drugs daclatasvir and sofosbuvir against SARS-CoV-2, the aetiological agent of COVID-19. J Antimicrob Chemother 76(7):1874–1885
Salaris C et al (2021) Protective effects of lactoferrin against SARS-CoV-2 infection in vitro. Nutrients 13(2):328
Samuel SM et al (2021) Therapeutic potential of metformin in COVID-19: reasoning for its protective role. Trends Microbiol 29(10):894–907
Sanchis-Gomar F et al (2020) Amiodarone in the COVID-19 era: treatment for symptomatic patients only, or drug to prevent infection? Am J Cardiovasc Drugs 20(5):413–418
Sarmah DT et al (2021) Tracing the footsteps of autophagy in computational biology. Brief Bioinform 22(4):bbaa286
Sayad B et al (2020) Sofosbuvir as repurposed antiviral drug against COVID-19: why were we convinced to evaluate the drug in a registered/approved clinical trial? Arch Med Res 51(6):577–581
Schmidt N et al (2021) The SARS-CoV-2 RNA–protein interactome in infected human cells. Nat Microbiol 6(3):339–353
Schoot TS et al (2020) Immunosuppressive drugs and COVID-19: a review. Front Pharmacol 11:1333
Sen A (2020) Repurposing prolactin as a promising immunomodulator for the treatment of COVID-19: are common Antiemetics the wonder drug to fight coronavirus? Med Hypotheses 144:110208
Shekhar N et al (2022) Indomethacin: an exploratory study of antiviral mechanism and host-pathogen interaction in COVID-19. Expert Rev Anti-Infect Ther 20(3):383–390
Shen B et al (2020) Proteomic and metabolomic characterization of COVID-19 patient sera. Cell 182(1):59–72
Smail SW et al (2021) Inflammation, immunity and potential target therapy of SARS-COV-2: a total scale analysis review. Food Chem Toxicol 150:112087

Sohn SY et al (2021) Interferon-Lambda intranasal protection and differential sex pathology in a murine model of SARS-CoV-2 infection. MBio 12(6):e02756–e02721
Song NJ et al (2022) Treatment with soluble CD24 attenuates COVID-19-associated systemic immunopathology. J Hematol Oncol 15:5
Stip E et al (2020) The large action of chlorpromazine: translational and transdisciplinary considerations in the face of COVID-19. Front Pharm 1826
Sukhatme VP et al (2021) Fluvoxamine: a review of its mechanism of action and its role in COVID-19. Front Pharmacol 12:652688
Sun L et al (2021) In vivo structural characterization of the SARS-CoV-2 RNA genome identifies host proteins vulnerable to repurposed drugs. Cell 184(7):1865–1883
Szolnoky G (2020) Sulodexide may be a real alternative to low molecular weight heparins in the prevention of COVID-19 induced vascular complications. Dermatol Ther
Tayara H et al (2021) Recent omics-based computational methods for COVID-19 drug discovery and repurposing. Brief Bioinform 22:22
Toft-Bertelsen TL et al (2021) Amantadine has potential for the treatment of COVID-19 because it inhibits known and novel ion channels encoded by SARS-CoV-2. Commun Biol 4(1):1347
Tomazou M et al (2021) Multi-omics data integration and network-based analysis drives a multiplex drug repurposing approach to a shortlist of candidate drugs against COVID-19. Brief Bioinform 22(6):bbab114
Tornling G et al (2021) Seven days treatment with the angiotensin II type 2 receptor agonist C21 in hospitalized COVID-19 patients; a placebo-controlled randomised multi-centre double-blind phase 2 trial. EClinicalMedicine 41:101152
Unal MA et al (2021) Ribavirin shows antiviral activity against SARS-CoV-2 and downregulates the activity of TMPRSS2 and the expression of ACE2 in vitro. Can J Physiol Pharmacol 99(5): 449–460
V'kovski P et al (2021) Coronavirus biology and replication: implications for SARS-CoV-2. Nat Rev Microbiol 19:155–170
Verma R et al (2021) RNA-protein interaction analysis of SARS-CoV-2 5′ and 3′ untranslated regions reveals a role of lysosome-associated membrane protein-2a during viral infection. Msystems 6(4):e00643–e00621
Volberding PA et al (1990) Zidovudine in asymptomatic human immunodeficiency virus infection: a controlled trial in persons with fewer than 500 CD4-positive cells per cubic millimeter. N Engl J Med 322(14):941–949
Walker AP et al (2020) Enisamium is a small molecule inhibitor of the influenza A virus and SARS-CoV-2 RNA polymerases. bioRxiv. 2020.04.21.053017
Wang M et al (2020) Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell Res 30(3):269–271
Weiss A et al (2021) Niclosamide shows strong antiviral activity in a human airway model of SARS-CoV-2 infection and a conserved potency against the Alpha (B. 1.1. 7), Beta (B. 1.351) and Delta variant (B. 1.617. 2). PLoS One 16(12):e0260958
WHO Weekly epidemiological update on COVID-19—5 March 2022, edition 83, https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports
Xiao X et al (2020) Identification of potent and safe antiviral therapeutic candidates against SARS-CoV-2. Front Immunol 11:586572
Xiong Y et al (2020) Transcriptomic characteristics of bronchoalveolar lavage fluid and peripheral blood mononuclear cells in COVID-19 patients. Emerg Microb Infect 9(1):761–770
Yan F et al (2020) Antihypertensive drugs are associated with reduced fatal outcomes and improved clinical characteristics in elderly COVID-19 patients. Cell Discov 6:77
Yan H et al (2021) Repurposing carrimycin as an antiviral agent against human coronaviruses, including the currently pandemic SARS-CoV-2. Acta Pharm Sin B 11(9):2850–2858
Yeh RF et al (2006) Lopinavir/ritonavir induces the hepatic activity of cytochrome P450 enzymes CYP2C9, CYP2C19, and CYP1A2 but inhibits the hepatic and intestinal activity of CYP3A as

measured by a phenotyping drug cocktail in healthy volunteers. J Acquir Immune Defic Syndr 42(1):52–60
Yong PF, D'Cruz DP (2008) Mycophenolate mofetil in the treatment of lupus nephritis. Biol: Targets Therapy 2(2):297
Yousefi H et al (2021) Repurposing existing drugs for the treatment of COVID-19/SARS-CoV-2 infection: a review describing drug mechanisms of action. Biochem Pharmacol 183:114296
Zafferani M et al (2021) Amilorides inhibit SARS-CoV-2 replication in vitro by targeting RNA structures. Sci Adv 7(48):eabl6096
Zhang LK et al (2020) Calcium channel blocker amlodipine besylate therapy is associated with reduced case fatality rate of COVID-19 patients with hypertension. Cell Discov 6(1):96
Zheng F et al (2020) SARS-CoV-2 clearance in COVID-19 patients with Novaferon treatment: a randomized, open-label, parallel-group trial. Int J Infect Dis 99:84–91

Chapter 20
2-Deoxy-D-Glucose: A Repurposed Drug for COVID-19 Treatment

Simran Chopra, Aditya Dahiya, Ashrit Nair, Navneet Sharma, and Rakesh Kumar Sharma

Abstract Coronaviruses is a broad group of viruses that has the potential to cause mild or severe respiratory infections. Currently, there is no specific treatment for the treatment of COVID-19. The symptomatic treatment is generally given on case-to-case basis along with basic life supportive measures for management of COVID-19. There is an acute urgency of evaluating the pre-existing drugs to develop a convincing treatment for COVID-19 or at least to reduce its severity. 2-DG being inhibitor of both glycolysis and glycosylation appears as a promising therapeutic option. In the present chapter, the rationale of repurposing of 2-DG as a potential treatment option for the management of COVID-19 has been discussed.

Keywords Drug repurposing · COVID-19 · 2-Deoxy-D-glucose · SARS-CoV-2 · Therapeutic management

20.1 Introduction

Local health facilities in Wuhan, China reported cases of pneumonia of unknown origin in the early December 2019. This infection was later recognized to be caused by the novel coronavirus—severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Park 2020). Coronaviruses is a broad group of viruses that has the potential to cause mild or severe respiratory infections. It is a beta coronavirus that has transmission through aerosols or fomites capability before the onset of symptoms (Pedersen and Ho 2020). The infection caused by the SARS-CoV-2 surpasses the severity and infectivity of both Middle East respiratory coronavirus syndrome (MERS) and severe acute respiratory syndrome (SARS) (Hu et al. 2021). This posed serious threats to the public health. COVID-19 disease induced by

S. Chopra · A. Dahiya · A. Nair · N. Sharma
Department of Textile and Fibre Engineering, Indian Institute of Technology Delhi, Delhi, India

R. K. Sharma (✉)
SGT University, Gurugram, Haryana, India
e-mail: prochancellor@sgtuniversity.org

R. C. Sobti et al. (eds.), *Drug Repurposing for Emerging Infectious Diseases and Cancer*, https://doi.org/10.1007/978-981-19-5399-6_20

SARS-CoV-2 was declared as a pandemic by the World Health Organization on March 11, 2020 (Hasöksüz et al. 2020). This class of virus is particularly known to cause various gastrointestinal and pulmonary infections (WHO 2020). The transmission of this novel viral strain began as a result of zoonotic transmission from animals to human with cats, camels and bats being the reservoir of the virus (WHO 2020; Balkrishna et al. 2020). The mode of transmission further changed to direct transmission in humans via respiratory droplets (Yuki et al. 2020). Respiratory droplets are the primary route of transmission of SARS-CoV-2. The conjunctival epithelium in ocular surface is also exposed to body fluid and the infectious droplets (Donald 2011). Angiotensin-converting enzyme-2 (ACE-2), the fusion receptor found in the lungs, heart, kidney, intestine, and endothelium, is responsible for the attachment of the SARS-CoV-2 with the host cells (Chavda et al. 2021; Hadizadeh 2021). On contacting infection, the common symptoms are cough (50–82%), fever (83–98%), muscle soreness (11–44%), fatigue (25–44%), and shortness of breath (19–55%) (Dawood 2021; Chen et al. 2020a). Along with these symptoms, some patients may also suffer from rhinorrhea, chest tightness, sputum production, sore throat, vomiting, diarrhea, nausea, headache, anosmia, and ageusia a few days before the onset of fever, suggesting that fever is critical parameter but not the only initial symptom of infection, whereas some patients only show a mild fever, mild fatigue, or and even may be asymptomatic (Wang et al. 2020).

In case of moderate-to-severe pneumonia, approximately about 15% of patients need inpatient care (Tsai et al. 2021). In case of hospitalized patients, the average time for the progression of symptoms to the occurrence of shortness of breath is 5 days (IQR, 1–10 days), where the median time to be hospitalized is 5 days.

To study the progression of disease, biomarkers such as hematological biomarkers (lymphocyte count, neutrophil count, neutrophil–lymphocyte ratio (NLR)), inflammatory biomarkers (C-reactive protein (CRP), procalcitonin (PCT), erythrocyte sedimentation rate (ESR), and immunological biomarkers (interleukin-6 (IL)-6 and biochemical (D-dimer, creatine kinase (CK), troponin, aspartate aminotransferase (AST)) have been found useful (Li et al. 2020).

Computed tomography revealed pneumonia has been observed along with a drop in oxygen saturation and patchy consolidations. Cytokine storm is recognized by the pro-inflammatory cytokine markers such as tumor necrosis factor -α (TNF-α), interferon-γ-induced protein 10 (IP-10) (Velavan 2020). This cytokine storm has the potential to cause hyperinflammation and oxidative stress. If not properly managed, COVID may result in viral sepsis, multiple organ failure and death (Zhou et al. 2020; Mohamed et al. 2021). If not managed with proper care, the course of disease may show exacerbated progression resulting in multiple organ failure and in worst cases death of critically ill patients. Admission to intensive care unit (ICU) may be required by around 3–29% of patients in order to manage complications, including hypotension or hypoxemic respiratory failure (Wang et al. 2020). In an average time of 8 days, some patients suffering from shortness of breath may develop blood clotting dysfunction, acute respiratory distress syndrome (ARDS), septic shock, or even multiple organ failure (Wu and McGoogan 2020). One of the features of COVID-19 is the high occurrence of multiple organ

failure. Most of the critically ill patients are related to comorbidities (pre-existing diseases), such as hypertension, cardiovascular disease, renal disease, and diabetes. Furthermore, the death rate is relatively high in patients with these comorbidities (Paraskevis et al. 2020). Since the death toll was focused among those aged ≥40 indicating the severity of COVID-19 patients is age-related (Vetter et al. 2020).

Currently, there is no specific treatment for the treatment of COVID-19. The symptomatic treatment is generally given on case-to-case basis along with basic life supportive measures for management of COVID-19. The lack of specific antiviral or standard strategy has resulted in an increase in the morbidity due to the COVID-19 infection. Researchers are working towards search of a clinically proven prophylactic or therapeutic strategy for this deadly virus. Drug discovery is a tedious, costly, and time-consuming procedure. Hence, drug repositioning also referred as drug repurposing has been used as an alternative method for designing effective treatment strategy (Pushpakom et al. 2018). Drug repurposing is the process of identifying new targets for existing medications. The in silico approach of drug repurposing uses two approaches, target-based and drug-based. The interaction between drug and target is explored in the target-based approach whereas in the drug-based approach new indications for already approved drugs are analyzed by comparing the characteristics of diseases (Zhou et al. 2020; Mohamed et al. 2021; Kulshrestha et al. 2021). There is an acute urgency of evaluating the pre-existing drugs to develop a convincing treatment for COVID-19 or at least to reduce its severity (Tan et al. 2020). Drug repurposing method utilizing risk-free compounds, is fast, cost-effective, and efficient as the safety, pharmacodynamics, and pharmacokinetic profile of the repositioned drugs have already been established (Hossen et al. 2020). Drug repurposing is an advantageous process because many different diseases are/may be influenced by common biological processes (Khan et al. 2020). Many established drug molecules have been engineered utilizing basic understanding of disease pathogenesis and drug pharmacodynamics, and tested as a part of drug repurposing against SARS-CoV-2 with their possible mode of action as shown in Table 20.1 (Chakotiya and Sharma 2020; Sarkar and das Mukhopadhyay 2022).

An antiviral drug named remdesivir that was originally designed to target Ebola virus is being repurposed for treatment of COVID-19 (Pareek et al. 2020). HIV protease inhibitors such as darunavir, lopinavir–ritonavir were also pursued (Grein et al. 2020; Cao et al. 2020). Moreover, the Food and Drug administration (FDA) even allowed the use of antimalarial drugs such as chloroquine and hydroxychloroquine which later showed no effect on clinical course and also elevated the side effects (Kalil 2020). Ivermectin, an antiparasitic drug has shown promising in vitro against SARS-CoV-2 (Chen et al. 2020b). Various antineoplastic drugs are also being studied for the management of COVID-19 due to the similarity in mechanism in the management of cancer that included controlled inflammation, inhibition of cell division along with modulation of the host–tumor microenvironment (Caly et al. 2020). In addition, many biologics and plant products targeting various stages of entry of SARS-CoV-2 and COVID-19 progression have been also repurposed (Balkrishna et al. 2020, 2021; Chakotiya and Sharma 2020).

Table 20.1 Repurposed drugs against COVID-19 and their possible mode of action

Category	Drug	Initially used against	Possible mode of action for COVID-19	Reference
Nucleotide based	Remdesivir	Hepatitis C, Ebola virus, and Marburg virus	Inhibit viral RNA polymerase	Srivastava and Singh (2021), Chakraborty et al. (2021)
	Favipiravir	Influenza	Inhibit viral RNA polymerase	Srivastava and Singh (2021), Chakraborty et al. (2021)
	Ribavirin	Hepatitis C	Inhibit viral RNA polymerase	Srivastava and Singh (2021)
Protease inhibitors	Lopinavir–ritonavir	HIV/AIDS	Inhibits the polypeptide proteolytic processing in virus	Srivastava and Singh (2021), Chakraborty et al. (2021)
	Darunavir	HIV/AIDS	Inhibits the polypeptide proteolytic processing in virus	Srivastava et al. (2021)
Anti-parasitic	Chloroquine and hydroxychloroquine	Malaria and Amoebic dysentery	Interrupts the viral binding to ACE2 receptor	Srivastava and Singh (2021), Chakraborty et al. (2021)
	Arbidol	Influenza A and B	Inhibits the viral membrane fusion	Srivastava and Singh (2021)
	Statins	High cholesterol	Upregulates the ACE2 activity	Srivastava and Singh (2021)
Cytokine storm inhibition	Tocilizumab	Rheumatoid arthritis, giant cell arteritis, and systemic juvenile idiopathic arthritis	Decreases the exaggerated immune system response	Srivastava et al. (2021), Chakraborty et al. (2021)
	Dexamethasone	Skin diseases, severe allergies, asthma etc.	Inhibition of pro-inflammatory gene, encoding for cytokines, cell adhesion molecules and acute inflammatory response	Ahmed and Hassan (2020)
	Mavrilimumab	Rheumatoid arthritis	Decreases the hyperinflammation	Bhatt et al. (2021)

(continued)

Table 20.1 (continued)

Category	Drug	Initially used against	Possible mode of action for COVID-19	Reference
			related with pneumonia	
	Baricitinib	Rheumatoid arthritis	Modulate down-stream inflammatory response	Richardson et al. (2020) Chakraborty et al. (2021)

Sustained aerobic glycolysis and elevated glucose in monocytes have been reported to directly promote cytokine production, viral replication, and the subsequent T cell dysfunction and lung epithelial cell death. Thus, to treat COVID-19 disease targeting, metabolic pathways may prove to be a new therapeutic strategy (Codo et al. 2020). 2-Deoxy-D-glucose (2-DG) that is known to inhibit glucose utilization, glycolysis, and glycosylation has been proved efficacious as an adjuvant to cancer radiotherapy (Ardestani and Azizi 2021). Computational techniques have been used for repurposing 2-DG and can pursue a different multitude of approaches such as target modeling, drug safety algorithms, or drug bank (Balkrishna et al. 2020). To make optimal conditions for their rapid, efficient replication, and spread, viruses alter the host cell metabolism. The host cells undergo metabolic reprogramming on entry of SARS-COV-2 to satisfy the amplified demand for nutrients and energy for viral replication, where 2-DG being inhibitor of both glycolysis and glycosylation appears as a promising therapeutic option (Balkrishna et al. 2020). In the present chapter, the rationale of repurposing of 2-DG as a potential treatment option for the management of COVID-19 has been discussed.

20.2 Drug–Target Interaction Profiles Are a Natural Extension of Molecular Docking

The drug–target interaction studies are widely researched topic and optimized for the repurposing of drugs. The main objective is primarily to identify novel targets for existing drugs and for identification of a new reputed drug (Borcherding et al. 2020). The identification of new drug–target interactions is relatively less costly and less time consuming in comparison to in vivo or biochemical experimental methods. The in silico or computational method enables the efficient identification of potential drug–target interaction contenders for guiding in vivo validation required for drug repositioning or discovery (Luo et al. 2017). The computational methods include ligand-based approaches and the molecular-docking-based approaches (Whitebread et al. 2005; Keiser et al. 2007).

Balkrishna and coworkers (2020) studied the analog of glucose, 2-DG, and its derivative (1, 3, 4, 6-tetra-O-acetyl-2-deoxy-D-glucopyranose), for tackling

COVID-19 by carrying out the in-silico docking and molecular simulations (Balkrishna et al. 2020). In this study, the active site mapping of the viral virulence factors was identified and the repurposing of 2-DG along with its derivative was examined via ligand–receptor docking method. The ADMETox values, drug likeliness and bioactivity indices were assessed using tools such as Molinspiration and Toxicity Estimation Software. It was observed that there is efficient docking of 2-DG with both viral protease 3CLpro and NSP15 endoribonuclease, resulting in efficient inactivation of viral receptors and ultimately leading to breakdown of the SARS-CoV-2 virus. This breakdown of virus was due to the hydrogen bond formed between 2-DG and proline residues of viral protease. There was hydrogen bond formation between the 2-DG derivative and the glutamine amino acid residues of the viral spike glycoprotein (Balkrishna et al. 2020).

20.3 Comparison of COVID-19 Progression with Cancer

In tumors or other proliferating cells, high rate of glucose uptake is reported that results in the production of lactate, even in the presence of oxygen and fully functioning mitochondria. This process is known as the Warburg effect (Ponti et al. 2020; Warburg 1925). Similarly, many viruses alter the host cell metabolism by enhancing glycolysis and consequently producing rapid energy for nucleotide replication and specific protein synthesis (Liberti and Locasale 2016; Singh et al. 2020). Since enhanced aerobic glycolysis maintains the replication of many viruses including MERS-CoV, it is pertinent that SARS-CoV-2 replication in host cells (especially the airway cells) is also reliant upon altered glucose metabolism, alike to the Warburg effect in cancer (Icard et al. 2021). Therefore, there appears to be a significant involvement of Warburg effect in the progression COVID-19 infection (Sanchez and Lagunoff 2015). In the lung endothelial cells, the Warburg effect becomes active due to the low oxygen levels in blood. Aerobic glycolysis supports the activation of pro-inflammatory cells such as M1 macrophages and neutrophils. The anti-inflammatory response and reparative process performed by M2 macrophages would not occur at an appropriate time due to the uncontrolled increase of M1 macrophages (Warburg 1925).

The progression of COVID-19 in the human body and how it affects various systems is depicted in Fig. 20.1.

20.4 2-DG Molecule as a Glucose Analog

2-DG is a synthetic analog of glucose in which a hydrogen is present at the second position of carbon in place of the hydroxyl group as shown in Fig. 20.2. 2-DG is (4R,5S,6R)-6-hydroxymethyltetrahydro-2H-2,4,5-pyrantriol with molecular formula $C_6H_{12}O_5$ and molecular weight 164.6 g/mol.

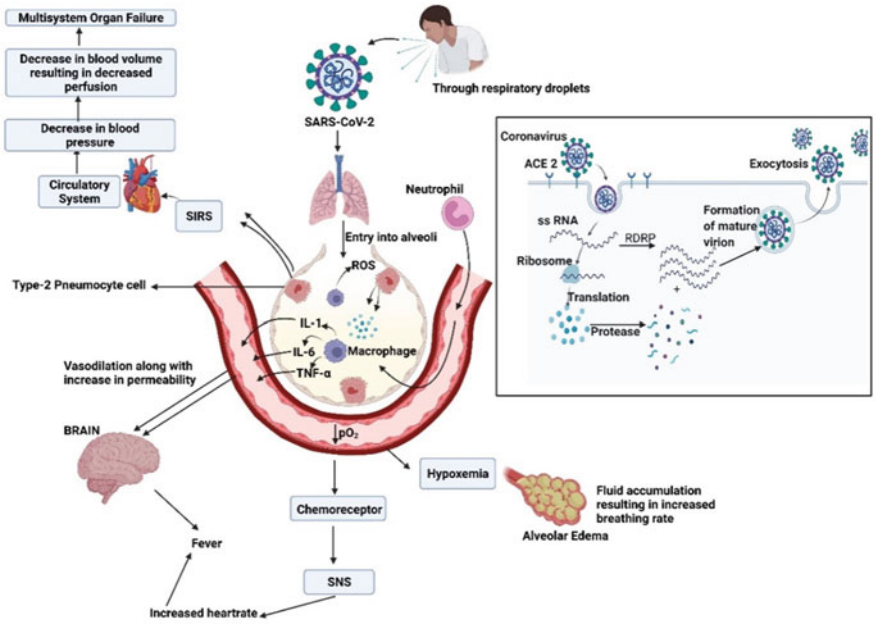


Fig. 20.1 COVID-19 progression in the human body

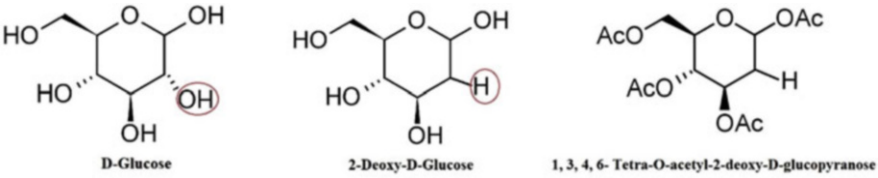


Fig. 20.2 Structure of glucose, 2-DG, and 1, 3, 4, 6-tetra-O-acetyl-2-deoxy-D-glucopyranose

20.5 Pharmacological Properties of 2-DG of Relevance to Cancer and COVID-19 Therapies

2-DG is a structurally similar to glucose differing only at the second carbon atom. 2-DG is soluble in water. 2-DG competes with glucose for transport into the cells and competitively inhibits transport of glucose. In cancer cells, the higher uptake of 2-DG is due to increase in the expression of glycolytic enzymes and glucose transporters. The glucose transporters are not able to differentiate between glucose and 2-DG. GLUTs aid the entry of 2-DG into the cell and also help in selective accumulation in cancer cell with higher glucose utilization. Upon entering the cells, 2-DG shows a range of biological effects by interacting with multiple cellular pathways as explained in the following subsections (Singh et al. 2020).

20.5.1 Glycolysis Inhibition

Inside the cell, 2-DG is phosphorylated to 2-DG-6-phosphate (2-DG-6-P) by hexokinase II enzyme. The 2-DG-6-P is not a substrate to dehydrogenases or isomerases. The 2-DG-6-P gets accumulated in the cell till it is dephosphorylated back to 2-DG via phosphorylase. Accumulation of 2-DG-6-P leads to competitive inhibition of hexokinase, depletion of ATPs, cell cycle arrest, cell growth inhibition, and, ultimately, cell death. The accumulation of 2-DG-6-P inhibits the glycolysis cycle. The extent of glycolysis inhibition is affected by the amount of 2-DG entering and accumulating in the cell (Aft et al. 2002). Tumors are generally oxygen deficient as there is no other source of ATP production using fatty acid or amino acids.

20.5.2 Autophagy Induction

Autophagy refers to the process of removal and degradation of intracellular bodies within autophagosomes and lysosomes respectively. The glycolysis inhibition causes ATP deficiency, which creates imbalance in the ATP/AMP ratio, leading to activation of AMPK (Aft et al. 2002; Pajak et al. 2020). The TSC1 proteins in the mTOR (mammalian target of rapamycin) kinase complex are phosphorylated by the active AMPK resulting in induction of autophagy (Hardie and Lin 2017).

Activation of AMPK can also lead to the expression of a tumor suppressor protein p53. In the presence of active p53, cell cycle gets arrested in the G1 checkpoint, which either allows cell damage repair or destroys the cell using Bcl-2 protein family via apoptosis process (Deretic et al. 2013).

The degradation products can be transported back for cellular metabolism in the cytoplasm. However, uncontrolled autophagy may lead to excessive self-degradation of cellular components leading to cell death. The autophagy can also be induced by endoplasmic reticulum as a stress response to glucose deficiency and decreased ATP levels (Xi et al. 2011; Pflaum et al. 2014).

20.5.3 Apoptosis Induction

Sustained autophagy and extensive self-degradation lead to apoptosis (Fan and Zong 2013). Depending on the cell type, different apoptotic pathways are induced in the cells by 2-DG (Aft et al. 2002). Study conducted by Munoz and coworkers reported that the ATP depletion makes cells sensitive to TNF superfamily death receptor-dependent extrinsic apoptosis (Muñoz-Pinedo et al. 2003). 2-DG can sensitize cells to TRAIL-induced apoptosis, at least in part through suppressing JNK-mediated cytoprotective autophagy processes (Xu et al. 2005). Several studies demonstrate the ability of ROS generation of 2-DG, leading to cell death (Aft et al. 2002).

20.5.4 Protein N-Glycosylation

N-glycans are membrane bound glycoprotein involved in various important functions like protein folding, stabilization, signal transmission, and intracellular interactions (Pelicano et al. 2006). All eukaryotic *N*-linked glycans are based on the $Man3GlcNAc_2$, a common core pentasaccharide, comprising two β-D-*N*-acetylglucosamine (GlcNAc) molecules and three D-mannose (Man). Through a co-translational process, the glycan $glucose_3$-$mannose_9$-*N*-$acetylglucosamine_2$ ($G_3Man_9GlcNAc_2$) is transferred from a lipid-linked oligosaccharide (LLO), $G_3Man_9GlcNAc_2$-P-P-dolichol, to Asn-X-Ser/Thr motifs and processed by various glycosyltransferases, giving rise to the three main types of glycans: hybrid, high-mannose, and complex *N*-glycans (Kim et al. 2009). Due to the similarity in the structure of 2-DG and the structure formed when hydroxyl is eliminated from C-2 of D-mannose, 2-DG is expected to interfere with the metabolism of D-mannose-related metabolic pathway. D-mannose is responsible for protein N-glycosylation and 2-DG interrupts the protein glycosylation in the presence of oxygen resulting in ER stress (Aft et al. 2002).

20.6 2-DG as an Adjuvant to Cancer Therapy

Damage to DNA is the major cause of cell death and cell loss induced by ionizing radiation (Bieberich 2014). However, this DNA damage can be repaired by various repair pathways which are further enhanced in tumor cells. In tumor cells, there is an increased glucose usage due to the continuous supply of metabolic energy in the form of ATP derived from the glycolytic pathway. In such cells, the inhibitors of the glucose transport and glycolysis have the potential to inhibit the repair processes resulting in enhancement of radiation damage and thereby increasing the efficacy of radiotherapy (Surova and Zhivotovsky 2013).

It was observed that administration of 2-DG as an adjuvant to radiation therapy could further enhance the inhibition of repair process and ultimately resulting in an overall increase in the efficiency of radiation damage in cells with high glycolytic rates such as cancer cells under both euoxic and hypoxic conditions (Jain and Pohlit 1972).

The first human trial of 2-DG was conducted at All India Institute of Medical Sciences, New Delhi. On administration of 2-DG to a thyroid patient (where the patient was given iodine-131 (I-131) along with two doses of 2-DG orally (10 g/dose in 150 mL water) after 16 h and 65 h), it was observed that there were no side effects observed and there was a marked reduction in the size of tumor. Even when the metastasis deposits were same, but the quality of life improved for the patient as the progress of the disease stopped (Jha and Pohlit 2009).

On administration of 2-DG to mice bearing Sarcoma 180 and solid Ehrlich Ascites (1–2 mg/g body weight) in combination with the gamma irradiation, it was

observed that there was a potential increase in the tumor cell loss and animal survival (Naqvi et al. 1974).

The effect of 2-DG on HeLa cell line showed a reduction in aerobic glycolysis by 62% along with inhibited DNA repair that resulted in enhanced radiation damage to cancer cell line (Latz et al. 1993).

Due to excellent DNA repair systems, glioblastoma multiforme (GBM), a fast-growing and aggressive brain tumor cells are reported as resistant to treatment in both hypoxic and euoxic conditions (Jain et al. 1985). After the surgical and/or radiotherapeutic excision of cancerous tissue, there still remains a possibility of local regrowth of human glioblastomas within the cranial cavity. In an in vitro study using organ cultures of human cerebral glioma, it was concluded that the higher concentrations of 2-DG (5 mM) may be required to induce radiation damage in the cerebral gliomas under euoxic conditions; however, this requirement may be decreased in hypoxic conditions (Dwarakanath et al. 2009).

For patients affected with GBM, Phase I/II clinical trials were conducted (Mohanti et al. 1996; Srinivasa Rao Dwarakanath and Kumar Jain 1987). Four weekly fractions of 5 Gy/fraction of gamma radiation to the whole brain were administered 2–3 weeks postsurgery. 2-DG was administered orally (200 mg/kg body weight) after overnight fasting and also 20–30 min prior to irradiation (5 Gy). This schedule of combination of 2-DG and radiotherapy was given once weekly on days 1, 8, 15, and 22. The total radiation dose of 20 Gy was given over a period of 21 days. Two weeks after the fourth week, a CT scan was carried out and radiotherapy was given to the residual tumor (plus 3 cm margin) at a dosage of 14 Gy in seven fractions at five fractions per week. There was no acute toxicity or late radiation damage observed. Moreover, a noteworthy improvement in the quality of life with a moderate increase in survival was observed (Srinivasa Rao Dwarakanath and Kumar Jain 1987). Dose optimization studies were undertaken to examine the tolerance and safety of escalating the 2-DG dosage during the combined treatment of 2-DG and radiotherapy in GBM patients. The dosage of 2-DG was administered in incremental doses of 200–250–300 mg/kg body weight. The dosage included seven weekly fractions of combined treatment of 2-DG along with radiation at a dose of 5 Gy/fraction including the postsurgery treatment of the residual tumor plus 3 cm margin. Patients with dosage up to 250 mg/kg showed excellent tolerance to the treatment with no toxicity (Mohanti et al. 1996).

An in vivo study was conducted by Maschek et al. (2004) in which the efficacy of 2-DG in combination with paclitaxel or adriamycin in nude mouse xenograft models of human osteosarcoma and non–small-cell lung cancer was observed. In both the cases, there was a significant reduction in the tumor growth in comparison to using either drug alone (Maschek et al. 2004).

To target the hypoxic cells in retinoblastoma, 2-DG was used as an adjuvant in another trial. Sixteen-week-old $LH_{BETA}T_{AG}$ mice were given carboplatin (31.25 μg/20 μL) along with 2-DG (500 mg/kg). It was observed that therapy alone with carboplatin resulted in 52% tumor size reduction, whereas 2-DG monotherapy resulted in 49% tumor size reduction. However, when 2-DG was used as an adjuvant along with carboplatin, then a reduction in tumor size was 86% (Boutrid et al. 2008).

A Phase I/II trial was conducted in 12 patients for the treatment of advanced solid tumor and hormone refractory prostate cancer. The drug was administered orally on daily basis for 2 weeks of every 3-week cycle. The dose was escalated from 30 mg/kg to 45 mg/kg and finally up to 60 mg/kg. The treatment was well tolerated with no mortality and maximum tolerance of dose not exceeding 60 mg/kg (Gounder et al. 2010).

In an in vitro study by Rae et al., 2018, 2-DG was used to sensitize prostate cancer cells to radiotherapy. Simultaneous administration of 1–10 mM 2-DG along with 1–4 Gy X-rays resulted in concentration-dependent decrease in clonogenic survival (Oncogenesis et al. 2018).

20.7 2-DG Against Various Viral Diseases

The in vitro multiplication of influenza virus when kept in the chorioallantoic membrane of the chick embryo was observed to be dependent on the presence of glucose in the medium. It was observed that 2-DG inhibits the synthesis of influenza virus (Kilbourne 1959).

In a study by Gallaher et al., the effect of 2-DG on the cell fusion induced by herpes simplex virus and Newcastle disease was analyzed. It was observed that the fusion from within induced by both the herpes simplex viruses and Newcastle disease is completely inhibited by treatment of the infected cells with 10 mM 2-DG (Gallaher et al. 1973).

The production of Newcastle disease virus and infectious vesicular stomatitis virus (VSV) was observed to be completely inhibited by 2-DG in pyruvate containing medium. This inhibition is observed when the virus is either grown in pyruvate-containing medium or dialyzed against phosphate-buffered saline is used for infection. Under these conditions, there is reduction in the synthesis of all VSV proteins. The VSV RNA synthesized at reduced rates is further unstable (Scholtissek et al. 1974).

The effect of 2-DG on porcine epidemic diarrhea virus (PEDV) was studied by Wang et al., and it was observed that 2-DG being an ER stress inducer possessed antiviral activity against the infection. It inhibited the virus infection by affecting the viral protein translation and also affects the virus assembly (Wang et al. 2014).

2-DG interfered with the genome replication of Kaposi's sarcoma-associated herpes virus (KSHV) and resulted in decreased virion production during the lytic phase of virus infection which is a requirement for KSHV tumorigenesis. Low doses of 2-DG targeted the KSHV replication in lytic phase cells without inducing cytotoxicity and at high doses killed the KSHV-infected cells at latent stage. Therefore, 2-DG targets the KSHV at viral and cellular level (Leung et al. 2012). The inhibitory effect of 2-DG has also been observed on human papillomavirus 18 (HPV 18) (Maehama et al. 1998).

Table 20.2 depicts various preclinical and clinical trials of 2-DG against cancer and virus-related diseases.

Table 20.2 Promising pre-clinical and clinical studies using 2-DG

Preclinical and clinical trials against cancer		
Trial design	Study	Endpoint
Effect of 2-DG on HeLa cell culture	In vitro	The radiation damage to cancer cells was enhanced due to the use of 2-DG.
Organ cultures of human cerebral glioma	In vitro	Radiation damage was induced by 2-DG in cerebral glioma cells.
Cell cultures of human prostate cancer cell line	In vitro	Radiation damage was induced by 2-DG in prostate cancer cells.
Sarcoma-180 and solid Ehrlich Ascites tumor bearing mice	In vivo	Improvised animal survival with a decrease in volume of tumor.
Nude mouse xenograft models of human osteosarcoma and non–small-cell lung cancer	In vivo	Reduction in the tumor growth as compared to using either drug alone.
Hypoxic cells in retinoblastoma	In vivo	Reduction in tumor size by 86% when 2-DG was used as an adjuvant along with carboplatin
Thyroid carcinoma	Human clinical trial	Significant enhancement in quality of life since the progress of the disease arrested and the metastasis deposits were same.
Supratentorial glioma patients	Phase I/II	Significant enhancement in quality of life along with no toxicity in multicenter, nonrandomized, open-label, single treatment arm (uncontrolled) trials.
Dose escalation trial in patients with malignant cerebral gliomas	Phase II	Significant tolerance to the treatment with no toxicity in multicenter, nonrandomized, open-label, single treatment group.
Solid tumor and hormone refractory prostate cancer	Phase I/II	Significant tolerance to the treatment with a maximum dose toleration of 60 mg/kg.
Pre-clinical against virus-related diseases		
Influenza virus kept in chorioallantoic membrane	In vitro	Inhibition of the influenza virus under the influence of 2-DG
Effect of 2-DG on cell fusion induced by Newcastle disease and herpes simplex virus	In vitro	Fusion from within induced by both Newcastle disease and herpes simplex virus is inhibited on treatment with 2-DG
Effect of 2-DG on vesicular stomatitis virus and Newcastle disease virus in pyruvate containing medium	In vitro	Inhibition of both the virus in pyruvate containing medium or when kept in phosphate buffered saline

20.8 Rationale for Using 2-DG as an Anti-COVID Drug

Approximately one-tenth of cancer incidences are virus-related, signifying that the increased uptake of glucose and aerobic glycolysis can be considered a common trait of viral infection (Xi et al. 2014). Thus, 2-DG may reduce various viral-induced tumors irrespective of the route it takes. Hence, the study of 2-DG on noncancerous viral infections is of vital importance. The metabolic demand of virally infected cells is upregulated primarily through high glucose uptake and glycolysis for rapid and higher viral replication (Ardestani and Azizi 2021). This change in metabolic state allows for selective accumulation of 2-DG in virally infected cells. Synthesis of anabolic intermediates required for viral replication process is inhibited by 2-DG (Bhatt et al. 2022). Evidence suggest that it interferes with N-linked glycosylation of viral proteins in the endoplasmic reticulum of infected cells, triggering misfolding of viral glycoproteins resulting in noninfective or nonvirulent virus particles (virions) (Codo et al. 2020; Bhatt et al. 2022; Bojkova et al. 2020). It has been hypothesized that 2-DG could also accumulate preferentially within infected lung cells and be effective in suppressing viral multiplication (Bere et al. 2021). Since 2-DG reduces glucose utilization, glycolysis, and glycosylation, in silico studies on molecular modeling were carried out to predict its efficacy against SARS-CoV-2. Toxicological evaluations have found 2-DG to not be genotoxic, possess very low acute toxicity and not affect the vital physiological functions at low-to-moderate doses. No observed adverse effects level (NOAEL) in mice was determined to be 1000 mg/kg/day, thereby providing a safety margin of 1.3 times based on body surface area, for the proposed human dose of 63 mg/kg/day (Bere et al. 2021). Given the need for effective treatments, to save lives of COVID-19 patients and reduce the burden on limited intensive care resources in hospitals in the face of this devastating pandemic currently ravaging the world, a comparative evaluation of risk to benefit would favor evaluation of 2-DG as adjunctive treatment to standard of care (SOC) in mild-to-severely ill COVID-19 patients. The increase uptake of glucose in SARS-CoV-2 and the possible use of 2-DG against an array of viral infections led to the use of 2-DG against SARS-CoV-2 (Codo et al. 2020).

In a study by Balkrishna et al., 2020, a derivative of 2-DG, 1, 3, 4, 6-tetra-O-acetyl-2-deoxy-D-glucopyranose was compared with other used antiretroviral drugs such as lopinavir, antiflu drug such as favipiravir, and antimalarial drugs such as hydroxychloroquine. The binding energy of this derivative was comparable to the hydroxychloroquine implying it to be a potential therapy for COVID-19. Thus, this derivative could apparently mitigate the virus completely. Also, 2-DG significantly inactivated the SARS-CoV-2 viral receptors and in fact the E-value of docking of 2-DG was better with lopinavir and favipiravir. As per Lipinski scores, 2-DG and 2-DG derivative, both possessed adequate oral bioavailability with no major side effects or signs of toxicity in ADMETox prediction (Balkrishna et al. 2020).

Therefore, the use of 2-DG against SARS-CoV-2 was hypothesized to reduce the supplemental oxygen dependence and lead to the faster recovery of patients. 2-DG is also expected to work against emerging new variants and breakthrough cases as was observed in the second wave due to the mechanistic-based approach of 2-DG (Mesri and Lampidis 2021).

20.9 Use of 2-DG Against SARS-CoV-2

Institute of Nuclear Medicine and Allied Sciences (INMAS), Defence Research and Development Organization, Delhi, and Dr. Reddy's Laboratories (DRL), Hyderabad, with the help of the Centre for Cellular and Molecular Biology (CCMB), Hyderabad developed the application of the 2-DG for the therapeutic management of COVID-19. DRL-INMAS conducted Phase II trials on 2-DG on COVID patients. On November 4, 2020, permission to conduct the Phase III clinical trials had been recommended by the subject expert committee, an advisory to the Drug Controller General of India (DCGI) on applications seeking approvals for new drugs, clinical trials, and vaccine. The permission for conducting Phase III clinical trials was granted on November 16, 2020. Table 20.3 summarizes studies on 2-DG for the management of COVID-19 in India (Narayan Bhatt et al. 2022).

On the basis of these trials, 2-deoxy-D-glucose received emergency use approval as an anti-COVID-19 by DCGI. Patented technology of 2-deoxy-D-glucose has been recently transferred to various Indian industries including Dr. Reddy's Laboratories, Mankind Pharma Limited, Bajaj Healthcare Limited, MSN Laboratories Limited, BDR Pharmaceuticals Internationals Pvt. Ltd., Granules India, Shilpa Medicare Limited, and Laurus Labs.

20.10 Possible Mechanism of Action of Use of 2-DG Against SARS-CoV-2

2-DG possibly acts at a number of points in the progression of the coronavirus. Balkrishna et al. suggest that the structure of 2-DG docks into 3CL protease and also with NSP15 endoribonuclease which results in inactivation of the SARS-CoV-2 binding receptors (Balkrishna et al. 2020). The NSP 15 is an interferon antagonist which inhibits the interferon-β production by an endoribonuclease activity-independent mechanism (Liu et al. 2019). The 3CLpro is vital for the post-

Table 20.3 Studies on 2-DG for the management of COVID-19

Preclinical studies	Phase I/II clinical trials	Phase III clinical trials
In vitro cell cultures studied at CCMB Hyderabad	Initiated by Dr. Reddy's Laboratory under CTRI number CTRI/2020/06/025664	Initiated in 27 COVID hospitals across India on 220 patients from Dec'20 to Mar'21 under CTRI number CTRI/2021/01/030231
Observation: Viral plaques in cells administered with 2-DG were significantly lower as compared to the control group	A clinical trial was conducted on 110 patients from May'20 to Oct'20. Patients showed better symptomatic cure as compared to patients who were not given 2-DG	There was a drastic reduction in the use of supplemental oxygen on administration of 2-DG. The drug showed promising results in patients aged 65 or above also.

translational processing of replicase gene (Sharma et al. 2020). The processing of viral RNAs responsible for viral replication is catalyzed by the endonucleases (Mesri and Lampidis 2021). Therefore, the 2-DG inactivates the viral proteases, inhibiting the process of viral capsid formation and further by retaining the action of endoribonuclease stops the viral replication process (Balkrishna et al. 2020). 2-DG is also known to have and anti-inflammatory effects and potential to reduce viral loads (Bojkova et al. 2020; Choi et al. 2020). A network-biology-led computational drug repurposing strategy by Khurana et al., 2021, suggests that increased AMP levels in the cells led to the activation of AMP-activated protein kinases that further inhibits the Janus kinase (JAK1). It is also responsible for the activation of the STAT transcription factors such as Signal Transducers and Activators of Transciptors-3 (STAT3). STAT 3 leads to apoptosis of cells and is responsible for the inhibition of replication of virus-infected cells (Khurana et al. 2022).

2-DG is believed as a broad-spectrum antiviral drug effective against different variants of COVID-19. It has been seen by Bhatt et al. (2021), that the 2-DG targets the metabolic requirements of the host cell and is effective against the B.6 and B.1.1.7 coronavirus variants (Bhatt et al. 2022).

As we know that the 2-DG competes with the D-glucose for the entry into the cell. Upon entry into the cell, it is phosphorylated by hexokinase to form 2-deoxy-D-glucose-6-phosphate (2-DG-6-P), which inhibits phosphohexose isomerase (El Mjiyad et al. 2011). The 2-DG-6-P cannot be further metabolized like glucose to glucose-6-phosphate to fructose-6-phosphate. This results in the inhibition of glycolysis and accumulation of 2-DG-6-P in the cytosol (Pelicano et al. 2006; Samal et al. 2021).

The inhibition of glycolytic pathway leads to the propagation of pentose phosphate pathway (PPP). The increase in PPP pathway leads to the increase in the NADPH production which aids in the reduction of glutathione by increasing the expression of the glutathione synthetase (Miwa et al. 2013).

The glycolysis inhibition causes ATP deficiency, which creates imbalance in the ATP/AMP ratio, leading to activation of AMPK. Active AMPK phosphorylates the TSC1 proteins in the mTOR kinase complex, resulting in induction of autophagy. Activation of AMPK can also lead to the expression of a tumor suppressor protein p53. In the presence of active p53, cell cycle gets arrested in the G1 checkpoint, which either allows cell damage repair or destroys the cell using Bcl-2 protein family via apoptosis process (Pajak et al. 2020). Another pathway for the autophagy of viral cell is the stress induction in the endoplasmic reticulum due to glucose deprivation and reduction in ATP levels. This stress stimulates the production of reactive oxygen species (ROS) that further catalyzes the process of cell death (Shutt et al. 2010).

The anti-inflammatory properties of 2-DG also inhibit the M2 macrophage polarization responsible for airway inflammation. The AMPK activation inhibited the Hif-1α expression that further inhibits the M2 macrophage polarization (Zhao et al. 2017).

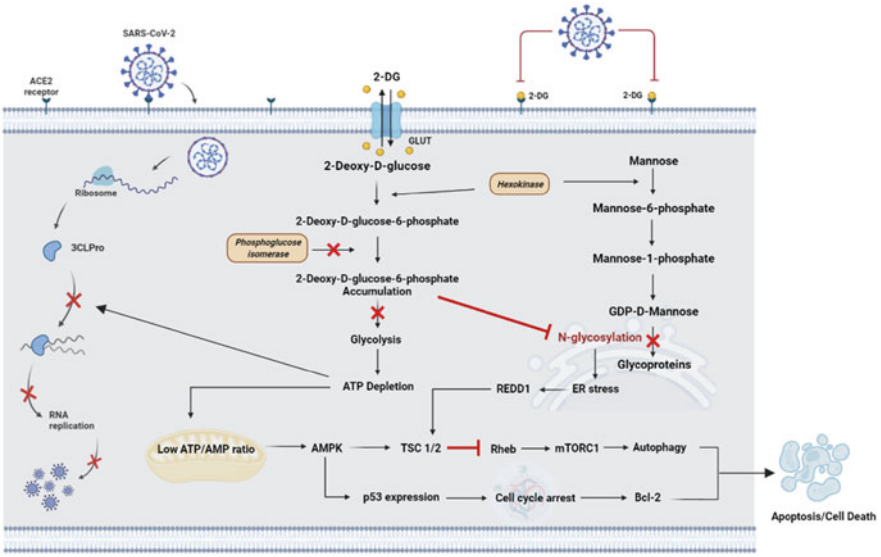


Fig. 20.3 Possible mechanism of 2-DG upon entry into the cell

Due to the similarity in the structure of 2-DG and the structure formed when hydroxyl is eliminated from C-2 of D-mannose, 2-DG is expected to interfere with the metabolism of D-mannose-related metabolic pathway. D-mannose is responsible for protein N-glycosylation and 2-DG interrupts the protein glycosylation in the presence of oxygen resulting in ER stress (Singh et al. 2020; Kilbourne 1959). 2-DG drug also leads to the formation of defective virions that lack the ability of infecting newer cells (Bhatt et al. 2022). Thus, the two main properties of 2-DG make it a plausible drug against COVID-19, as it inhibits the glycolysis in hypoxic conditions and glycosylation in the presence of oxygen.

Figure 20.3 represents the possible mechanisms of actions of 2-DG against coronavirus.

20.11 Future Perspective

2-DG is a promising adjuvant to Standard of Care (SOC) in mitigating the COVID-19 infection. Using Toxicity Estimation Software Tool (T.E.S.T), it was also observed that it had no major signs of toxicity or side effects (Balkrishna et al. 2020). If any, the side effects of 2-DG are short-lived and temporary due to its half-life of approximately 90 mins (Dwarakarnath and Jain 2009). 2-DG was found to be effective against B.6 and B.1.1.7 coronavirus variants by Bhatt et al. since it targets

metabolism of the infected host cells. Therefore, 2-DG is hypothesized to be a broad-spectrum antiviral drug that can be used against various variants of COVID-19 and other viral infections (Bhatt et al. 2022). In a retrospective case series by Chandra et al., it was concluded that on administration of 2-DG, the oxygen levels improved to more than 93% in 70% of the patients within 7 days. There was a significant reduction in the time taken for viral clearance along with that short duration to negative RT-PCR results was also observed (Chandra et al. 2021). The utility of 2-DG in other viral diseases including emerging infections needs to be systematically explored.

20.12 Conclusion

In moderate-to-severely ill patients, 2-DG is found to be effective in reducing oxygen dependence along with the standard of care as compared to standard of care alone. It also benefitted the patients by reducing the time in viral clearance and reduction in duration of time to test RT-PCR negative. Therefore, 2-DG can be a promising adjuvant in alleviating the COVID-19 symptoms along with the standard of care.

Acknowledgement The authors thank their respective organizations for scientific support.

Conflict of Interest The authors of the current review article declare no conflict of interest.

References

Aft RL, Zhang FW, Gius D (2002) Evaluation of 2-deoxy-D-glucose as a chemotherapeutic agent: mechanism of cell death. Br J Cancer 87:805–812

Ahmed MH, Hassan A (2020) Dexamethasone for the treatment of Coronavirus Disease (COVID-19): a review. SN Comp Clin Med 12(2):2637–2646

Ardestani A, Azizi Z (2021) Targeting glucose metabolism for treatment of COVID-19. Signal Transduct Target Ther 6:1–2

Balkrishna A, Thakur P, Singh S, Dev S, Jain V, Varshney A, Sharma R (2020) Glucose antimetabolite 2-deoxy-d-glucose and its derivative as promising candidates for tackling COVID-19: insights derived from in silico docking and molecular simulations. Authorea Preprints. https://doi.org/10.22541/AU.158567174.40895611/V2

Balkrishna A, Thakur P, Singh S, Singh N, Tanwar A, Sharma RK (2021) In silico screening for investigating the potential activity of phytoligands against SARS-CoV-2. J Life Sci. https://doi.org/10.36069/JOLS/20210302

Bere J, Dharani Jonnalagadda N, Kappari L, Karangula J, Boggula N, Kappari V (2021) 2-Deoxy-D-glucose: an update review. J Innov Dev Pharm Technical Sci 4(5):68–78

Bhatt AN, Kumar A, Rai Y, Kumari N, Vedagiri D, Harshan KH, Kumar CV, Chandna S (2022) Glycolytic inhibitor 2-Deoxy-D-glucose attenuates SARS-CoV-2 multiplication in host cells and weakens the infective potential of progeny virions. Life Sci 295:120411

Bhatt K, Garimella R, Taugir R et al (2021) Effectiveness of mavrilimumab in viral infections including SARS-CoV-2 infection—a brief review. Infect Chemother 53:1

Bieberich E (2014) Synthesis, Processing, and Function of N-glycans in N-glycoproteins. Adv Neurobiol 9:47–70
Bojkova D, Klann K, Koch B, Widera M, Krause D, Ciesek S, Cinatl J, Münch C (2020) Proteomics of SARS-CoV-2-infected host cells reveals therapy targets. Nature 583:469–472
Borcherding N, Jethava Y, Vikas P (2020) Repurposing anti-cancer drugs for COVID-19 treatment. Drug Des Devel Ther 14:5045
Boutrid H, Jockovich ME, Murray TG, Piña Y, Feuer WJ, Lampidis TJ, Cebulla CM (2008) Targeting hypoxia, a novel treatment for advanced retinoblastoma. Invest Ophthalmol Vis Sci 49:2799–2805
Caly L, Druce JD, Catton MG, Jans DA, Wagstaff KM (2020) The FDA-approved drug ivermectin inhibits the replication of SARS-CoV-2 in vitro. Antivir Res 178:104787
Cao B, Wang Y, Wen D et al (2020) A trial of lopinavir–ritonavir in adults hospitalized with severe Covid-19. N Engl J Med 382:1787–1799
Chakotiya AS, Sharma RK (2020) Phytoconstituents of Zingiber officinale targeting host viral protein interaction at entry point of SARS CoV 2 A molecular docking study. Defence Life Sci J 5:268–277
Chakraborty C, Sharma AR, Bhattacharya M, Agoramoorthy G, Lee SS (2021) The drug repurposing for COVID-19 clinical trials provide very effective therapeutic combinations: lessons learned from major clinical studies. Front Pharmacol 12:2942
Chandra P, Dutta B, Mittal S, Gupta N, Kataria S (2021) 2-Deoxy-D-glucose (2-DG) for treatment of moderate to severely ill COVID-19 patients: a retrospective case series. J Med Case Rep Case Ser 2:15. https://doi.org/10.38207/JMCRCS/2021/0215220
Chavda VP, Vora LK, Pandya AK, Patravale VB (2021) Intranasal vaccines for SARS-CoV-2: from challenges to potential in COVID-19 management. Drug Discov Today 26:2619–2636
Chen J, Liu D, Liu L et al (2020b) A pilot study of hydroxychloroquine in treatment of patients with moderate COVID-19. J Zhejiang Univ Med Sci 49:215–219
Chen N, Zhou M, Dong X et al (2020a) Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 395:507–513
Choi SY, Heo MJ, Lee C, Choi YM, An I-S, Bae S, An S, Jung JH (2020) 2-deoxy-D-glucose ameliorates animal models of dermatitis. Biomedicine. https://doi.org/10.3390/BIOMEDICINES8020020
Codo AC, Davanzo GG, Monteiro L de B, et al (2020) Elevated glucose levels favor SARS-CoV-2 infection and monocyte response through a HIF-1α/glycolysis-dependent axis. Cell Metab 32: 437-446 .e5
Dawood AA (2021) Transmission of SARS CoV-2 virus through the ocular mucosa worth taking precautions. Vacunas (English Edition) 22:56
Deretic V, Saitoh T, Akira S (2013) Autophagy in infection, inflammation and immunity. Nat Rev Immunol 13(10):722–737
Donald BR (2011) Algorithms in structural molecular biology. MIT Press, p. 429
Dwarakanath BS, Singh D, Banerji AK et al (2009) Clinical studies for improving radiotherapy with 2-deoxy-D-glucose: present status and future prospects. J Cancer Res Ther. https://doi.org/10.4103/0973-1482.55136
Dwarakarnath BS, Jain V (2009) Targeting glucose metabolism with 2-deoxy-D-glucose for improving cancer therapy. Future Oncol 5:581–585
El Mjiyad N, Caro-Maldonado A, Ramírez-Peinado S, Mũoz-Pinedo C (2011) Sugar-free approaches to cancer cell killing. Oncogene 30:253–264
Fan YJ, Zong WX (2013) The cellular decision between apoptosis and autophagy. Chin J Cancer 32:121
Gallaher WR, Levitan DB, Blough HA (1973) Effect of 2-deoxy-D-glucose on cell fusion induced by Newcastle disease and Herpes simplex viruses. Virology 55:193–201
Gounder MK, Lin H, Stein MN, Goodin S, Bertino JR, DiPaola RS (2010) Abstract 2756: Phase I trial of 2-deoxyglucose for treatment of advanced solid tumors and hormone refractory prostate cancer: a pharmacokinetics (PK) assessment. Cancer Res 70:2756–2756

Grein J, Ohmagari N, Shin D et al (2020) Compassionate use of remdesivir for patients with severe Covid-19. N Engl J Med 382:2327–2336
Hadizadeh F (2021) Supplementation with vitamin D in the COVID-19 pandemic? Nutr Rev 79: 200–208
Hardie DG, Lin SC (2017) AMP-activated protein kinase—not just an energy sensor F1000Res:10.12688/F1000RESEARCH.11960.1/DOI
Hasöksüz M, Kiliç S, Saraç F (2020) Coronaviruses and SARS-COV-2. Turkish J Med Sci 50:549–556
Hossen MS, Barek MA, Jahan N, Safiqul Islam M (2020) A review on current repurposing drugs for the treatment of COVID-19: reality and challenges. SN Comp Clin Med 10(2):1777–1789
Hu B, Guo H, Zhou P (2021) Characteristics of SARS-CoV-2 and COVID-19. Nat Rev Microbiol 19(3):141–154
Icard P, Lincet H, Wu Z, Coquerel A, Forgez P, Alifano M, Fournel L (2021) The key role of Warburg effect in SARS-CoV-2 replication and associated inflammatory response. Biochimie 180:169
Jain VK, Kalia VK, Sharma R, Maharajan V, Menon M (1985) Effects of 2-deoxy-D-glucose on glycolysis, proliferation kinetics and radiation response of human cancer cells. Int J Radiat Oncol Biol Phys 11:943–950
Jain VK, Pohlit W (1972) Influence of energy metabolism on the repair of X-ray damage in living cells. Biophysik 8(3):254–263
Jha B, Pohlit W (2009) Reversibility of inhibition of DNA double strand break repair by 2-deoxy-D-glucose in Ehrlich ascites tumour cells. Int J Radiat Biol 63:459–467. https://doi.org/10.1080/09553009314550611
Kalil AC (2020) Treating COVID-19—off-label drug use, compassionate use, and randomized clinical trials during pandemics. JAMA 323:1897–1898
Keiser MJ, Roth BL, Armbruster BN, Ernsberger P, Irwin JJ, Shoichet BK (2007) Relating protein pharmacology by ligand chemistry. Nat Biotechnol 25:197–206
Khan HJ, Rohondia SO, Othman Ahmed ZS, Zalavadiya N, Dou QP (2020) Increasing opportunities of drug repurposing for treating breast cancer by the integration of molecular, histological, and systemic approaches. Drug Repurpos Cancer Ther:121–172
Khurana P, Varshney R, Gupta A (2022) A network-biology led computational drug repurposing strategy to prioritize therapeutic options for COVID-19 (preprint). Heliyon 8(5):e09387
Kilbourne ED (1959) Inhibition of influenza virus multiplication with a glucose antimetabolite (2-deoxy-D-glucose). Nature 183(4656):271–272
Kim PJ, Lee DY, Jeong H (2009) Centralized modularity of N-linked glycosylation pathways in mammalian cells. PLoS One. https://doi.org/10.1371/JOURNAL.PONE.0007317
Kulshrestha S, Arora T, Sengar M, Sharma N, Chawla R, Bajaj S, Raghav PK (2021) Advanced approaches and in silico tools of chemoinformatics in drug designing. In: Chemoinformatics and bioinformatics in the pharmaceutical sciences, Academic Press. pp. 173–206
Latz D, Thonke A, Juling-Pohlit L, Pohlit W (1993) Tumor response to ionizing radiation and combined 2-deoxy-D-glucose application in EATC tumor bearing mice: monitoring of tumor size and microscopic observations. Strahlentherapie und Onkologie: Organ der Deutschen Rontgengesellschaft 169:405–411
Leung HJ, Duran EM, Kurtoglu M, Andreansky S, Lampidis TJ, Mesri EA (2012) Activation of the unfolded protein response by 2-deoxy-D-glucose inhibits Kaposi's sarcoma-associated herpesvirus replication and gene expression. Antimicrob Agents Chemother 56:5794–5803
Li SY, Tang YS, Chan YJ, Tarng DC (2020) Impact of the COVID-19 pandemic on the management of patients with end-stage renal disease. J Chinese Med Assoc 83:628–633
Liberti MV, Locasale JW (2016) The Warburg effect: how does it benefit cancer cells? Trends Biochem Sci 41:211–218
Liu X, Fang P, Fang L, Hong Y, Zhu X, Wang D, Peng G, Xiao S (2019) Porcine deltacoronavirus nsp15 antagonizes interferon-β production independently of its endoribonuclease activity. Mol Immunol 114:100–107

Luo Y, Zhao X, Zhou J, Yang J, Zhang Y, Kuang W, Peng J, Chen L, Zeng J (2017) A network integration approach for drug-target interaction prediction and computational drug repositioning from heterogeneous information. Nat Commun 8(1):1–13

Maehama T, Patzelt A, Lengert M, Hutter K-J, Kanazawa K, Hausen H, Rösl F (1998) Selective down-regulation of human papillomavirus transcription by 2-deoxyglucose. J Cancer 76:639–646

Maschek G, Savaraj N, Priebe W, Braunschweiger P, Hamilton K, Tidmarsh GF, de Young LR, Lampidis TJ (2004) 2-deoxy-D-glucose increases the efficacy of adriamycin and paclitaxel in human osteosarcoma and non-small cell lung cancers in vivo. Cancer Res 64:31–34

Mesri EA, Lampidis TJ (2021) 2-Deoxy-D-glucose exploits increased glucose metabolism in cancer and viral-infected cells: relevance to its use in India against SARS-CoV-2. IUBMB Life 73:1198–1204

Miwa H, Shikami M, Goto M et al (2013) Leukemia cells demonstrate a different metabolic perturbation provoked by 2-deoxyglucose. Oncol Rep 29:2053–2057

Mohamed K, Yazdanpanah N, Saghazadeh A, Rezaei N (2021) Computational drug discovery and repurposing for the treatment of COVID-19: a systematic review. Bioorg Chem 106:104490

Mohanti BK, Rath GK, Anantha N et al (1996) Improving cancer radiotherapy with 2-deoxy-D-glucose: phase I/II clinical trials on human cerebral gliomas. Int J Radiat Oncol Biol Phys 35:103–111

Muñoz-Pinedo C, Ruiz-Ruiz C, de Almodóvar CR, Palacios C, López-Rivas A (2003) Inhibition of glucose metabolism sensitizes tumor cells to death receptor-triggered apoptosis through enhancement of death-inducing signaling complex formation and apical procaspase-8 processing. J Biol Chem 278:12759–12768

Naqvi S, Joshi KN, Basu AK, Jain VK (1974) Optimizing cancer therapy—Studies on the combination of 131-I with 2-deoxy-D-glucose in the treatment of thyroid cancer. In Bombay, India: Abstr. VI Ann. Conf. Soc. Nucl. Med., p. 3

Narayan Bhatt A, Shenoy S, Munjal S, Chinnadurai V, Vinoth Kumar A, Shanavas A, Kanwar R, Chandna S (2022) 2-Deoxy-D-glucose as an adjunct to standard of care in the medical management of COVID-19: a proof-of-concept dose-ranging randomised clinical trial. BMC Infect Dis 22(1):669. https://doi.org/10.1186/s12879-022-07642-6

Oncogenesis MJ, Rae C, Sey CH, Mairs RJ (2018) Radiosensitization of prostate cancer cells by 2-deoxyglucose. Madridge J Oncogenesis 2:30–34

Pajak B, Siwiak E, Sołtyka M et al (2020) 2-Deoxy-D-glucose and its analogs: from diagnostic to therapeutic agents. Int J Mol Sci. https://doi.org/10.3390/IJMS21010234

Paraskevis D, Kostaki EG, Magiorkinis G, Panayiotakopoulos G, Sourvinos G, Tsiodras S (2020) Full-genome evolutionary analysis of the novel corona virus (2019-nCoV) rejects the hypothesis of emergence as a result of a recent recombination event. Infect Genet Evol 79:104212. https://doi.org/10.1016/J.MEEGID.2020.104212

Pareek S, Huang Y, Nath A, Huang RS (2020) The success story of drug repurposing in breast cancer. Drug Repurpos Cancer Ther:173–190

Park SE (2020) Epidemiology, virology, and clinical features of severe acute respiratory syndrome -coronavirus-2 (SARS-CoV-2; Coronavirus Disease-19). Clin Exp Pediatr 63:119

Pedersen SF, Ho YC (2020) SARS-CoV-2: a storm is raging. J Clin Invest 130:2202–2205

Pelicano H, Martin DS, Xu RH, Huang P (2006) Glycolysis inhibition for anticancer treatment. Oncogene 25(34):4633–4646

Pflaum J, Schlosser S, Müller M (2014) p53 Family and cellular stress responses in cancer. Front Oncol. https://doi.org/10.3389/FONC.2014.00285

Ponti G, Maccaferri M, Ruini C, Tomasi A, Ozben T (2020) Biomarkers associated with COVID-19 disease progression. Crit Rev Clin Lab:389–399. https://doi.org/10.1080/1040836320201770685

Pushpakom S, Iorio F, Eyers PA et al (2018) Drug repurposing: progress, challenges and recommendations. Nat Rev Drug Discov 18:41–58

Richardson P, Griffin I, Tucker C, Smith D, Oechsle O, Phelan A, Stebbing J (2020) Baricitinib as potential treatment for 2019-nCoV acute respiratory disease. Lancet (London, England) 395: e30–e31

Samal KC, Panda B, Behera L (2021) Anti-covid drug: 2-deoxy-D-glucose and Its mechanism of action. Biot Res Today 3:345–347

Sanchez EL, Lagunoff M (2015) Viral activation of cellular metabolism. Virology 479–480:609–618

Sarkar PK, das Mukhopadhyay C (2022) Mechanistic insights from the review and evaluation of ayurvedic herbal medicines for the prevention and management of COVID-19 patients. J Herbal Med 32:100554

Scholtissek C, Rott R, Hau G, Kaluza G (1974) Inhibition of the multiplication of vesicular stomatitis and newcastle disease virus by 2-deoxy-D-glucose. J Virol 13:1186–1193

Sharma A, Goyal S, Yadav AK, Kumar P, Gupta L (2020) In-silico screening of plant-derived antivirals against main protease, 3CLpro and endoribonuclease, NSP15 proteins of SARS-CoV-2. J Biomol Struct Dyn. https://doi.org/10.1080/07391102.2020.1808077/SUPPL_FILE/TBSD_A_1808077_SM2135.DOCX

Shutt DC, O'Dorisio MS, Aykin-Burns N, Spitz DR (2010) 2-Deoxy-D-glucose induces oxidative stress and cell killing in human neuroblastoma cells. Cancer Biol Ther 9:853

Singh S, Singh PK, Suhail H, Arumugaswami V, Pellett PE, Giri S, Kumar A (2020) AMP-activated protein kinase restricts zika virus replication in endothelial cells by potentiating innate antiviral responses and inhibiting glycolysis. J Immunol 204:1810–1824

Srinivasa Rao Dwarakanath B, Kumar Jain V (1987) Modification of the radiation induced damage by 2-deoxy-D-glucose in organ cultures of human cerebral gliomas. Int J Radiat Oncol Biol Phys 13:741–746

Srivastava K, Singh MK (2021) Drug repurposing in COVID-19: a review with past, present and future. Metab Open 12:100121

Surova O, Zhivotovsky B (2013) Various modes of cell death induced by DNA damage. Oncogene 32(33):3789–3797

Tan M, Liu Y, Zhou R, Deng X, Li F, Liang K, Shi Y (2020) Immunopathological characteristics of coronavirus disease 2019 cases in Guangzhou, China. Immunology 160:261–268

Tsai PH, Lai WY, Lin YY et al (2021) Clinical manifestation and disease progression in COVID-19 infection. J Chin Med Assoc 84:3–8

Velavan T (2020) The COVID-19 epidemic. Trop Med Int Health 25(3):278–280

Vetter P, Vu DL, L'Huillier AG, Schibler M, Kaiser L, Jacquerioz F (2020) Clinical features of covid-19. BMJ. https://doi.org/10.1136/BMJ.M1470

Wang D, Hu B, Hu C et al (2020) Clinical characteristics of 138 hospitalized patients with 2019 Novel coronavirus–infected pneumonia in Wuhan, China. JAMA 323:1061–1069

Wang Y, Li JR, Sun MX, Ni B, Huan C, Huang L, Li C, Fan HJ, Ren XF, Mao X (2014) Triggering unfolded protein response by 2-deoxy-D-glucose inhibits porcine epidemic diarrhea virus propagation. Antivir Res 106:33–41

Warburg O (1925) The metabolism of carcinoma cells. J Cancer Res 9:148–163

Whitebread S, Hamon J, Bojanic D, Urban L (2005) Keynote review: in vitro safety pharmacology profiling: an essential tool for successful drug development. Drug Discov Today 10:1421–1433

WHO Director-General's opening remarks at the media briefing on COVID-19 - 11 March 2020. https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19%2D%2D-11-march-2020. Accessed 22 Dec 2021

Wu Z, McGoogan JM (2020) Characteristics of and important lessons from the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. JAMA 323:1239–1242

Xi H, Kurtoglu M, Lampidis TJ (2014) The wonders of 2-deoxy-D-glucose. IUBMB Life 66:110–121

Xi H, Kurtoglu M, Liu H, Wangpaichitr M, You M, Liu X, Savaraj N, Lampidis TJ (2011) 2-Deoxy-D-glucose activates autophagy via endoplasmic reticulum stress rather than ATP depletion. Cancer Chemother Pharmacol 67:899–910

Xu R, Pelicano H, Zhou Y, Carew JS, Feng L, Bhalla KN, Keating MJ, Huang P (2005) Inhibition of glycolysis in cancer cells: a novel strategy to overcome drug resistance associated with mitochondrial respiratory defect and hypoxia. Cancer Res 65:613–621

Yuki K, Fujiogi M, Koutsogiannaki S (2020) COVID-19 pathophysiology: a review. Clin Immunol 215:108427

Zhao Q, Chu Z, Zhu L et al (2017) 2-Deoxy-D-glucose treatment decreases anti-inflammatory M2 macrophage polarization in mice with tumor and allergic airway inflammation. Front Immunol. https://doi.org/10.3389/FIMMU.2017.00637

Zhou P, Yang X, Wang X, Hu B, Zhang L et al (2020) A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 579(7798):270–273

Chapter 21
Repurposing Methylene Blue for the Management of COVID-19: Prospects, Paradox, and Perspective

Sandeep Sharma, Viney Jain, and Rakesh Kumar Sharma

Abstract COVID-19 onslaught has led to widespread morbidity and mortality globally. Another major concern, especially in developing countries like India, has been the development of fungal superinfection and colonization of other pathogens in hospitalized COVID-19 patients. Even though an armamentarium of repurposed, antiviral, anticytokine, and antifungal drugs is available to manage the disease progression, no single drug and/or therapy has provided positive clinical outcomes with efficacy and affordability. Therefore, it is imperative to explore innovative approaches for standalone treatment and/or adjunct therapeutic regimes based on our current understanding of disease prognosis. Low-income and emerging economies have less resources to protect themselves against the COVID-19-induced health and economic crisis. With the continuously evolving nature of coronavirus, a cost-effective strain independent mechanism that could be delivered easily even in a nonhealthcare setting is an urgent need of the hour. Methylene blue appears an apt candidate as it is an FDA-approved safe drug that is economically viable and easily available. Since MB has a long-standing history of being used in clinical setup for diverse medical applications and possesses intrinsic anti-inflammatory, anticytokine, and antifungal properties, this study analyzes prospects of its use in the management of COVID-19. Paradox and prospects of MB applications for the management of COVID-19, with or without fungal superinfections, are also discussed.

Keywords Antifungal · Antiviral · Coronavirus · COVID-19 · Methylene Blue · Superinfection

S. Sharma
I.K. Gujral Punjab Technical University, Jalandhar, Punjab, India

V. Jain
Jain Vishva Bharti University, Ladnun, Rajasthan, India

R. K. Sharma (✉)
SGT University, Gurugram, Haryana, India
e-mail: prochancellor@sgtuniversity.org

R. C. Sobti et al. (eds.), *Drug Repurposing for Emerging Infectious Diseases and Cancer*, https://doi.org/10.1007/978-981-19-5399-6_21

21.1 Introduction

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) presents a series of progressive pneumonia-like diseases that primarily affect the respiratory tract. The World Health Organization declared the SARS-CoV-2 outbreak a pandemic on March 11, 2020. Most of the time, the COVID-19 infection commences when an uninfected individual inhales virus-laden droplets or aerosols containing the virus in the air passage. The cells of the nasal pathway have a disproportionately high cell surface receptor called angiotensin converting enzyme 2 (ACE2). The virus gets attached to this receptor through its spike glycoprotein and enters the cell, where it uses the machinery of the cell to make numerous copies of itself and invade more normal cells. The terminal alveoli in the lungs, which are about 600 million, also contain cells rich in ACE2 receptors where the virus attacks as the disease progress. ACE2 receptors are also present on the vascular endothelial cells of all organs. Due to this, endothelial inflammation is prominent across vascular beds in various organs of COVID-19 patients (Varga et al. 2020). With the further progression of the disease, the production of free radicals like the reactive oxygen species (ROS), reactive nitrogen species (RNS), and cytokines exceed the ability of tissues to cope up with the virus and neutralize them. This induces oxidative stress, necrosis, or apoptosis, and finally causes excessive damage to the lung alveoli and vascular endothelium (Varga et al. 2020; Carsana et al. 2020). Figure 21.1 summarizes the stage-wise progression of COVID-19, symptoms, and the diagnosis. With the deadly subsequent waves of COVID-19, a rush of cases involving "black fungus" or

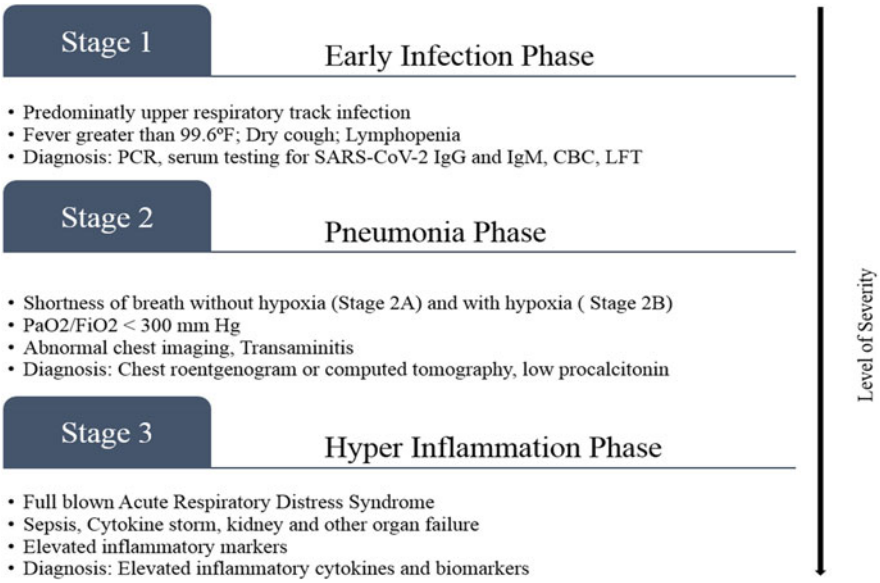


Fig. 21.1 Stage-wise progression of COVID-19, symptoms, and the diagnosis

mucormycosis had also been reported among recovering and recovered COVID-19 patients in India (Divakar 2021; Mahalaxmi et al. 2021). This aggressive infection mainly affected the nose, eye, sinuses, brain, and lungs. This infection had been of major concern and life-threatening for diabetic patients or severely immunocompromised individuals, including severe and critically ill COVID-19 patients on steroid medications that weaken the immune system.

The current sophisticated therapy regime and the hospital stay of patients having COVID-19 may lead to an inordinate amount of medical expenditure and comorbidities. Owing to these constraints, particularly for the developing economies having low-resourced settings, there is an ardent need for new cost-effective therapeutic options that can be delivered effectively for COVID-19 patients and also address the associated conditions. One of the important aspects of effective management of COVID-19 patients involves the concurrent management of ROS, RNS, and bradykinin, which are the major determinants in COVID-19 progression (Karamyan 2021; Hosseini et al. 2020). Methylene blue (MB) offers the solution to this triad problem and also addresses the associated infections.

Methylene blue (MB) or methylthionine chloride, chemically (3,7-bis (dimethyl amino) phenothiazine-5-ium chloride), a phenothiazine derivative, is an FDA- and EMA-approved drug and offers an excellent safety profile. It can play a pivotal role in treating disease progression as it inhibits the formation of superoxide anion (ROS precursor) by blocking the xanthine oxidase pathway (Salaris et al. 1991), counteracts the synthesis of nitric oxide (RNS precursor) by direct inhibition of nitric oxide (NO)-synthase (Mayer et al. 1993) and may terminate effects of bradykinin by inhibition of nitric oxide synthase inhibitor, with the overall result of prevention of excess inflammation (Denny et al. 2015; Wang et al. 2016; Ghahestani et al. 2020). As several bradykinin functions in the body are exerted by nitric oxide (NO), the inhibition of NO-synthase inhibitor by MB aborts the inflammatory effects of bradykinin and stimulates oxygen saturation and hemoglobin recruitment.(Mayer et al. 1993; Rhaleb et al. 1989). Figure 21.2 provides an insight into the inhibitory effect of MB on major determinants of COVID-19 progression.

MB is mainly indicated in methemoglobinemia wherein it acts by converting (reducing) ferric iron in methemoglobin (met-Hb) to the ferrous ion of normal hemoglobin (Clifton and Leikin 2003). It also exerts broad-based antiviral activity with and without light (Cagno et al. 2021). MB has a reputation as a strong protein binder owing to its structural space (Fletcher and Hamilton 2006) and has a potent antifungal activity (Guffey et al. 2017; Ansari et al. 2016). MB has also been used for making blood products virus-free before transfusions and malaria treatment (Bojadzic et al. 2021; Lu et al. 2018). Owing to this multitude of properties of MB, the current article attempts to explore the existing corpus of information available on MB and set forth an evidence-based hypothesis, prospects, and paradox governing its use for treating COVID-19 and its associated comorbidities.

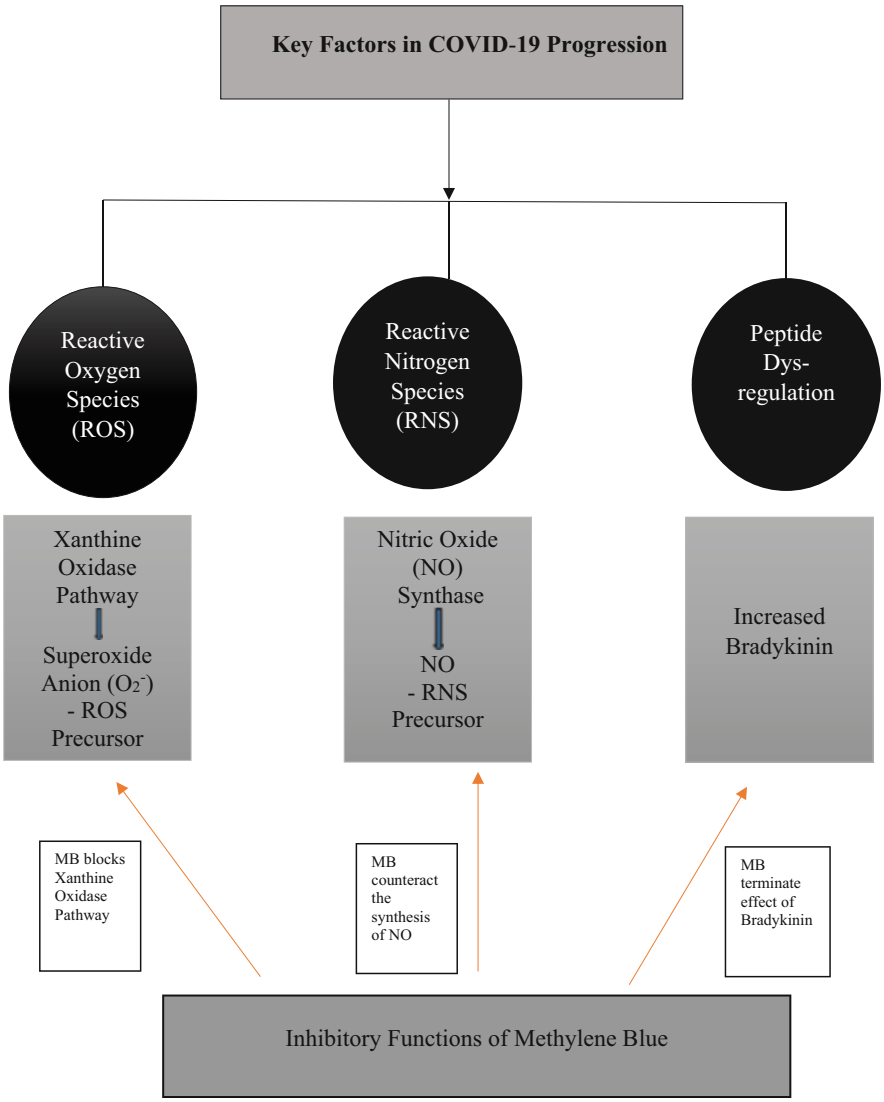


Fig. 21.2 Intricate relationship between various factors in COVID-19 disease progression and the inhibitory effect of MB on them

21.2 Problems with Conventional Therapy

SARS-CoV-2 is a progressive degenerative disease involving multiple mechanisms and an array of immunological responses leading to acute respiratory distress syndrome (ARDS). As symptoms of COVID-19 infection like ARDS take some time to occur, most of the available antiviral drugs fail to fight it. This is because by

that time, the virus has already triggered an enhanced anti-inflammatory response and the overall viral load has significantly decreased (Wang et al. 2020; Cao et al. 2020). Using anticytokine drugs is also not recommended as these drugs act on one or only a few of a range of cytokines involved in the host inflammatory response and other important mediators of inflammation like ROS and RNS are not targeted (Scigliano and Scigliano 2021; Lang et al. 2002). Both antiviral and anticytokine drugs cannot inhibit the production of free radicals and cytokines at the same time, which is of paramount importance in treating disease progression (Dabholkar et al. 2021). Using nitric oxide (RNS precursor) inhibitor is also not recommended owing to their lack of specificity in blocking the various isoform of NOS namely L – NMMA and L – NAME, which may cause unwanted broad tissue necrosis (Evora 2016). Thus, with the onset of the ARDS phase, various mediators of inflammation, such as ROS, RNS, and cytokine production become intractable and any attempt to control it with conventional therapy does not yield favorable results.

21.3 Rationale and Hypothesis of Methylene Blue as an Adjunct to Standard of Care

SARS-CoV-2 virus after entering the host leads to multiple clinical manifestations. Therefore, it is imperative to target the treatment depending upon the patient exposure to the virus and the stage of disease progression. Figure 21.3 presents the different mechanistic approaches during treatment of the SARS-CoV-2 using MB.

Inhibition of Virus Entry: A recent study has shown that MB has inhibitory activity against the interaction between SARS-CoV-2 spike protein and its cognate receptor ACE2 (Bojadzic et al. 2021). This process is the first and the most critical step in initiating the viral attachment and the simultaneous entry of coronavirus inside the host cell. Also, the endosome and lysosomes are critical components that are involved in the entry of SARS-CoV-2 into the cells. MB, upon protonation, actively accumulates in the lysosomes and raises its pH. Because of this, the low pH-dependent hydrolases gets blocked and might lead to the inhibition of virus uncoating and membrane fusion and ultimately the inhibition of endosome differentiation at intermediate stages of endocytosis (Wainwright and Amaral 2005).

Inhibition of Virus Replication: MB is a zinc ionophore and helps in transporting Zn^{2+} ions across the lipid membrane in the cell. However, Zn^{2+} possesses an inherent property to inhibit the elongation of RNA-dependent RNA polymerase, which is a critical component in the replication process of the virus within the host (Te Velthuis et al. 2010). In this way, MB could significantly inhibit the progression and replication of the SARS-CoV-2 virus.

Respiratory Control: Nitric oxide is one of the key determinants in COVID-19 progression and plays a major role in viral-induced pneumonia (Fang et al. 2021). Nitric oxide causes the suppression of mitochondrial respiration through cytochrome oxidase and NADH ubiquinone oxidoreductase targeting. This causes an

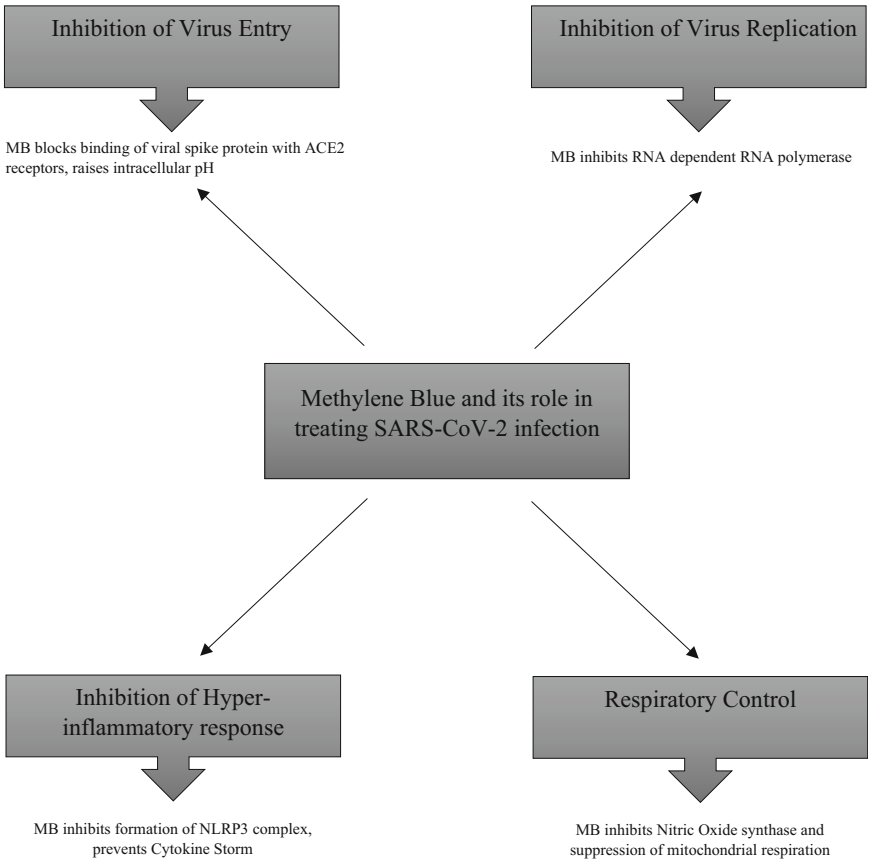


Fig. 21.3 Mechanistic approaches for treating SARS-CoV-2 virus using methylene blue

amplification of the hypoxic state and oxygen debt during the viral attack. MB directly acts with nitric oxide synthase, thereby inhibiting the production of nitric oxide. MB could also be used in slowing the progression of the disease and increasing the recruitment of Hb, as well as oxygen saturation (Mayer et al. 1993; Rhaleb et al. 1989). By reducing met-Hb, MB increases the SpO_2% and activity of the normally slow NADPH–methemoglobin reductase pathway, causing a decrease in the hypoxic state (Alamdari et al. 2020a).

Inhibition of Inflammatory Response: Viral infection in the host body is triggered by the formation and activation of NLRP3 (NOD-like receptor protein3) inflammasome. In COVID-19 patients, activation of the NLRP3 complex causes macrophages to release interleukins, a key component of the inflammatory response (Chen et al. 2019). This causes an onset of hyperinflammatory response, which may further lead to cytokine storm in advanced cases. MB has an inhibitory action on the

Table 21.1 Recent clinical studies showing prospects of MB for COVID-19 and the associated complications

Clinical manifestation/study	Therapy	Outcome
A COVID-19 patient was suffering from ulcerative lip lesions which could be observed as extensive crusted ulcers on both sides of the lip vermilion	Combination of antimicrobial photodynamic therapy (aPDT) using MB and photobiomodulation therapy (PBMT)	Following two sessions of aPDT and one session of PBMT, complete healing of lip lesion was observed within 4 days (Ramires et al. 2021)
A study on postoperative delirium (POD) and early postoperative cognitive dysfunction (POCD) on elderly patients was done. The patients were on non-cardiac major surgery	Intraoperative IV 2 mg/kg MB within 60 min immediately after anesthetic induction	A marked decline in POD and POCD was observed in the patients (Deng et al. 2021)
A cohort of 2500 French patients having cancer	Combination therapy comprising standard therapy and α-lipoic acid (800 mg twice a day), hydroxy-citrate (500 mg three times a day) and MB (75 mg three times a day) and a low carb diet	No influenza-like illnesses were observed in any of the patients (Henry et al. 2020)
Combination therapy of methylene blue-vitamin C–N-acetyl cysteine was given to five COVID-19 patients who were admitted to ICU	Patients were administered MB (1 mg/kg) along with vitamin C (1500 mg/kg) and N-acetyl Cysteine (1500 mg/kg) orally or intravenously	A marked increase in SPO_2% was observed along with the acceleration of normally slow NADPH–met-Hb reductase (Alamdari et al. 2020a)
80 patients suffering from severe COVID-19	Patients were given oral MB in one subset, and the other subset was given standard of care	Adding MB to treatment protocols for COVID-19 patients greatly improved SpO_2 and respiratory distress, leading to decreased hospital stay and mortality (Hamidi-Alamdari et al. 2021)

NLRP3 complex on macrophages and may help in preventing hyperinflammatory response associated with SARS-CoV-2 infection (van den Berg and te Velde 2020).

Recent clinical trials and explorative studies have shown the remarkable capability of MB, either alone or in a combination, to significantly provide the desired clinical results for patients suffering from COVID-19 and its associated comorbidities. Table 21.1 shows the various human studies using MB and the study outcome for COVID-19 and its associated complications. These studies emphasize the multidimensional possibility of using MB in COVID-19 patients and associated complications, warranting the use of MB on a commercial scale.

21.4 Value Addition by Photoirradiation

Photodynamic therapy (PDT) uses a simple concept of preferentially localizing a nontoxic dye, known as a photosensitizer (PS) in the target tissues or cells, followed by activating it using harmless visible light. This activation of PS causes the production of ROS which has lethal effects on cells and tissues that had bound the PS. MB is a photosensitizer with a maximum absorption peak of 668 nm (Nadtoka et al. 2020). MB, as an intercalator (owing to the positive charge on its surface), intercalates within nucleic acid strands by combining with negatively charged G-C base pairs of viral nucleic acid. Subsequent application of light causes its excitation and leads to the production of reactive singlet oxygen. This singlet oxygen is highly reactive and causes the oxidation of guanosine to guanine peroxide, breaking the nucleic strands and providing the desired virucidal activity (Lozano et al. 2013). Experiments have shown that MB upon photoactivation could completely inactivate the virus in plasma before therapy, rendering it free from virus and ensuring patient safety in plasma therapy of COVID-19 patients (Jin et al. 2021).

The fungal burden in skin abrasion wounds infected with *Candida albicans* is also considerably removed by combining photoirradiation with MB. The ratio of dye concentration to fungal cell density has a direct relationship with the efficacy of PDT. Experiments at high density of Candida albicans have proved that for achieving a therapeutically efficient PDT effect, both MB and light sources at higher concentrations are required (Demidova and Hamblin 2005). However, it is to be noted that too high a concentration of PS causes the attenuation of light penetrating the infected wounds by fruitlessly absorbing the light by the dye molecules not bound to any microbial cell. This may hamper the desired efficacy of PDT. Experiments by Dai et al. had revealed that with an initial average *C. albicans* cell density of 10^7 CFU/ml and at MB concentration of 20 μM, there was approximately 4.43 log CFU inactivation after an exposure of 9.75 J/cm^2 light. At 10^8 CFU/ml and 20 μM MB, the surviving fraction of *C. albicans* was reduced by 30% at the same light dose (Dai et al. 2011). Apart from *Candida* genus, combination therapy of MB and PDT has also been reported to provide fungicidal efficacy against *Sporothrix globosa* in vitro and in vivo (Li et al. 2019). Further studies have confirmed that photosensitized MB can also act against *Trichophyton rubrum* (Valkov et al. 2021). Based on this analogy, MB PDT can be used for treating black fungus, yellow fungus, and white fungus disease.

21.5 Utility of MB in Fungal Superinfections Associated With COVID-19

Even though a range of antifungal compounds like azoles, polyenes, allylamines, and echinocandins are available commercially for treating COVID-19-related fungal complications, their side effect, affordability, and multidrug resistance cannot be

ignored (White et al. 1998; Farmakiotis et al. 2014). For example, the use of potent compound like Amphotericin B as an antifungal agent in COVID-19 may predispose the patients to toxicity and may not be affordable to general masses owing to its high cost. MB has been reported to provide a critical advantage in treating such fungal superinfection as it causes mitochondrial dysfunction, disruption of redox, and membrane homeostasis of the fungus, particularly those belonging to *Candida* genus (Ansari et al. 2016) and further impede the yeast to hyphal transition which causes the virulence of fungus in the host body (Lu and Su 2014; Thomas et al. 2013). The development of resistance to PDT by microbes is an unlikely event because, unlike most other antifungal drugs, PDT is typically a multitarget process (Lyon et al. 2011). Therefore, the potent antifungal activity exhibited by MB with or without photoirradiation is of value in tackling fungal superinfections associated with COVID-19 (Li et al. 2021; Cieplik et al. 2018).

21.6 Risk–Benefit Analysis

MB has been an established drug used in the clinical setting for decades (Schirmer et al. 2011). MB offers an excellent safety profile for treating methemoglobinemia, malaria, and cyanide poisoning. MB can be administered through a range of routes viz. intraduodenally, orally, and intravenously (O'leary et al. 1968; Walter-Sack et al. 2009). Table 21.2 provides a detailed insight into the multifarious applications of MB for treating a plethora of diseases, besides its use in COVID-19 and associated complications.

Owing to its safety profile and a long-standing history of use in clinical practice, few veteran pulmonologists in developing countries like India have used MB on their patients for treating the COVID-19 and the associated complications (Sharma 2021). The results were encouraging where it was revealed that around 7000 COVID-19 patients did not develop mucormycosis, a secondary complication of COVID-19, on treatment with MB. Apart from mucormycosis, the patients on MB also did not suffer from post-COVID-19 fibrosis of the lungs owing to the strong antifibrotic action of MB (Golwalkar 2020). The treatment regime comprises using MB in nebulized form and sublingually together. MB in the sublingual and nebulized form helps remarkably in clearing alveolar-capillary blocks. Intravenous administration may be required for patients with severe cyanosis (oxygen levels less than 85%). The primary line of treatment is using 0.1% MB as inhalation through a nebulizer using nasal mask while the supportive line of treatment comprises using MB sublingually. Putting few drops of MB in the humidifier, moisturizing jar, or concentrators through which oxygen passes can even help in preventing almost all types of contamination, including mucormycosis, present in oxygen (Shukla 2021). MB has also been reported in successful pretreatment of grafts during transplantation, where it significantly reduced the risk of virus transmission (Helfritz et al. 2018). The extensive use of personal protective equipment (PPE) by the healthcare workers, frontline workers, and other stakeholders has led to their supply

Table 21.2 Diverse application of MB for treating an array of diseases besides COVID-19 and associated complications

Disease/ complication	Treatment/study	Outcome
Antiviral activity		
Genital herpes	MB in 0.1% concentration was applied topically to the infected part followed by exposure to 15-watt fluorescent lamp for 15 minutes	Disease eradication in about 70% of instances by topically using MB and light exposure of the lesions (Weinstein 1975)
Acute hemorrhagic conjunctivitis	Experiments on virus inhibition of E70 and CA24v acute hemorrhagic conjunctivitis (AHC) epidemic isolates in human corneal epithelial cells using 0.25-1% MB were done. AHC virus cytopathy inhibition by MB was investigated	MB pretreatment significantly inhibited virus-induced caspase-3 activation and DNA fragmentation (Langford et al. 2020)
CNS-related disorders		
Depression and anxiety	A clinical trial in which 31 bipolar manic-depressive subjects were treated with 300 mg/day MB	MB helped in reducing the amount of illness by almost half (Naylor et al. 1986)
Alzheimer disease	Paired helical filaments (PHFs) from microtubule associated protein tau were treated with 0.01%–1% MB	MB reverses the proteolytic stability of protease-resistant PHFs by blocking the tau-tau binding interaction through the repeat domain (Wischik et al. 1996)
Pain	1 ml of 1% MB was injected into the diseased disc for treating chronic discogenic low back pain	Based on the Oswestry Disability Index, marked improvement was observed in 87% of the patients and a median improvement of 68% in disability was obtained (Kim et al. 2012)
Antifungal activity		
Toenail onychomycosis	Using 2% MB aqueous solution and irradiation with light-emitting diode device	Patients on MB treatment achieved a clinical cure rate of 90% (Figueiredo Souza et al. 2014)
Trichophyton rubrum infection	10 mL of a 2.0 μmol/L solution of MB was allowed to be in contact with *Trichophyton rubrum* followed by light irradiation at 625 nm	A rate of delivery of 7.80 mW/cm^2 significantly inhibited T. rubrum when applying 625 nm light in PDT using MB (Guffey et al. 2017)
Antibacterial activity		
Hidradenitis suppurativa	MB in niosomel gel was used in conjunction with intense pulsed light for topical treatment	A marked decrease in Hidradenitis Suppurativa Lesion, Area and Severity Index (HS-LASI) after treatment was elicited. The subjects also did not suffer from pain, erythema or hyper- pigmentation (Fadel and Tawfik 2015)

(continued)

Table 21.2 (continued)

Disease/ complication	Treatment/study	Outcome
Rickettsial infection	Vero cells infected with rickettsia were treated with MB and red light	48 h post-treatment with MB followed by exposure of red light for 30 min, the viable *Rickettsia slovaca* count was reduced by 96% (Špitalská et al. 2018)
Root canal disinfection	Root canals of extracted teeth were incubated with *Enterococcus faecalis* for three weeks and further treated with NaOCl irrigation followed by MB-PDT and observed for colony-forming units (CFU)	Mean values of CFU decreased by 99% compared to the control group (Mozayeni et al. 2020)
Other diseases		
Progeria, a genetic premature ageing disease	Studies on dermal fibroblast lines using MB solution for ROS scavenging effect and Nrf2 expression were conducted. The effect of MB on skin thickness, skin hydration and upregulation of elastin and collagen were also investigated	MB was effective in stimulating skin fibroblast proliferation and delaying cellular senescence (Mei Xiong et al. 2017)
Cyanide poisoning	Potassium cyanide infusion at 0.75 mg/kg/min was given to rats, followed by MB at 20 mg/kg. The human equivalent dose was about 4 mg/kg. In a separate study group, MB was administered 5 min after the end of sub-lethal exposure to a toxic concentration of cyanide	MB given instantaneously after cyanide infusion restored blood pressure, cardiac contractility and limited the O_2 deficit which resulted in the survival of all animals. Giving MB 5 minutes after a non-lethal dose of CN also accelerated lactate recovery and O_2 deficit. A marked decline in ROS production and restoration of ATP/ADP ratio was also obtained (Haouzi et al. 2019)
Transplant operations		
Heart transplantation	Cardiac patient with a history of myocarditis underwent an orthotopic bicaval cardiac transplantation and explantation of the automatic implantable cardioverter-defibrillator device. The underlying disease was dilative cardiomyopathy. MB was given IV (2 mg/kg body weight) over 30 min	Hemodynamic status remained stable with no fever or inflammatory response evidenced by normal leukocyte counts and C-reactive protein. MB prevented the possible case of malperfusion and exhibited a catecholamine-saving effect (Kofidis et al. 2001)
Liver transplantation	Infusion of MB was done at 100 mg bolus for 12 h/1.5 mg/kg postoperation	Initially, in absence of MB, the patient developed symptoms of primary graft dysfunction and did not show a good response to vasopressor support. However, MB infusion greatly controlled the situation, leading to increased urine output. Further, the patient

(continued)

Table 21.2 (continued)

Disease/ complication	Treatment/study	Outcome
		was weaned from vasoactive support and mechanical ventilation (Vilalva et al. 2018)
Lung transplantation	Before graft implantation, 2 ml of MB at 1% concentration was intraperitoneally injected into the animal	Minimization of Ischemia-reperfusion injury, which is one of the most common obstacles for lung transplantation, was successfully obtained (Abreu et al. 2014)

shortage and disposal issues, particularly in low budget economies. MB treatment has been reported to decontaminate PPE containing coronaviruses with the maintenance of integrity and fit even after five cycles of treatment (Lendvay et al. 2019).

The normal recommended dose of MB is 1–2 mg/kg usually given as 0.1–0.2 mL/kg of a 1% (10 mg/mL) solution intravenously administered over 5–10 min (Clifton and Leikin 2003). However, an increase in dose beyond this therapeutic dose may lead to dose-dependent toxic manifestations. At 2–4 mg/kg, MB may cause hemolytic anemia and skin desquamation in infants, while a dose of 7 mg/kg may cause nausea, pain in the abdominal region and chest region, increased body temperature, and hemolysis. A still higher dose of 20 mg/kg may cause hypotension (Bilgin et al. 1998; Porat et al. 1996). The clinically used dose of MB as oral administration ranges between 50 and 300 mg (Oz et al. 2011). At this oral dose, its absorption is reported to be 53–97% with plasma peak concentrations achieved at around half an hour to 1 h (Disanto and Wagner 1972). MB undergoes multicompartmental pharmacokinetics with both intravenous and oral administrations having a terminal plasma half-life of 5–7 h (Peter et al. 2000). MB is a potent reversible inhibitor of monoamine oxidase (MAO), particularly MAO-A, and using it concomitantly with selective serotonin reuptake inhibitors (SSRIs) at doses exceeding 5 mg/kg can lead to inhibition of serotonin degradation (Gillman 2006). This causes serotonin syndrome, a state in which there is a marked increase in the toxic concentration of serotonin.(Oz et al. 2011; Ramsay et al. 2007). It is imperative to note here that the overdoses of SSRIs produce only mild-to-moderate degrees of serotonin toxicity. Moreover, it is the concomitant use of MAO inhibitors with SSRIs that may precipitate severe serotonergic side effects which can further progress to the dose-dependent serotonin toxicity (Gillman 2008). The serotonin toxicity can be found by using MB not only by the parenteral route but also by the oral intake of MB containing agents (Zuschlag et al. 2018). Administration of MB to patients with glucose-6-phosphate dehydrogenase deficiency may lead to the development of hemolytic anemia (Rosen et al. 1971). This condition is further characterized by the formation of Heinz bodies with no reduction in met-Hb levels (Clifton and Leikin 2003). An important thing to be borne in mind while using MB in COVID-19 is that the treatment course should start early, much before the onset of inflammation, as it is impossible to reverse tissue damage caused by the virus

inflammatory response (Scigliano and Scigliano 2021). Another critical aspect is the use of MB in reduced form along with antioxidants, as the oxidized form may increase oxidative stress and consequently inflammation (Alamdari et al. 2020b).

21.7 Prospects, Paradox, and Perspective for COVID-19 and the Associated Complications

Owing to the lack of awareness about the significant positive clinical aspects of MB, its use was limited in the first and subsequent waves of COVID-19. Irrational use of steroids for treating the COVID-19 patients coupled with a large population with comorbidities, and use of industrial oxygen or oxygen concentrators in an unhygienic way, greatly led to the onset of black fungus disease (Mahalaxmi et al. 2021; Bhogireddy et al. 2021). This is incongruent with the fact that the sources of black fungus are saprophytes and humans usually had no danger from them. The cases of black fungus were more apparent in adult patients with COVID-19 and acute hypoxemic respiratory failure. These patients were on conventional oxygen therapy or enhanced respiratory support that included high-flow nasal cannula, noninvasive positive-pressure ventilation, intubation, and invasive mechanical ventilation. Because of this, such patients got maximum exposure to contaminated oxygen, thus contributing toward "Hospital Acquired Infection of Mucormycosis" and a sudden spike in cases of black fungus disease (Bhatia 2021; Stone et al. 2021). Given the fact that COVID-19 patients may slowly progress into fungal infections due to irrational use of antiviral and anticytokine drugs, it is postulated that use of MB, either alone or in combination with PDT, especially during the early course of disease progression may render significant positive clinical outcome. It is recommended to add MB either as compassionate and/or informed voluntary treatment for patients suffering from COVID-19 and its associated complication. Even though few veteran specialists are using MB in their patients, though sporadically in various countries, studies and randomized clinical trials highlighting its multifarious uses are still missing. No big pharmaceutical firm seems interested in investing in this freely available drug, which makes this drug highly neglected. Even medical practitioners have not explicitly used and prescribed MB to their patients owing to the lack of knowledge and awareness regarding its multifarious application. Since MB has a proven track record of safety for decades, its commercial exploitation as an adjunct to the Standard of Care cannot be overruled. MB can be used equally well for prophylaxis and therapeutic purpose. Using MB as inhalation or by a simple technique of nebulization can prevent an array of viral and fungal diseases, including COVID-19 complications, while its therapeutic use can be exploited under medical supervision. As the third wave of COVID-19 pandemic is just around the corner and nothing can be predicted about the clinical manifestation of this disease, it is high time government authorities and stakeholders should follow these anecdotes from the experts and facilitate efforts to qualify MB as a key component in the fight

against COVID-19 pandemic. Clinical trials using MB in the general populace, either alone or in combination with PDT, are necessary to avert and thwart the future clinical progression of COVID-19-related disorders and the associated mortality and morbidity.

21.8 Conclusion

Ardent efforts by government officials, DRDO, and the NGOs have resulted in an adequate supply of medical oxygen in India and other developing countries, which was a far-fetched thing a few months ago. This, however, does not mean that the threat and concern of COVID-19 have subsided, as it can still wreak havoc. More efforts are required to focus on ways of prevention of virus attack and the associated superinfections. Even though the current vaccination drive has provided much relief, it has the inherent drawback of being strain specific. Of particular concern is the emergence of new variants for which vaccination may not deliver the desired protection. MB has been indicated for use for an array of diseases like common influenza, urinary tract infection, central nervous system-related disorders, antifungal agent, antiviral agent, and for treating methemoglobinemia and cyanide poisoning. This warrants its use as a broad-based antiviral and antifungal agent not specific for COVID-19 patients but a plethora of other commonly encountered diseases and for unanticipated pandemics and epidemics, the most recent being COVID-19. As the majority of the medical use of MB can be explored by simple way of steam inhalation or nebulization, it provides an easy option for use by masses at home in a cost-effective manner even in nonhospital or home care setting.

Financial Support & Sponsorship

None.

Conflicts of Interest All authors declare no competing interests.

References

Abreu MDM, Pazetti R, De Almeida FM, Correia AT, Parra ER, Da Silva LP et al (2014) Methylene blue attenuates ischemia-reperfusion injury in lung transplantation. J Surg Res 192:635–641

Alamdari DH, Moghaddam AB, Amini S, Keramati MR, Zarmehri AM, Alamdari AH et al (2020a) Application of methylene blue -vitamin C –N-acetyl cysteine for treatment of critically ill COVID-19 patients, report of a phase-I clinical trial. Eur J Pharmacol 885:173494

Alamdari DH, Moghaddam AB, Amini S, Alamdari AH, Damsaz M, Yarahmadi A (2020b) The application of a reduced dye used in orthopedics as a novel treatment against coronavirus (COVID-19): a suggested therapeutic protocol. Arch Bone Jt Surg 8:291–294

Ansari MA, Fatima Z, Hameed S (2016) Antifungal action of Methylene Blue involves mitochondrial dysfunction and disruption of redox and membrane homeostasis in *C. albicans*. Open Microbiol J 10:12–22

Bhatia M (2021) The rise of mucormycosis in Covid-19 patients in India. Expert Rev Anti Infect Ther:1–2

Bhogireddy R, Krishnamurthy V, Jabaris SSL, Pullaiah CP, Manohar S (2021) Is Mucormycosis an inevitable complication of Covid-19 in India? Braz J Infect Dis 25:101597

Bilgin H, Ozcan B, Bilgin T (1998) Methemoglobinemia induced by methylene blue pertubation during laparoscopy. Acta Anaesthesiol Scand 42:594–595

Bojadzic D, Alcazar O, Buchwald P (2021) Methylene Blue inhibits the SARS-CoV-2 Spike–ACE2 protein-protein interaction—a mechanism that can contribute to its antiviral activity against COVID-19. Front Pharmacol 11:1–9

Cagno V, Medaglia C, Cerny A, Cerny T, Zwygart ACA, Cerny E et al (2021) Methylene Blue has a potent antiviral activity against SARS-CoV-2 and H1N1 influenza virus in the absence of UV-activation in vitro. Sci Rep 11:1–8. Nature Publishing Group UK

Cao B, Wang Y, Wen D, Liu W, Wang J, Fan G et al (2020) A trial of lopinavir–ritonavir in adults hospitalized with severe Covid-19. N Engl J Med 382:1787–1799

Carsana L, Sonzogni A, Nasr A, Rossi RS, Pellegrinelli A, Zerbi P et al (2020) Pulmonary post-mortem findings in a series of COVID-19 cases from northern Italy: a two-centre descriptive study. Lancet 20:1135–1140

Chen IY, Moriyama M, Chang MF, Ichinohe T (2019) Severe acute respiratory syndrome coronavirus viroporin 3a activates the NLRP3 inflammasome. Front Microbiol 10:1–9

Cieplik F, Deng D, Crielaard W, Buchalla W, Hellwig E, Al-Ahmad A et al (2018) Antimicrobial photodynamic therap—what we know and what we don't. Crit Rev Microbiol 44:571–589

Clifton J, Leikin JB (2003) Methylene blue. Am J Ther 10:289–291

Dabholkar N, Gorantla S, Kumar S, Alexander A (2021) Repurposing methylene blue in the management of COVID-19: mechanistic aspects and clinical investigations. Biomed Pharmacother 142:112023

Dai T, Bil De Arce VJ, Tegos GP, Hamblin MR (2011) Blue dye and red light, a dynamic combination for prophylaxis and treatment of cutaneous Candida albicans infections in mice. Antimicrob Agents Chemother 55:5710–5717

Demidova TN, Hamblin MR (2005) Effect of cell-photosensitizer binding and cell density on microbial photoinactivation. Antimicrob Agents Chemother 49:2329–2335

Deng Y, Wang R, Li S, Zhu X, Wang T, Wu J et al (2021) Methylene blue reduces incidence of early postoperative cognitive disorders in elderly patients undergoing major non-cardiac surgery: an open–label randomized controlled clinical trial. J Clin Anesth 68:110108

Denny JT, Burr AT, Balzer F, Tse JT, Denny JE, Chyu D (2015) Methylene blue treatment for cytokine release syndrome-associated vasoplegia following a renal transplant with rATG infusion: a case report and literature review. Exp Ther Med 9:1915–1920

Disanto AR, Wagner JG (1972) Pharmacokinetics of highly ionized drugs II: methylene blue—absorption, metabolism, and excretion in man and dog after oral administration. J Pharm Sci 61: 1086–1090

Divakar PK (2021) Fungal taxa responsible for mucormycosis/"black fungus" among covid-19 patients in India. J Fungi 7:1–6

Evora PRB (2016) Methylene Blue is a guanylate cyclase inhibitor that does not interfere with nitric oxide. Texas Hear Inst J 43:103

Fadel MA, Tawfik AA (2015) New topical photodynamic therapy for treatment of hidradenitis suppurativa using methylene blue niosomal gel: a single-blind, randomized, comparative study. Clin Exp Dermatol 40:116–122

Fang W, Jiang J, Su L, Shu T, Liu H, Lai S (2021) The role of NO in COVID-19 and potential therapeutic strategies. Free Radic Biol Med 163:153–162

Farmakiotis D, Tarrand JJ, Kontoyiannis DP (2014) Drug-resistant Candida glabrata infection in cancer patients. Emerg Infect Dis 20:1833–1840

Figueiredo Souza LW, Souza SVT, Botelho ACC (2014) Randomized controlled trial comparing photodynamic therapy based on methylene blue dye and fluconazole for toenail onychomycosis. Dermatol Ther 27:43–47

Fletcher S, Hamilton AD (2006) Targeting protein-protein interactions by rational design: mimicry of protein surfaces. J R Soc Interface 3:215–233

Ghahestani SM, Shahab E, Karimi S, Madani MH (2020) Methylene blue may have a role in the treatment of COVID-19. Med Hypotheses 144:110163

Gillman PK (2006) Methylene blue implicated in potentially fatal serotonin toxicity. Anaesthesia 61:1007–1019

Gillman PK (2008) Methylene blue is a potent monoamine oxidase inhibitor. Can J Anesth 55:311–312

Golwalkar D. Treatment for COVID-19 using Methylene Blue. 2020.

Guffey JS, Payne W, Roegge W (2017) In vitro fungicidal effects of methylene blue at 625-nm. Mycoses 60:723–727

Hamidi-Alamdari D, Hafizi-Lotfabadi S, Bagheri-Moghaddam A, Safari H, Mozdourian M, Javidarabshahi Z, Peivandi-Yazdi A, Ali-Zeraati A, Sedaghat A, Poursadegh F, Barazandeh-Ahmadabadi F, Agheli-Rad M, Tavousi SM, Vojouhi S, Amini S, Amini M, Majid-Hosseini KG (2021) Methylene Blue for treatment of hospitalized Covid-19 patients: a randomized, controlled, open-label clinical trial, phase 2. Rev Invest Clin 73:190–198

Haouzi P, Gueguinou M, Sonobe T, Judenherc-haouzi A, Tubbs N, Trebak M et al (2019) Revisiting the physiological effects of methylene blue as a treatment of cyanide intoxication. Clin Toxicol (Phila) 56:828–840

Helfritz FA, Bojkova D, Wanders V, Kuklinski N, Westhaus S, Von Horn C et al (2018) Methylene blue treatment of grafts during cold ischemia time reduces the risk of hepatitis C virus transmission. J Infect Dis 218:1711–1721

Henry M, Summa M, Patrick L, Schwartz L (2020) A cohort of cancer patients with no reported cases of SARS-CoV-2 infection: the possible preventive role of Methylene Blue. Subst An Int J Hist Chem 4(1):888

Hosseini L, Mahmoudi J, Sadigh-Eteghad S, Ziaee M (2020.; In Press) Bradykinin system: a therapeutic goal for COVID-19. Arch Pediatr Infect Dis:e108554

Jin C, Yu B, Zhang J et al (2021) Retraction note to: methylene blue photochemical treatment as a reliable SARS-CoV-2 plasma virus inactivation method for blood safety and convalescent plasma therapy for COVID-19. BMC Infect Dis 21:672

Karamyan VT (2021) Between two storms, vasoactive peptides or bradykinin underlie severity of COVID-19? Physiol Rep 9:1–9

Kim SH, Ahn SH, Cho YW, Lee DG (2012) Effect of intradiscal methylene blue injection for the chronic discogenic low back pain: one year prospective follow-up study. Ann Rehabil Med 36: 657–664

Kofidis T, Strüber M, Wilhelmi M, Anssar M, Simon A, Harringer W et al (2001) Reversal of severe vasoplegia with single-dose methylene blue after heart transplantation. J Thorac Cardiovasc Surg 122:823–824

Lang JD, McArdle PJ, O'Reilly PJ, Matalon S (2002) Oxidant-antioxidant balance in acute lung injury. Chest 122:314S–320S

Langford MP, Sebren AR, Burch MA, Redens TB (2020) Methylene blue inhibits acute hemorrhagic conjunctivitis virus production and induction of caspase-3 mediated human corneal cell cytopathy. Clin Ophthalmol 14:4483–4492

Lendvay TS, Chen J, Harcourt BH, Scholte FEM, Lin YL, Kilinc-Balci FS et al (2019) Addressing personal protective equipment (PPE) decontamination: methylene blue and light inactivates SARS-COV-2 on N95 respirators and medical masks with maintenance of integrity and fit. Infect Control Hosp Epidemiol 2021:1–10

Li J, Zhu M, An L, Chen F, Zhang X (2019) Fungicidal efficacy of photodynamic therapy using methylene blue against Sporothrix globosa in vivo and in vivo. Eur J Dermatology 29:160–166

Li C, Jia X, Bian Y, Qi D, Wu J (2021) Different susceptibility of spores and hyphae of Trichophyton rubrum to methylene blue mediated photodynamic treatment in vitro. Mycoses 64:48–54

Lozano M, Cid J, Müller TH (2013) Plasma treated with methylene blue and light: clinical efficacy and safety profile. Transfus Med Rev 27:235–240

Lu Y, Su CLH (2014) Candida albicans hyphal initiation and elongation. Trends Microbiol 22:707–714

Lu G, Nagbanshi M, Goldau N, Mendes Jorge M, Meissner P, Jahn A, Mockenhaupt FP, Mueller O (2018) Efficacy and safety of methylene blue in the treatment of malaria: a systematic review. BMC Med 16:1–16

Lyon JP, Moreira LM, de Moraes PCG, dos Santos FV, de Resende MA (2011) Photodynamic therapy for pathogenic fungi. Mycoses 54:e265–e271

Mahalaxmi I, Jayaramayya K, Venkatesan D (2021) Mucormycosis: an opportunistic pathogen during COVID-19. Environ Res 201:111643

Mayer B, Brunner F, Schmidt K (1993) Inhibition of nitric oxide synthesis by methylene blue. Biochem Pharmacol 45:367–374

Mei Xiong Z, O'Donovan M, Sun L, Young Choi J, Ren M, Cao K (2017) Anti-aging potentials of methylene blue for human skin longevity. Sci Rep 7:1–12

Mozayeni MA, Vatandoost F, Asnaashari M, Shokri M, Azari-Marhabi S, Asnaashari N (2020) Comparing the efficacy of toluidine blue, methylene blue and curcumin in photodynamic therapy against Enterococcus Faecalis. J Lasers Med Sci 11:S49–S54

Nadtoka O, Virych P, Nadtoka S, Kutsevol N (2020) Synthesis and performance of hybrid hydrogels loaded with methylene blue and its use for antimicrobial photodynamic inactivation. J Chem 2020:1–10

Naylor GJ, Martin B, Hopwood SE, Watson Y (1986) A two-year double-blind crossover trial of the prophylactic effect of methylene blue in manic-depressive psychosis. Biol Psychiatry 21:915–920

O'leary JL, Petty J, Harris AB, Inukai J (1968) Supravital staining of mammalian brain with intraarterial methylene blue followed by pressurized oxygen. Biotech Histochem 43:197–201

Oz M, Lorke DE, Hasan M, Petroianu GA (2011) Cellular and molecular actions of methylene blue in the nervous system. Med Res Rev 31:93–117

Peter C, Hongwan D, Küpfer A, Lauterburg BH (2000) Pharmacokinetics and organ distribution of intravenous and oral methylene blue. Eur J Clin Pharmacol 56:247–250

Porat R, Gilbert S, Magilner D (1996) Methylene blue-induced phototoxicity: an unrecognized complication. Pediatrics 97:717–721

Ramires MCCH, Hernandes C, Barbara M, Yudi R, Felipe L, Campos L (2021) A combination of phototherapy modalities for extensive lip lesions in a patient with SARS-CoV-2 infection. Photodiagnosis Photodyn Ther 33:102196

Ramsay RR, Dunford C, Gillman PK (2007) Methylene blue and serotonin toxicity: inhibition of monoamine oxidase A (MAO A) confirms a theoretical prediction. Br J Pharmacol 152:946–951

Rhaleb NE, Dion S, Barabé J, Rouissi N, Jukic D, Drapeau G et al (1989) Receptors for kinins in isolated arterial vessels of dogs. Eur J Pharmacol 162:419–427

Rosen PJ, Johnson C, McGehee WG, Beutler E (1971) Failure of methylene blue treatment in toxic methemoglobinemia. Association with glucose-6-phosphate dehydrogenase deficiency. Ann Intern Med 75:83–86

Salaris SC, Babbs CF, Voorhees WD (1991) Methylene blue as an inhibitor of superoxide generation by xanthine oxidase. A potential new drug for the attenuation of ischemia/reperfusion injury. Biochem Pharmacol 42:499–506

Schirmer RH, Adler H, Pickhardt M, Mandelkow E (2011) Lest we forget you—methylene blue. Neurobiol Aging 32:2325.e7–2325.e16

Scigliano G, Scigliano GA (2021) Methylene blue in covid-19. Med Hypotheses 146:110455

Sharma S (2021) Gujarat: after Corona, methylene blue finds takers in mucormycosis too. Times of India:2–4

Shukla R. Mucormycosis: as India struggles with Black Fungus , doctors say Methylene Blue can be a solution , demand detailed study on priority. Financial Express; 2021.
Špitalská E, Špitalský Z, Markovic Z, Štefanidesová K, Škultéty Ľ (2018) Low-cost light-induced therapy to treat rickettsial infection. Photodiagnosis Photodyn Ther 24:150–152
Stone N, Gupta N, Schwartz I (2021) Mucormycosis: time to address this deadly fungal infection. Lancet Microbe 2:e343–e344
Te Velthuis AJ, van den Worm SH, Sims AC, Baric RS, Snijder EJ, van Hemert MJ (2010) Zn2+ inhibits coronavirus and arterivirus RNA polymerase activity in vitro and zinc ionophores block the replication of these viruses in cell culture. PLoS Pathog 6:1–10
Thomas E, Roman E, Claypool S, Manzoor N, Pla J, Panwar SL (2013) Mitochondria influence CDR1 efflux pump activity, Hog1-mediated oxidative stress pathway, iron homeostasis, and ergosterol levels in Candida albicans. Antimicrob Agents Chemother 57:5580–5599
Valkov A, Zinigrad M, Nisnevitch M (2021) Photodynamic eradication of trichophyton rubrum and candida albicans. Pathogens 10:1–13
van den Berg DF, te Velde AA (2020) Severe COVID-19: NLRP3 inflammasome dysregulated. Front Immunol 11:1–6
Varga Z, Flammer AJ, Steiger P, Haberecker M, Andermatt R, Zinkernagel A (2020) Endothelial cell infection and endotheliitis in COVID-19. Lancet 395:1417–1418
Vilalva KH, Mumic FT, Da Silveira MRG, Mente ED, Evora PRB, e Silva OC. Use of methylene blue to treat hypovolemic shock followed by ischemia-reperfusion injury in the postoperative orthotopic liver transplant patient: a case report. Exp Clin Transplant 2018; 16: 511–514.
Wainwright M, Amaral L (2005) The phenothiazinium chromophore and the evolution of antimalarial drugs. Trop Med Int Heal 10:501–511
Walter-Sack I, Rengelshausen J, Oberwittler H, Burhenne J, Mueller O, Meissner P et al (2009) High absolute bioavailability of methylene blue given as an aqueous oral formulation. Eur J Clin Pharmacol 65:179–189
Wang J, Zhao C, Kong P, Bian G, Sun Z, Sun Y et al (2016) Methylene blue alleviates experimental autoimmune encephalomyelitis by modulating AMPK/SIRT1 signaling pathway and Th17/Treg immune response. J Neuroimmunol 299:45–52
Wang M, Cao R, Zhang L, Yang X, Liu J, Xu M et al (2020) Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell Res 30: 269–271
Weinstein L (1975) Genital Herpes: treatment with methylene blue and light exposure. Pharmacol Treat 14:69–72
White TC, Marr KA, Bowden RA (1998) Clinical, cellular, and molecular factors that contribute to antifungal drug resistance. Clin Microbiol Rev 11:382–402
Wischik CM, Edwards PC, Lai RYK, Roth M, Harrington CR (1996) Selective inhibition of Alzheimer disease-like tau aggregation by phenothiazines. Proc Natl Acad Sci U S A 93: 11213–11218
Zuschlag ZD, Warren MW, Schultz SK (2018) Serotonin toxicity and urinary analgesics: a case report and systematic literature review of methylene blue-induced serotonin syndrome. Psychosomatics 59:539–546

Chapter 22
Drug Repurposing in COVID-19 and Cancer: How Far Have We Come?

Yasir Bashir, Nabeela Farhat, and Asad U. Khan

Abstract Drug repurposing is a strategy for ascertaining new implications for already approved drugs. Historically, this field started with the serendipitous and inadvertent findings of a drug that was found to have an effect other than its original indication that was previously unrecognized and that had potential application in an entirely different disease. The fact that the rate of failure associated with the development of new drugs is high and the funds needed are enormous, it has compelled the scientific fraternity to look for alternatives and thus the drug repurposing approach has gained traction in the scientific community. The havoc that COVID-19 wreaked is unprecedented and till date it has led to the death of around 5.7 million people worldwide. The scientific fraternity, the world over, has embarked on the journey of getting a sure shot treatment for this deadly disease and till date many studies have been published discussing the role of various repurposed drug candidates in COVID-19 treatment. A majority of these studies have been carried out using structural bioinformatics and have not been validated by in vitro experiments. There is a pressing need for the treatment of COVID-19 disease using repurposed drugs by experimental validation and clinical testing, and augmented by the modern Machine Learning (ML)- and Artificial Intelligence (AI)- based approaches. A number of drug candidates have been investigated for their potential applications in cancer therapy, however the conundrum about the utility of either repurposed drug candidates or only active anti-cancer drugs for cancer therapy is to be pursued thoroughly so that mankind gets the most out of whatever potential the drug candidates, whether old or new, have in store for us. This chapter discusses the utility of drug repurposing approach as an alternative strategy for drug discovery that is intended to find treatment for new and emerging infectious diseases, viz. COVID-19 and cancer.

Keywords Drug repurposing · COVID-19 · Cancer · Drug resistance

Y. Bashir · N. Farhat · A. U. Khan (✉)
Medical Microbiology and Molecular Biology Lab., Interdisciplinary Biotechnology Unit, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

R. C. Sobti et al. (eds.), *Drug Repurposing for Emerging Infectious Diseases and Cancer*, https://doi.org/10.1007/978-981-19-5399-6_22

22.1 Introduction

Drug repurposing (DR) is a strategy for ascertaining new implications for already approved drugs or the application of established drug compounds to new therapeutic indications (Langedijk et al. 2015; Ballard et al. 2020). Though the break-through discoveries related to DR have mostly been serendipitous in the initial phase, this field of science has progressed leaps and bounds in the last three decades. Moreover, the fact that the rate of failure is high (~90%) and the funds that need to be allocated for the development of new drugs are enormous, it has compelled the scientific fraternity to look for alternatives and thus the DR approach has gained traction in the scientific community. A number of advantages of DR can be enumerated over developing an entirely new drug which include the following:

- The drug candidates for repurposing have already been proven safe for applications in other indications, so there are bleak chances of their failure from a safety point of view in the downstream trials regarding their efficacy, thus there is a lower risk of failure.
- The preclinical evaluation including toxicity testing, validation of the target, hit-to-lead optimization, early clinical trials, safety assessment, etc. will already have been completed, thus circumventing the need for preclinical development phase, thus there is a reduced time frame for drug development.
- Although the downstream regulatory and phase III clinical trial costs for a drug being repurposed may not differ from the investment on a novel drug candidate in the same disease condition, significant savings could be managed in costs at preclinical and early phase stages (Fig. 22.1).

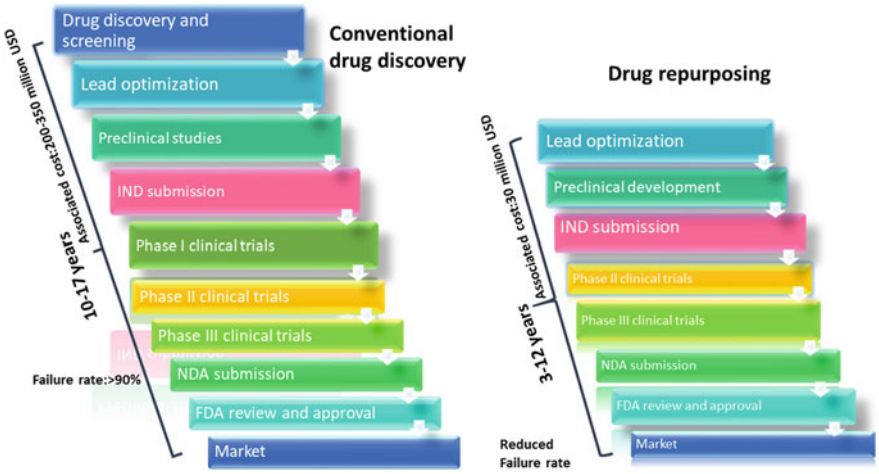


Fig. 22.1 Time and resources associated with conventional drug development versus drug repurposing are depicted schematically

22.2 Success Stories of Drug Repurposing

Historically, the field of DR started with the serendipitous and inadvertent findings of a drug that was found to have an effect other than its original indication that was previously unrecognized and that had potential application in an entirely different disease. Some of the most famous successful DR examples are presented in Table 22.1. These success stories speak volumes about the feasibility and effectiveness of the DR strategy and thus this approach has gained a considerable amount of attention in the public and private sectors. Researchers across the globe are increasingly searching for drugs to repurpose and also open up ways to mitigate the crisis of antimicrobial resistance that is considered a potential threat for humankind in the near future (O'Neill 2014).

Table 22.1 List of approved repurposed drugs on various diseases

Drug	Original application	New application	Year
Zidovudine	Anticancer	Antiviral	1987
Minoxidil	Hypertension	hair loss	1988
Eflornithine	Antitumor agent	Human African trypanosomiasis	1990
Paromomycin	Antibiotic	Visceral leishmaniasis	1994
Finasteride	Benign prostatic hyperplasia	Alopecia	1997
Amphotericin B	Antifungal	Visceral leishmaniasis	1997
Sibutramine	Depression	Obesity	1997
Bupropion	Depression	smoking cessation	1997
Sildenafil	Hypertension	erectile dysfunction and pulmonary arterial hypertension	1998
Methotrexate	Cancer	Rheumatoid arthritis	1999
Fluoxetine	Depression	Premenstrual dysphoric disorder	2000
Atomoxetine	Parkinson disease	Attention deficit hyperactivity disorder	2002
Thalidomide	Morning sickness	multiple myeloma	2003
Paclitaxel	Cancer	Restenosis	2004
Topiramate	Epilepsy	Migraine	2004
Cymbalta	Depression	Diabetic peripheral neuropathy	2004
Rituximab	Cancer	Rheumatoid arthritis	2006
Raloxifene	Osteoporosis	Breast cancer	2007
Lumigan	Glaucoma	Hypotrichosis simplex	2009
Lidocaine	Local anesthetic	Arrhythmia	2010
Topiramate	Epilepsy	Obesity	2012
Dapoxetine	Analgesia and depression	Premature ejaculation	2012
Ketoconazole	Fungal infections	Cushing syndrome	2014
Miltefosine	Skin metastases	Visceral leishmaniasis	2014
Aspirin	Analgesia	Colorectal cancer	2015

Like all other fields of science, the DR approach is also amenable to failure and thus there are reports of unsuccessful repurposing of drugs as well. Latrepirdine, a drug originally used as an Antihistamine, was repurposed to treat Huntington disease but the phase III clinical trials were unsuccessful (Bezprozvanny 2010); Ceftriaxone, an antibiotic, was investigated to treat Amyotrophic lateral sclerosis but phase III trial failed to show efficacy (Cudkowicz et al. 2014); Topiramate, originally used to treat epilepsy, was repurposed for inflammatory bowel disease (IBD). The study, though successful in a rodent, failed in a retrospective cohort study (Crockett et al. 2014). These failures were not due to toxicity as the said drugs had already cleared the initial characterization phase and were pursued based on their successful pre-clinical evaluation. These drugs failed in their phase III trials; however, there are also other reasons for failure in the DR approach and they include intellectual property considerations, regulatory considerations, and organizational hurdles (Pushpakom et al. 2019).

22.3 Drug Repurposing and Infectious Diseases

22.3.1 COVID-19

Of late, the world has been left devastated by the coronavirus disease-19 (COVID-19) caused by SARS-CoV-2, a novel severe acute respiratory syndrome coronavirus-2. It originated from Wuhan, China in December 2019 and quickly spread throughout the globe, becoming a global pandemic by March 2020 (Xie et al. 2020). COVID-19 has not only affected the global public health but the economy of the world as well (World Health Organization 2020). Till date, five different variants of SARS-CoV-2 have been found viz. Alpha, Beta, Gamma, Delta, and the latest of them all is the Omicron variant. The rate at which this variant transmits into the populations is alarming and as such the scientific community, the world over, has launched an unprecedented research in various fields to alleviate the COVID-19 effects and come up with a sure-shot treatment for such a deadly disease.

Although a number of different drugs are being administered to COVID-19-affected patients, there are no clinical studies that prove the efficacy of these drugs as yet. For example, the derivatives of chloroquine (CQ) tested on coronaviruses had earlier exhibited significant in vitro anti-coronavirus effect (Keyaerts et al. 2004), which were further corroborated by the findings in which hydroxychloroquine (HCQ) inhibited SARS-CoV-2 replication in vitro (Liu et al. 2020; Yao et al. 2020). When the COVID-19 pandemic struck the world, HCQ initially showed promising anti-coronavirus effects in SARS-CoV-2-infected patients (Mehra et al. 2020; Boulware et al. 2020; Geleris et al. 2020), leading to its fast-track approval as anti-coronavirus drug by the US Food and Drug administration (USFDA). The Solidarity trial by World Health Organization (WHO) investigated four drugs viz. remdesivir, lopinavir, hydroxychloroquine, and interferon for their effect on SARS-CoV-2-infected patients (WHO 2020). The studies showed that these drugs had little

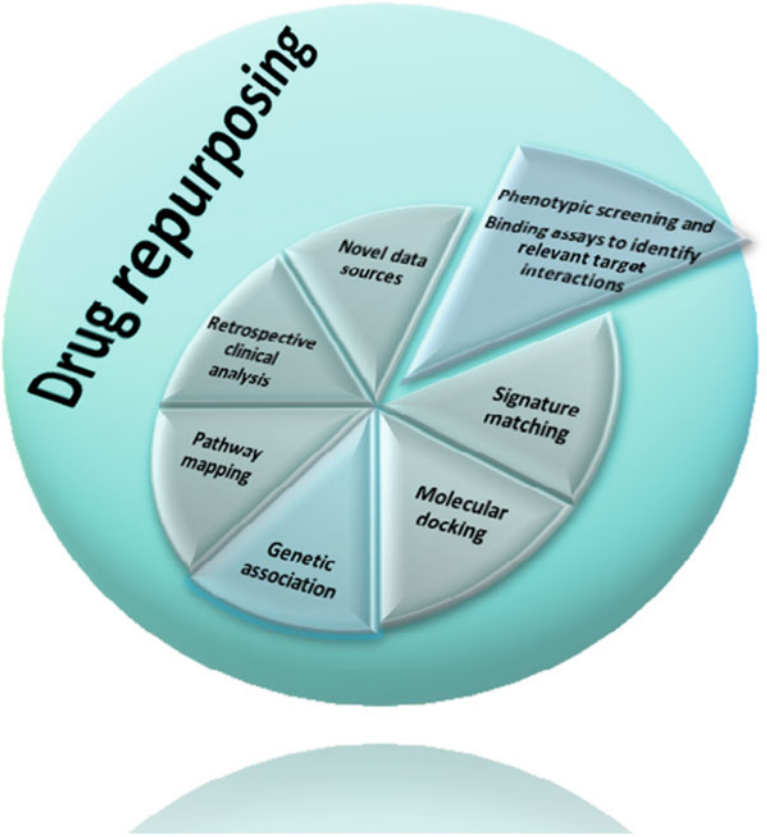


Fig. 22.2 Drug repurposing approaches: Computational approaches, either alone or in combination, can be used to systematically analyze large-scale data in order to extract the meaningful interpretations for DR hypotheses

or no effect on COVID-19 patients leading to the retraction of these drugs for their use against COVID-19.

DR approach involving anti-hepatitis C virus molecules as potential drugs against COVID-19 is under investigation (El Fiky 2020). In addition, many other candidate drugs being repurposed include the protease inhibitors (Kumar et al. 2020), anti-inflammatory drugs (Zhang et al. 2020a), and anti-aging drugs (Sargiacomo et al. 2020) which are under investigation as effective therapeutic agents for COVID-19. Various computational approaches can be used to systematically analyze large-scale data in order to extract the meaningful interpretations for DR hypotheses (Fig. 22.2).

Table 22.2 lists the drug candidates at various stages of their development as anti-COVID-19 therapeutic agents.

In this regard, the initiatives started by the World Health Organization (WHO) have been of paramount significance to prioritize the use of target-specific compounds. Worth to mention is the Solidarity PLUS trial wherein three drugs—

Table 22.2 List of various drug candidates being repurposed for COVID-19 treatment

Intervention	Study title	NCT number[a]
Favipiravir	Clinical Study To Evaluate The Performance And Safety Of Favipiravir in COVID-19	NCT04336904
Remdesivir	PK and Safety of Remdesivir for Treatment of COVID-19 in Pregnant and Non-Pregnant Women in the US	NCT04582266
AZD7442	Phase III Study of AZD7442 for Treatment of COVID-19 in Outpatient Adults	NCT04723394
Ritonavir	Evaluation of Protease Inhibition for COVID-19 in Standard-Risk Patients (EPIC-SR).	NCT05011513
Aprotinin	An Open Non-comparative Study of the Efficacy and Safety of Aprotinin in Patients Hospitalized With COVID-19	NCT04527133
AZD1222	Phase II/III Study of AZD2816, for the Prevention of COVID-19 in Adults	NCT04973449
Hydroxychloroquine Azithromycin	Hydroxychloroquine vs. Azithromycin for Hospitalized Patients With Suspected or Confirmed COVID-19	NCT04329832
Leflunomide	Targeting de Novo Pyrimidine Biosynthesis by Leflunomide for the Treatment of COVID-19 Virus Disease	NCT05007678
Nafamostat Mesilate TD139	DEFINE—Evaluating Therapies for COVID-19	NCT04473053
Almitrine	Efficacy of Intravenous Almitrine in Reducing the Need for Mechanical Ventilation in Patients With Hypoxemic Acute Respiratory Failure Due to Covid-19-related Pneumonia	NCT04357457
Dexamethasone	The Effect of Dexamethasone 12 mg vs 6 mg on Thromboembolic Events in Patients With Critical COVID-19	NCT05195242
Molnupiravir	Efficacy and Safety of Molnupiravir (MK-4482) in Non-Hospitalized Adult Participants With COVID-19 (MK-4482-002)	NCT04575597
Mavrilimumab	Study of Mavrilimumab (KPL-301) in Participants Hospitalized With Severe Corona Virus Disease 2019 (COVID-19) Pneumonia and Hyper-inflammation	NCT04447469
Cannabidiol	Cannabidiol for COVID-19 Patients With Mild to Moderate Symptoms	NCT04467918
Azoximer bromide	Efficacy and Safety of Polyoxidonium® in Hospitalized Patients With Coronavirus Disease COVID-19	NCT04381377
Sarilumab	Cohort Multiple Randomized Controlled Trials Open-label of Immune Modulatory Drugs and Other Treatments in COVID-19 Patients—Sarilumab Trial—CORIMUNO-19—SARI	NCT04324073
Pitavastatin	Evaluating the Use of Pitavastatin to Reduce the Risk of Cardiovascular Disease in HIV-Infected Adults	NCT02344290

[a]Source: https://clinicaltrials.gov/

artesunate, imatinib, and infliximab—are to be tested in hospitalized SARS-CoV-2-infected patients (WHO 2020). These drugs being repurposed have been envisioned for their immense potential in alleviating the risk of death in SARS-CoV-2-infected patients.

22.3.2 Cancer

Cancer therapy, for long, has been a research-intensive field and a significant amount of funding has been diverted for research in this field in order to mitigate the crisis that this deadly disease has given rise to. Globally, cancer is the leading cause of mortality with around ten million deaths in 2020 (https://www.who.int/news-room/fact-sheets/detail/cancer) despite the plethora of studies undertaken to discover drugs that are novel and more effective. Given the fact that the cost of development of a new drug de novo averages between 1 and 2 billion US$ (Nosengo 2016), it is no surprise that DR approach is gaining traction in cancer therapy as well. Table 22.3 enlists the various drug candidates that have been used for repurposing against various cancers.

The utility of this approach to develop innovative treatments in oncology based on repurposing the non-cancer medications for anti-cancer potential has only increased (Pantziarka et al. 2020). Researchers and clinicians, across the globe, are turning to this approach to alleviate the crisis of drug shortage by repurposing old drugs for new cancer therapies (Corsello et al. 2017). It is believed that almost all the drugs that are used in human therapy are multi-target drugs, i.e., they have the potential to act on many targets (Huang et al. 2020; Dallavalle et al. 2020), so, if these drug-targets have molecular signatures consistent with those of cancer, there is a huge possibility of finding novel anti-cancer therapeutic (Wang et al. 2019; Patel et al. 2013). Table 22.4 summarizes the repurposed drug candidates used for targeting the various hallmarks of cancer along with the therapy approach used (Zhang et al. 2020b).

22.4 Challenges and Future Perspectives

The havoc that COVID-19 wreaked is unprecedented and till date it has led to the death of around 5.7 million people worldwide (https://covid19.who.int/). The scientific fraternity, the world over, has embarked on the journey of getting a sure shot treatment for this deadly disease and till date many studies have been published discussing the role of various repurposed drug candidates in COVID-19 treatment. A majority of these studies have been carried out using structural bioinformatics. Though these studies have contributed substantially towards our understanding of the bonding between viral proteins and various lead compounds that may be potential drug candidates for COVID-19, nonetheless, a majority of them have not been validated by in vitro experiments. To add to that, various studies predicted the

Table 22.3 Drug candidates used for repurposing against various cancers and their original medical indication

Drug	Original indication	New indication
Artemisinin and related derivatives	Malaria	Lung, breast, colorectal cancer
Aspirin	Pain Fever	Gastrointestinal, esophageal cancer
Berberine	Bacterial diarrhea	Gastric, lung, colorectal cancer
Chloroquine	Malaria Rheumatoid arthritis	Pancreatic, breast cancer, chondrosarcoma
Curcumin	Dermatological diseases	Prostate, breast cancer, multiple myeloma
Disulfiram	Alcohol-aversion drug	Prostate, breast cancer, melanoma
Genistein	Menopause Osteoporosis obesity	Breast, bladder, colorectal cancer
Indomethacin	Rheumatic disease	Colorectal, esophageal, ovarian cancer
Itraconazole	Antifungal agent	Prostate, lung cancer
Mebendazole and related derivatives	Intestinal helminthiasis	Medulloblastoma, glioma, astrocytoma
Metformin	Type 2 diabetes	Breast, colorectal, prostate cancer
Niclosamide	Antihelminthic drug	Colorectal, prostate cancer
Prazosin	Hypertension	Adrenal incidentaloma
Quinacrine	Malaria Giardiasis Rheumatoid arthritis	Non–small-cell lung, prostatic cancer
Rapamycin	Immunosuppressant Antirestenosis agent	Rectum, breast, prostate cancer
Ritonavir	HIV	Kaposi's sarcoma, breast cancer
Thalidomide	Sedative Antiemetic	Prostate, colorectal, ovarian cancer
Triamterene	Diuretic	Acute myelocytic leukemia

usefulness of drug candidates that failed to demonstrate their efficacy when subjected to clinical trials. So, there is a pressing need for the treatment of COVID-19 disease using repurposed drugs by experimental validation and clinical testing, and augmented by the modern machine learning (ML)- and artificial intelligence (AI)-based approaches, so that this battle is won against the COVID-19 disease.

While a number of drug candidates have been investigated for their potential applications in cancer therapy, there is also a counter-narrative to drug repurposing for cancer therapeutics. Some researchers while focusing on different aspects of repurposing viz. single-agent activity, costs on repurposing, sample size in clinical trials, etc. come to a conclusion that the testing of repurposed drugs bearing no

Table 22.4 List of drugs targeting the cancer hallmarks using DR approach

Cancer hallmark	Therapy approach	Drug candidates
Proliferative signaling sustenance	Monotherapy	Prazosin Rapamycin Indomethacin
Growth suppressor evasion	Combinatorial	Quinacrine Ritonavir
Cell death resistance	Monotherapy	Chloroquine Artemisinin
Enabling replicative immortality	Combinatorial	Genistein Curcumin
Mutation and genomic instability	Combinatorial	Mebendazole Spironolactone
Energy metabolism reprogramming	Monotherapy	Disulfiram Metformin
Inducing angiogenesis	Combinatorial	Itraconazole Thalidomide
Metastasis and invasion activation	Combinatorial	Niclosamide Berberine
Tumor-promoting inflammation	Combinatorial	Thiocolchicoside Aspirin
Immuno-destruction evasion	Monotherapy	Vaccines for Infectious disease

single-agent activity in large, randomized trials, shouldn't be pursued and instead these funds could be diverted to testing known anti-cancer drugs in trials for which results could be extrapolated in a global context (Tran and Prasad 2020). The same is corroborated by their inability to be pursued in uncontrolled, phase II studies, owing to the fact that these drugs lack single-agent activity in cancer. So the conundrum about the utility of either repurposed drug candidates or only active anticancer drugs for cancer therapy is to be pursued thoroughly so that mankind gets the most out of whatever potential the drug candidates, whether old or new, have in store for us.

In summary, drug repurposing approach has emerged as an alternative strategy for drug discovery and is intended to find treatment for new and emerging infectious diseases including, but not limited to, COVID-19 and cancer therapy. James Black, a 1988 Nobel laureate in physiology and medicine, once said: "the most fruitful basis for the discovery of a new drug is to start with an old drug." DR has unfurled as a promising therapeutic strategy for the effective mitigation of COVID-19 as well as cancer, and is believed to be our savior in tackling the emerging infectious diseases.

References

Ballard C et al (2020) Drug repositioning and repurposing for Alzheimer disease. Nat Rev Neurol 16(12):661–673. https://doi.org/10.1038/s41582-020-0397-4

Bezprozvanny I (2010) The rise and fall of Dimebon. Drug News Perspect 23:518–523

Boulware DR et al (2020) A randomized trial of hydroxychloroquine as post-exposure prophylaxis for Covid-19. New Engl J Med 383:517–525

Corsello SM et al (2017) The drug repurposing Hub: a next-generation drug library and information resource. Nat Med 23:405–408
Crockett SD et al (2014) Topiramate use does not reduce flares of inflammatory bowel disease. Dig Dis Sci 2014(59):1535–1543
Cudkowicz ME et al (2014) Safety and efficacy of ceftriaxone for amyotrophic lateral sclerosis: a multistage, randomised, double-blind, placebo-controlled trial. Lancet Neurol 13:1083–1091
Dallavalle S et al (2020) Improvement of conventional anticancer drugs as new tools against multidrug resistant tumors. Drug Resist Updates 50:100682
El Fiky AA (2020) Anti-HCV, nucleotide inhibitors, repurposing against COVID-19. Life Sci 248: 117477
Geleris J et al (2020) Observational Study of Hydroxychloroquine in hospitalized patients with Covid-19. N Engl J Med 382:2411–2418
Huang A et al (2020) Synthetic lethality as an engine for cancer drug target discovery. Nat Rev Drug Discov 19:23–38
Keyaerts E et al (2004) In vitro inhibition of severe acute respiratory syndrome coronavirus by chloroquine. Biochem Biophys Res Commun 323:264–268
Kumar S et al (2020) Repurposing antiviral protease inhibitors using extracellular vesicles for potential therapy of COVID-19. Viruses 12:486
Langedijk J et al (2015) Drug repositioning and repurposing: terminology and definitions in literature. Drug Discov Today 20:1027–1034
Liu J et al (2020) Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection in vitro. Cell Discov 6:1–4
Mehra MR et al (2020) Hydroxychloroquine or chloroquine with or without a macrolide for treatment of COVID-19: a multinational registry analysis. Lancet S0140
Nosengo N (2016) Can you teach old drugs new tricks? Nature 534(7607):314–316
O'Neill J (2014) Antimicrobial resistance: tackling a crisis for the health and wealth of nations. Review on Antimicrobial Resistance, London
Pantziarka, P., et al. (2020). Drug repurposing for cancer therapy—an introduction. In: Drug repurposing in cancer therapy approaches and applications. pp. 1–14
Patel MN et al (2013) Objective assessment of cancer genes for drug discovery. Nat Rev Drug Discov 12:35–50
Pushpakom S et al (2019) Drug repurposing: progress, challenges and recommendations. Nat Rev Drug Discov 18(1):41–58. https://doi.org/10.1038/nrd.2018.168
Sargiacomo C et al (2020) COVID-19 and chronological aging: senolytics and other anti-aging drugs for the treatment or prevention of corona virus infection? Aging 12:6511–6517
Tran AA, Prasad V (2020) Drug repurposing for cancer treatments: a well-intentioned, but misguided strategy. Lancet Oncol 21(9):1134–1136
Wang S et al (2019) Structural simplification of natural products. Chem Rev 119:4180–4220
WHO (2020) "Solidarity PLUS" clinical trial for COVID-19 treatments. Available at: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/global-research-on-novel-coronavirus-2019-ncov/solidarity-clinical-trial-for-covid-19-treatments
World Health Organization (2020) WHO clinical management of severe acute respiratory infection (SARI) when COVID-19 disease is suspected. WHO, London
Xie P, Ma W, Tang H, Liu D (2020) Severe COVID-19: a review of recent progress with a look toward the future. Front Public Heal 21:77–94. https://doi.org/10.5353/th_b4501128
Yao X et al (2020) In vitro antiviral activity and projection of optimized dosing design of hydroxychloroquine for the treatment of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Clin Infect Dis 71:732–739
Zhang W et al (2020a) The use of anti-inflammatory drugs in the treatment of people with severe coronavirus disease 2019 (COVID-19): the perspectives of clinical immunologists from China. Clin Immunol 214:108393
Zhang Z et al (2020b) Overcoming cancer therapeutic bottleneck by drug repurposing. Signal Transduction Target Ther 5(1):1–25

Chapter 23
Repurposing of Doxycycline to Attenuate Influenza Virus Pathogenesis Via Inhibition of Matrix Metalloproteinases in Neutrophils

T. Narasaraju, Charlene Fong, Sunil K. Lal, and Vincent T. K. Chow

Abstract Influenza viruses constitute a significant threat to public health worldwide over many decades, and continue to inflict significant morbidity and mortality. Previous studies show that infection of mice with mouse-adapted influenza A/Aichi/2/1968(H3N2) passage 10 (P10) virus can elicit exuberant inflammatory responses in the lungs with extensive infiltration of macrophages and neutrophils (which are sources of gelatinases) that contribute to pulmonary damage. The lungs of mice with severe influenza pneumonitis also reveal extensive neutrophilic infiltration, neutrophil extracellular traps (NETs), alveolar damage, heightened viral load, and pathologic features of acute respiratory distress syndrome (ARDS). Excessive neutrophil and matrix metalloproteinase (MMP) activities are implicated in the pathogenesis of acute lung injury (ALI) and ARDS. Hence, an objective of this study was to investigate the production of MMP-2 and MMP-9 in neutrophils differentiated from the MPRO murine pro-myelocytic cell line, following infection with mouse-adapted influenza H3N2 virus. Another objective was to investigate the effects of doxycycline on expression of MMP-2 and MMP-9 at transcriptional and translational levels in neutrophils in the context of influenza virus infection. MMP-2 and MMP-9 production and gelatinase activity were found to be induced by infection of differentiated neutrophils with mouse-adapted influenza H3N2 virus.

T. Narasaraju
NUHS Infectious Diseases Translational Research Program, Department of Microbiology and Immunology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore

Adichunchanagiri Institute of Medical Sciences, Adichunchanagiri Centre for Research and Innovation, Adichunchanagiri University, BG Nagara, Karnataka, India

C. Fong · V. T. K. Chow (✉)
NUHS Infectious Diseases Translational Research Program, Department of Microbiology and Immunology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore
e-mail: micctk@nus.edu.sg

S. K. Lal
School of Science, Monash University Malaysia, Subang Jaya, Selangor, Malaysia
e-mail: sunil.lal@monash.edu

R. C. Sobti et al. (eds.), *Drug Repurposing for Emerging Infectious Diseases and Cancer*, https://doi.org/10.1007/978-981-19-5399-6_23

Furthermore, doxycycline treatment was able to abrogate this increase in MMP-2 and MMP-9 expression and gelatinase activity. Given that excessive neutrophil infiltration and uncontrolled neutrophil gelatinase activity in pulmonary tissues play pivotal roles in the pathogenesis of ALI and ARDS, this study offers insights into the mechanism of excessive MMP-2 and MMP-9 production and activity in neutrophils during influenza virus infection. Thus, repurposing doxycycline as an MMP inhibitor represents a potential therapeutic strategy to ameliorate influenza-associated pulmonary injury by targeting MMPs, gelatinase production and activity in neutrophils. Doxycycline therapy (alone or in combination with other drugs) has also been considered and explored for the management of other infectious diseases such as coronavirus infections and tuberculosis.

Keywords Influenza · H3N2 virus · Neutrophils · MPRO cell line · Gelatinases · Matrix metalloproteinases · MMP-2 · MMP-9 · MMP inhibitor · Doxycycline · Drug repurposing

Influenza constitutes a significant threat to public health worldwide over many decades. Influenza A viruses cause significant morbidity and mortality over widespread geographical distances (Ivan et al. 2020). The world has experienced several major pandemics caused by influenza A viruses, including the 1918 H1N1, 1957 H2N2, 1968 H3N2, and 2009 H1N1 strains. Moreover, outbreaks due to highly pathogenic avian influenza viruses (such as H5N1 and H7N9) have occurred in many countries all over the world (Chow et al. 2008; Sakharkar et al. 2009; Zhou et al. 2018).

23.1 Neutrophils and Influenza Virus-Induced Lung Injury

Influenza virus infection induces the mobilization of neutrophil effector systems—the virus and virus-infected cells are engulfed and phagocytosed by neutrophils to form a lysosome, leading to neutrophil activation. The neutrophil then undergoes a respiratory burst, leading to reactive oxygen species (ROS) generation, and release of contents from the azurophil and specific granules into the phagolysosome—thus creating a toxic microenvironment that kills the virus (Smith 1994; Hashimoto et al. 2007). In addition, neutrophils are also implicated in the inflammatory response that characterizes acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) during severe influenza virus infection (Abraham 2003; Quispe-Laime et al. 2010; Narasaraju et al. 2011).

ALI is a complex clinical syndrome which is characterized by pulmonary edema, capillary leakage, and pneumonia. ARDS is the most severe form of ALI, and is characterized by diffuse alveolar damage and leukocytic inflammation of the lung parenchyma, epithelial damage, and hypoxemia. Neutrophil-predominant host inflammatory responses are essential for the development of ALI and ARDS. The

inappropriate release of the proteolytic enzymes contained in the neutrophil granules into the extracellular space can cause host tissue injury via proteolytic activity and release of ROS. This occurs in the case of excessive neutrophil infiltration, premature activation of neutrophils during migration, and/or formation of neutrophil extracellular traps or NETs (Narasaraju et al. 2011). During neutrophil migration into the lung airways, uncontrolled activation of neutrophils may occur in response to certain microbial or host-derived stimuli—excessive release of the proteolytic enzymes then culminates in damage and sloughing of pulmonary epithelial and endothelial cells (Ware and Matthay 2000; Xu et al. 2006).

In the early phase of ALI and ARDS, the release of pro-inflammatory mediators from monocytes, alveolar macrophages, and vascular endothelial cells results in neutrophil migration and sequestration. Activated neutrophils release terminal effectors such as ROS, neutrophil elastases, and matrix metalloproteinases (MMPs) to cause lung tissue injury, leading to leakage of proteinaceous fluid into the alveolar spaces and airways. The intense inflammatory response leads to pulmonary endothelial and epithelial cell damage, and disruption of the capillary-alveolar barrier function (Taubenberger and Morens 2008).

The MPRO cell line is derived from murine bone marrow cells via the transduction of a dominant-negative retinoic acid receptor. The MPRO line is dependent on granulocyte-macrophage colony-stimulating factor (GM-CSF), is arrested at a pro-myelocytic stage, and can morphologically differentiate into neutrophils following treatment with 10 μM all-*trans* retinoic acid (ATRA)—rendering it a useful cell line for in vitro neutrophil studies (Lawson et al. 1998; Johnson et al. 1999).

23.2 Functions of Matrix Metalloproteinases

MMPs carry out essential functions in the form of extracellular matrix (ECM) degradation, which is necessary for ECM turnover and tissue remodeling in various processes such as cell migration, embryonic development, and angiogenesis. MMPs have a wide range of targets, including non-ECM proteins such as growth factors and various cytokines—thus also affecting various processes in cellular proliferation, cell migration, and apoptosis. MMPs are naturally regulated at various levels including gene expression, zymogen activation, mRNA stability, enzyme inactivation, and compartmentalization. Most MMPs are inducibly transcribed, with the exception of MMP-2 which is constitutively expressed (Sternlicht and Werb 2001; Parks et al. 2004; Snoek-van Beurden and Von den Hoff 2005).

Within the MMP family, gelatinases are considered an integral subclass due to their ability to degrade major constituents of the basement membrane, including type IV collagen, laminin, and gelatin. Gelatinases include MMP-2 (gelatinase A) and MMP-9 (gelatinase B). MMP-2 can also digest type I, II, and III collagens, while MMP-9 can digest type V collagen (Murphy and Crabbe 1995; Sternlicht and Werb 2001; Parks et al. 2004). However, the two gelatinases differ in certain ways. MMP-2 is synthesized by a broad range of cells, including alveolar epithelial cells, endothelial cells, fibroblasts, macrophages, and dendritic cells. MMP-9 is mainly

produced by inflammatory cells such as neutrophils, monocytes, macrophages, and lymphocytes (Murphy and Crabbe 1995; Corbel et al. 2000). Both gelatinases are differentially regulated at transcriptional and extracellular levels. MMP-9 is transcriptionally regulated by cytokines and growth factors, whereas MMP-2 is only mildly responsive to these molecules.

Given that various MMPs, especially MMP-2 and MMP-9, are involved in pulmonary pathology, MMP inhibitors may be therapeutically exploited for ameliorating influenza-induced immunopathology. MMP inhibitors function in several ways, such as by inhibiting RNA synthesis, chelating zinc, and binding to the MMP active site.

MMP inhibitors have been shown to be effective therapeutic agents in animal models in which they can prevent pathologic changes of emphysema and ARDS (Carney et al. 2001). However, the nonspecificity of MMP inhibitors may lead to dose-limiting adverse effects. Periostat® or doxycycline hyclate is an example of MMP inhibitor which is clinically approved for use in periodontal disease (Corbitt et al. 2007; Fingleton 2007).

23.3 Repurposing Doxycycline to Mitigate Influenza-Induced Tissue Injury

Doxycycline is a broad-spectrum tetracycline antibiotic whose mode of action is to prevent access of acyl transfer-RNA to the acceptor site on the mRNA-30S ribosomal subunit complex, thus inhibiting the elongation process of bacterial protein synthesis. It possesses bacteriostatic, antiprotozoal, and antihelmintic effects (Smith and Cook 2004; Batty et al. 2007). Doxycycline also acts as a nonspecific MMP inhibitor, and its roles in MMP-2 and MMP-9 inhibition have been extensively studied. Some proposed mechanisms of MMP inhibition by doxycycline include downregulating MMP gene expression, chelating to zinc at the catalytic site, inhibiting pro-MMP activation, or scavenging of ROS (Curci et al. 2000; Cena et al. 2010; Chang et al. 2010). Ng et al. (2012) showed that oral administration of a low dose of doxycycline not only reduces inflammation following influenza virus infection in mice but also leads to significant reduction of host lung injury by minimizing the destruction of pulmonary epithelium and endothelium, and by decreasing leakage of proteinaceous material into the airways. Influenza-induced host lung injury is effectively improved by lower doses of doxycycline. However, higher doses of the drug substantially reduce inflammation to render viral clearance inefficient, thus resulting in high virus load, direct cytopathic effects on the host cells, and eventually aggravating pulmonary damage. It is thus vital to use an optimal (but not excessive) dosage of doxycycline to mitigate inflammation and gelatinase activities in influenza virus infection to alleviate acute lung injury.

23.4 Study Objectives

The first objective of this study was to analyze the in vitro production of MMP-2 and MMP-9 gelatinases following influenza H3N2 virus infection of neutrophils. The main rationale of this aim was predicated on previous in vivo studies showing that mouse-adapted influenza A/Aichi/2/1968(H3N2) passage 10 (P10) virus infection elicits an exaggerated inflammatory response in the lungs with extensive infiltration of macrophages and neutrophils (which are sources of gelatinases) that contribute to pulmonary damage (Narasaraju et al. 2009). Subsequent studies examining severe influenza pneumonitis in mice revealed excessive neutrophilic infiltration, NETs, alveolar damage, heightened viral load, and ARDS-like pathology (Narasaraju et al. 2011). This objective was to focus on the production of MMP-2 and MMP-9 in neutrophils differentiated from the MPRO murine pro-myelocytic cell line, following infection with mouse-adapted influenza H3N2 virus.

The second objective was to investigate the effects of doxycycline on expression of MMP-2 and MMP-9 at transcriptional and translational levels in neutrophils in the context of influenza virus infection. Since excessive neutrophil and MMP activities are implicated in the pathogenesis of ALI and ARDS (Narasaraju et al. 2011), doxycycline may serve as a potential therapeutic to ameliorate host damage caused by neutrophil gelatinases during severe pulmonary influenza infection.

23.5 Materials and Methods

Mouse lung-adapted influenza A/Aichi/2/1968(H3N2) virus was prepared as described previously (Narasaraju et al. 2009; Ivan et al. 2012). One batch of BALB/c mice was infected with mouse lung-adapted passage 14 (P14) H3N2 virus, and their lung homogenates harvested to generate passage 15 (P15) virus. P15 virus was then used for infecting a larger batch of mice to generate passage 16 (P16) virus. Automated cycle sequencing of the P16 virus hemagglutinin (HA) and nonstructural 1 (NS1) genes amplified by reverse transcription-polymerase chain reaction (RT-PCR) confirmed the presence of mutations previously identified in P10 virus (i.e., G218E in HA and D125G in NS1). Virus plaque assay using MDCK cells was performed for viral quantification of P16-infected lung homogenates which were used for infection of MPRO cells.

MPRO cell culture and differentiation into neutrophils were carried out as described previously (Ivan et al. 2013). Total cell count and differential cell count were determined using trypan blue exclusion and Giemsa staining, respectively. MPRO cells were treated with 10 μM ATRA to induce differentiation into neutrophils. Figure 23.1 shows that MPRO cell differentiation peaked at day 6, during which the majority of differentiated cells acquired neutrophil-like morphologic features such as multilobed or segmented nucleus with granulated cytoplasm (Fig. 23.2).

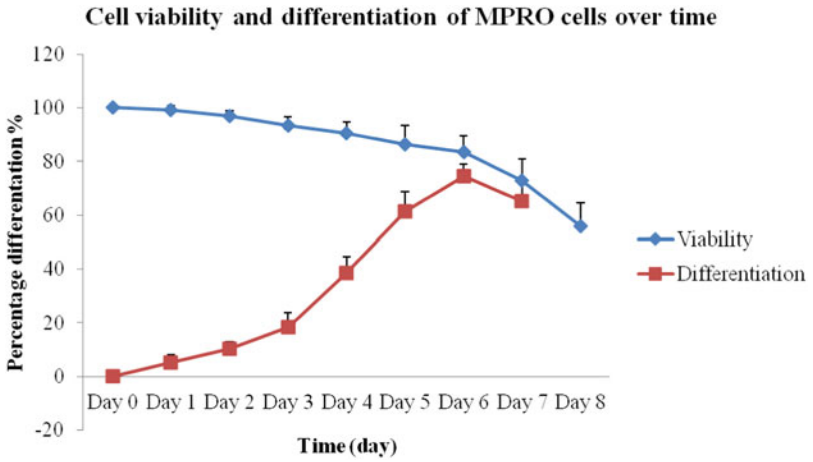


Fig. 23.1 Comparison of percentage of MPRO cell viability and cell differentiation over time. Cells were subjected to trypan blue staining for cell viability, and to Giemsa staining for neutrophil differentiation, and counted by microscopy. The graphs display the mean and standard deviation for each time-point ($n = 12$ each). Day 0 indicates time of addition of all-trans retinoic acid (ATRA). Neutrophil differentiation peaked at day 6, while cell viability decreased progressively over time

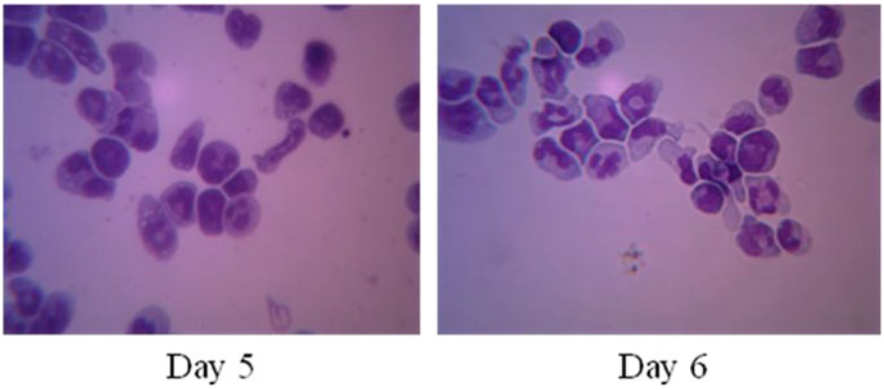


Fig. 23.2 Giemsa staining of differentiating MPRO cells at days 5 and 6 after the addition of ATRA. Representative images to exemplify MPRO cells differentiating and acquiring neutrophil-like morphologic characteristics such as segmented nucleus and granulated cytoplasm on day 5 and especially on day 6. Differentiated MPRO cells at day 6 following ATRA treatment were used for influenza virus infection

MPRO cells at day 6 following ATRA treatment were used for virus infection at multiplicity of infection (MOI) of 0.1. Three million cells were seeded in each well of 24-well plates. The four experimental groups were: control uninfected and untreated cells; infected but untreated cells; uninfected cells treated with 50 μM doxycycline (DOX); infected cells treated with 50 μM doxycycline. For the infected

and DOX-treated group, neutrophils were incubated at 37 °C for 1 h before addition of virus. Cells were incubated at various time-points of 1, 7, and 10 h postinfection before harvesting samples for analyses.

Cell pellets were subjected to RNA extraction followed by reverse transcription and real-time quantitative PCR using SYBR Green marker to analyze mRNA levels of MMP-2 and MMP-9, as described previously (Ng et al. 2012).

Culture supernatants were harvested and subjected to Western blot analyses to evaluate expression levels of MMP-2 and MMP-9 proteins; and to gelatinase zymography to assess gelatinase activity, as described previously (Ng et al. 2012).

Statistical analyses. Results were expressed as mean value ± standard deviation. Statistical analyses and comparisons of samples were performed using Student's *t*-test. Values of $P < 0.05$ were considered to be statistically significant.

23.6 Results and Discussion

In this study, it was hypothesized that influenza H3N2 virus could induce the production and gelatinase activity of MMP-2 and MMP-9 in neutrophils in vitro. Another hypothesis was that doxycycline could inhibit the expression and activity of MMP-2 and MMP-9 at protein and transcriptional levels.

23.6.1 Mouse-Adapted Influenza H3N2 P16 Virus Infection of MPRO Neutrophils Enhances MMP-2 and MMP-9 Protein Expression, Gelatinase Activity, and MMP-9 Transcription

Western blot analyses showed that mouse-adapted influenza A/H3N2 P16 virus infection of MPRO neutrophils was indeed able to induce and elevate protein expression of both MMP-2 and MMP-9 at all time-points. However, this degree of enhanced expression of MMP-2 and MMP-9 over time was somewhat different.

Furthermore, gelatinase zymography also revealed that influenza H3N2 P16 virus could also induce an overall significant increase in both MMP-2 and MMP-9 gelatinolytic activities.

Real-time qRT-PCR also demonstrated that H3N2 P16 virus could significantly induce MMP-9 mRNA expression, which exhibited a time-dependent increase in transcriptional response to influenza virus infection.

The above findings are summarized in Tables 23.1 and 23.2.

Table 23.1 Comparison of percentage change in MMP-9 expression by Western blotting, gelatinase zymography, and real-time quantitative RT-PCR in test versus control neutrophils at different time-points

	Time-points	Infected versus uninfected	Uninfected control	Infected
		No DOX	DOX versus No DOX	DOX versus No DOX
Western blot (WB)	1 h	↑ 87%*	↓ 60%*	↓ 20%*
	7 h	↑ 26%*	↓ 60%*	↓ 23%*
	10 h	↑ 64%*	↓ 23%*	↓ 48%*
Gelatinase zymography (GZ)	1 h	↑ 30%*	↓ 80%*	↓ 40%*
	7 h	↑ 150%*	NS	↓ 60%*
	10 h	↑ 80%*	↓ 10%*	↓ 50%*
Real-time quantitative RT-PCR	1 h	↑ 30%*	↓ 10%	↓ 8%
	7 h	↑ 70%*	↓ 20%*	↓ 20%*
	10 h	↑ 100%*	↓ 40%*	↓ 40%*

First, comparison of influenza virus-infected neutrophils versus uninfected control neutrophils without doxycycline (DOX) treatment (at 1, 7, and 10 h postinfection). Second, comparison of uninfected control neutrophils treated with 50 μM DOX versus uninfected control neutrophils without DOX treatment. Third, comparison of infected neutrophils treated with 50 μM DOX versus infected neutrophils without DOX treatment

*Denotes statistically significant difference of $P < 0.05$ as determined by two-sample, two-tailed test with equal variances. *NS* no significant difference

Table 23.2 Comparison of percentage change in MMP-2 expression by Western blotting and gelatinase zymography in test versus control neutrophils at different time-points

	Time-points	Infected versus uninfected	Uninfected control	Infected
		No DOX	DOX versus No DOX	DOX versus No DOX
Western blot (WB)	1 h	↑ 30%*	↓ 40%*	↓ 66%*
	7 h	↑ 100%*	↓ 20%*	↓ 100%*
	10 h	↑ 150%*	↓ 14%*	↓ 25%*
Gelatinase zymography (GZ)	1 h	↑ 30%*	↓ 20%*	↓ 20%*
	7 h	↑ 150%*	NS	↓ 20%*
	10 h	↑ 60%*	↓ 30%*	↓ 20%*

First, comparison of influenza virus-infected neutrophils versus uninfected control neutrophils without doxycycline (DOX) treatment (at 1, 7, and 10 h postinfection). Second, comparison of uninfected control neutrophils treated with 50 μM DOX versus uninfected control neutrophils without DOX treatment. Third, comparison of infected neutrophils treated with 50 μM DOX versus infected neutrophils without DOX treatment

*Denotes statistically significant difference of $P < 0.05$ as determined by two-sample, two-tailed test with equal variances. *NS* no significant difference

23.6.2 *Doxycycline Treatment Inhibits MMP-2 and MMP-9 Protein Expression, Gelatinase Activity, and MMP-9 Gene Expression in Neutrophils Infected With Influenza H3N2 P16 Virus*

Doxycycline treatment of uninfected and virus-infected MPRO neutrophils resulted in inhibition of expression of both MMP-2 and MMP-9 proteins, although to varying extents at different time-points (Figs. 23.3 and 23.4). Doxycycline also suppressed MMP-9 mRNA levels, with greater inhibition of gene expression the longer the incubation with doxycycline. Doxycycline treatment also decreased the gelatinolytic activity of both MMP-2 and MMP-9, with the latter exhibiting a more marked and time-dependent reduction (Tables 23.1 and 23.2). These findings indicate that the

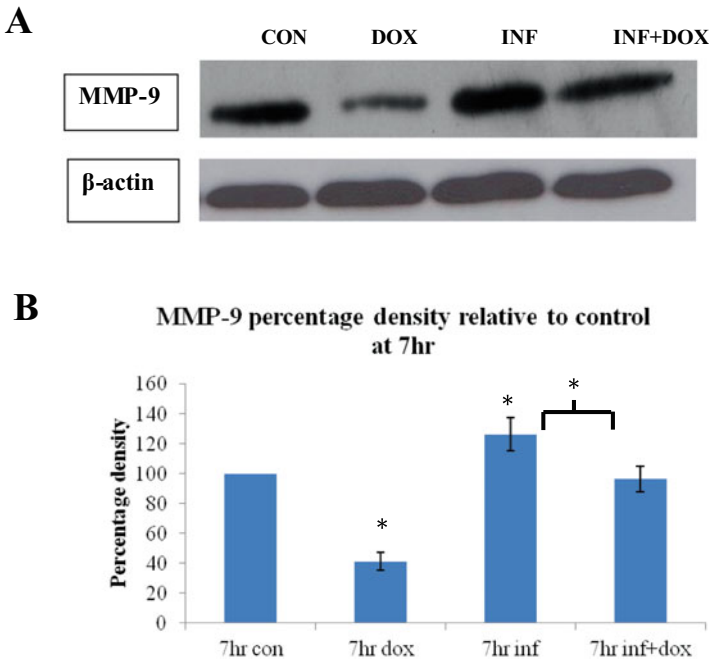


Fig. 23.3 Western blot analyses depicting MMP-9 protein expression in cell supernatant samples at 7-h time-point. (**a**) Representative immunoblots of supernatant samples from control and infected neutrophils at 7 h postinfection, showing both MMP-9 and housekeeping β-actin expression. There were four experimental groups ($n = 4$ per group). CON: uninfected and untreated control neutrophils. DOX: uninfected neutrophils with doxycycline (DOX) treatment. INF: influenza infection without DOX treatment. INF + DOX: influenza infection with DOX treatment. (**b**) Densitometric analyses of each MMP-9 protein band normalized against β-actin, and then expressed as a percentage of the band density relative to the control at 7 h (which was designated as 100%). *Denotes the statistically significant difference of $P < 0.05$ as determined by two-sample, two-tailed test with equal variances

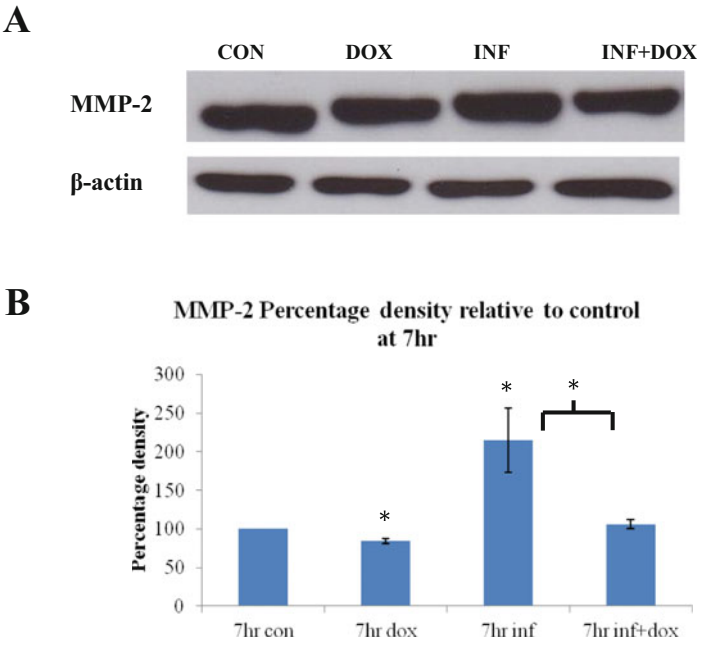


Fig. 23.4 Western blot analyses depicting MMP-2 protein expression in cell supernatant samples at 7-h time-point. (**a**) Representative immunoblots of supernatant samples from control and infected neutrophils at 7 h postinfection, showing both MMP-2 and housekeeping β-actin expression. There were four experimental groups ($n = 4$ per group). CON: uninfected and untreated control neutrophils. DOX: uninfected neutrophils with doxycycline (DOX) treatment. INF: influenza infection without DOX treatment. INF + DOX: influenza infection with DOX treatment. (**b**) Densitometric analyses of each MMP-2 protein band normalized against β-actin, and then expressed as a percentage of the band density relative to the control at 7 h (which was designated as 100%). *Denotes the statistically significant difference of $P < 0.05$ as determined by two-sample, two-tailed test with equal variances

expression of MMP-2 and MMP-9 in influenza virus-infected neutrophils could be affected by doxycycline via different mechanisms. Such differences between MMP-2 and MMP-9 may include their constitutive expression, mRNA and protein stability and half-life, negative feedback loops among others (Ben-Yosef et al. 2005).

23.6.3 Future Perspectives and Repurposing Doxycycline for Other Infections

This study focused on neutrophils, and further investigations are warranted on other relevant tissues and cell types. For example, in endothelial cells, doxycycline can affect MMP-9 production but not MMP-2 production (Hanemaaijer et al. 1998). The

effects of doxycycline on other MMPs in influenza virus-infected neutrophils should also be investigated, such as MMP-7, MMP-8, MMP-13, MMP-19, MMP-25, and MMP-27. Interestingly, MMP-25 is also an activator of pro-MMP-2, and may potentially be involved in the mechanism underpinning doxycycline's effects on gelatinase expression in infected neutrophils.

This study only analyzed the impact of doxycycline treatment on MMP-2 and MMP-9 inhibition, but there are likely to be other known and unknown molecular mechanisms of doxycycline. Further analyses of doxycycline treatment of infected versus uninfected neutrophils by harnessing transcriptomics and proteomics may elucidate additional genes, pathways, and networks that mediate the underlying molecular mechanisms. It would also be interesting to explore whether combination therapy with doxycycline together with antiviral agents such as oseltamivir can confer synergistic effects to ameliorate influenza pathogenesis.

Doxycycline therapy has also been considered and explored for the management of other infectious diseases, including COVID-19 (Narendrakumar et al. 2021)—one study found circulating MMP-9 as an early biomarker of respiratory failure in COVID-19 (Ueland et al. 2020). Doxycycline can inhibit feline coronavirus replication in vitro (Dunowska and Ghosh 2021), and can act synergistically with remdesivir antiviral to significantly reduce murine coronavirus replication in macrophages (Tan et al. 2021). A randomized controlled trial exploring doxycycline (versus placebo) when added to standard antituberculous therapy for pulmonary tuberculosis can ameliorate immunopathology and disease parameters in doxycycline-treated patients (Miow et al. 2021). Much remains to be explored to harness the repurposing of doxycycline in the management of MMP-related pathological disorders and other microbial infections (Liu and Khalil 2017).

23.7 Summary

In conclusion, MMP-2 and MMP-9 production and gelatinase activity were induced by infection of differentiated neutrophils with mouse-adapted influenza A/Aichi/2/1968(H3N2) virus. Furthermore, doxycycline treatment was able to abrogate this increase in MMP-2 and MMP-9 expression and gelatinase activity. Given that excessive neutrophil infiltration and uncontrolled neutrophil gelatinase activity in pulmonary tissues play critical roles in the pathogenesis of ALI and ARDS, this study provides insights into the mechanism of excessive MMP-2 and MMP-9 production and activity in neutrophils during influenza virus infection. Thus, harnessing doxycycline as an MMP inhibitor represents a potential therapeutic strategy to mitigate influenza-associated pulmonary injury by targeting MMPs, gelatinase production, and activity in neutrophils. Doxycycline therapy (alone or in combination with other agents) has also been considered and explored for the management of other infectious diseases such as coronavirus infections and tuberculosis.

Acknowledgments This study was funded by a research grant from the National University of Singapore. We gratefully acknowledge the assistance of K.S. Tan, Edwin Yang, F.X. Ivan, H.H. Ng, Kelly Lau, M.C. Phoon, and J.P. Hsu.

References

Abraham E (2003) Neutrophils and acute lung injury. Crit Care Med 31(4 Suppl):S195–S199

Batty KT, Law AS, Stirling V, Moore BR (2007) Pharmacodynamics of doxycycline in a murine malaria model. Antimicrob Agents Chemother 51:4477–4479

Ben-Yosef Y, Miller A, Shapiro S, Lahat N (2005) Hypoxia of endothelial cells leads to MMP-2-dependent survival and death. Am J Physiol Cell Physiol 289:C1321–C1331

Carney DE, McCann UG, Schiller HJ, Gatto LA, Steinberg J, Picone AL, Nieman GF (2001) Metalloproteinase inhibition prevents acute respiratory distress syndrome. J Surg Res 99:245–252

Cena JJ, Lalu MM, Cho WJ, Chow AK, Bagdan ML, Daniel EE, Castro MM, Schulz R (2010) Inhibition of matrix metalloproteinase activity in vivo protects against vascular hyporeactivity in endotoxemia. Am J Physiol Heart Circ Physiol 298:H45–H51

Chang WY, Clements D, Johnson SR (2010) Effect of doxycycline on proliferation, MMP production, and adhesion in LAM-related cells. Am J Physiol Lung Cell Mol Physiol 299: L393–L400

Chow VT, Tambyah PA, Goh KT (2008) To kill a mocking bird flu? Ann Acad Med Singap 37: 451–453

Corbel M, Boichot E, Lagente V (2000) Role of gelatinases MMP-2 and MMP-9 in tissue remodeling following acute lung injury. Braz J Med Biol Res 33:749–754

Corbitt CA, Lin J, Lindsey ML (2007) Mechanisms to inhibit matrix metalloproteinase activity: where are we in the development of clinically relevant inhibitors? Recent Pat Anticancer Drug Discov 2:135–142

Curci JA, Mao D, Bohner DG, Allen BT, Rubin BG, Reilly JM, Sicard GA, Thompson RW (2000) Preoperative treatment with doxycycline reduces aortic wall expression and activation of matrix metalloproteinases in patients with abdominal aortic aneurysms. J Vasc Surg 31:325–342

Dunowska M, Ghosh S (2021) *In vitro* effects of doxycycline on replication of feline coronavirus. Pathogens 10:312

Fingleton B (2007) Matrix metalloproteinases as valid clinical targets. Curr Pharm Des 3:333–346

Hanemaaijer R, Visser H, Koolwijk P, Sorsa T, Salo T, Golub LM, van Hinsbergh VW (1998) Inhibition of MMP synthesis by doxycycline and chemically modified tetracyclines (CMTs) in human endothelial cells. Adv Dent Res 12:114–118

Hashimoto Y, Moki T, Takizawa T, Shiratsuchi A, Nakanishi Y (2007) Evidence for phagocytosis of influenza virus-infected, apoptotic cells by neutrophils and macrophages in mice. J Immunol 178:2448–2457

Ivan FX, Rajapakse JC, Welsch RE, Rozen SG, Narasaraju T, Xiong GM, Engelward BP, Chow VT (2012) Differential pulmonary transcriptomic profiles in murine lungs infected with low and highly virulent influenza H3N2 viruses reveal dysregulation of TREM1 signaling, cytokines, and chemokines. Funct Integr Genomics 12:105–117

Ivan FX, Tan KS, Phoon MC, Engelward BP, Welsch RE, Rajapakse JC, Chow VT (2013) Neutrophils infected with highly virulent influenza H3N2 virus exhibit augmented early cell death and rapid induction of type I interferon signaling pathways. Genomics 101:101–112

Ivan FX, Zhou X, Lau SH, Rashid S, Teo JSM, Lee HK, Koay ES, Chan KP, Leo YS, Chen MIC, Kwoh CK, Chow VT (2020) Molecular insights into evolution, mutations and receptor-binding specificity of influenza A and B viruses from outpatients and hospitalized patients in Singapore. Int J Infect Dis 90:84–96

Johnson BS, Chandraratna RA, Heyman RA, Allegretto EA, Mueller L, Collins SJ (1999) Retinoid X receptor (RXR) agonist-induced activation of dominant-negative RXR-retinoic acid receptor alpha403 heterodimers is developmentally regulated during myeloid differentiation. Mol Cell Biol 19:3372–3382

Lawson ND, Krause DS, Berliner N (1998) Normal neutrophil differentiation and secondary granule gene expression in the EML and MPRO cell lines. Exp Hematol 26:1178–1185

Liu J, Khalil RA (2017) Matrix metalloproteinase inhibitors as investigational and therapeutic tools in unrestrained tissue remodeling and pathological disorders. Prog Mol Biol Transl Sci 148: 355–420

Miow QH, Vallejo AF, Wang Y, Hong JM, Bai C, Teo FS, Wang AD, Loh HR, Tan TZ, Ding Y, She HW, Gan SH, Paton NI, Lum J, Tay A, Chee CB, Tambyah PA, Polak ME, Wang YT, Singhal A, Elkington PT, Friedland JS, Ong CW (2021) Doxycycline host-directed therapy in human pulmonary tuberculosis. J Clin Invest 131:e141895

Murphy G, Crabbe T (1995) Gelatinases A and B. Methods Enzymol 248:470–484

Narasaraju T, Sim MK, Ng HH, Phoon MC, Shanker N, Lal SK, Chow VT (2009) Adaptation of human influenza H3N2 virus in a mouse pneumonitis model: insights into viral virulence, tissue tropism and host pathogenesis. Microbes Infect 11:2–11

Narasaraju T, Yang E, Samy RP, Ng HH, Poh WP, Liew AA, Phoon MC, van Rooijen N, Chow VT (2011) Excessive neutrophils and neutrophil extracellular traps contribute to acute lung injury of influenza pneumonitis. Am J Pathol 179:199–210

Narendrakumar L, Joseph I, Thomas S (2021) Potential effectiveness and adverse implications of repurposing doxycycline in COVID-19 treatment. Expert Rev Anti-Infect Ther 19:1001–1008

Ng HH, Narasaraju T, Phoon MC, Sim MK, Seet JE, Chow VT (2012) Doxycycline treatment attenuates acute lung injury in mice infected with virulent influenza H3N2 virus: involvement of matrix metalloproteinases. Exp Mol Pathol 92:287–295

Parks WC, Wilson CL, López-Boado YS (2004) Matrix metalloproteinases as modulators of inflammation and innate immunity. Nat Rev Immunol 4:617–629

Quispe-Laime AM, Bracco JD, Barberio PA, Campagne CG, Rolfo VE, Umberger R, Meduri GU (2010) H1N1 influenza A virus-associated acute lung injury: response to combination oseltamivir and prolonged corticosteroid treatment. Intensive Care Med 36:33–41

Sakharkar MK, Sakharkar KR, Chow VT (2009) Human genomic diversity, viral genomics and proteomics, as exemplified by human papillomaviruses and H5N1 influenza viruses. Hum Genomics 3:320–331

Smith JA (1994) Neutrophils, host defense, and inflammation: a double-edged sword. J Leukoc Biol 56:672–686

Smith VA, Cook SD (2004) Doxycycline-a role in ocular surface repair. Br J Ophthalmol 88:619–625

Snoek-van Beurden PA, Von den Hoff JW (2005) Zymographic techniques for the analysis of matrix metalloproteinases and their inhibitors. BioTechniques 38:73–83

Sternlicht MD, Werb Z (2001) How matrix metalloproteinases regulate cell behavior. Annu Rev Cell Dev Biol 17:463–516

Tan YL, Tan KSW, Chu JJH, Chow VT (2021) Combination treatment with remdesivir and ivermectin exerts highly synergistic and potent antiviral activity against murine coronavirus infection. Front Cell Infect Microbiol 11:700502

Taubenberger JK, Morens DM (2008) The pathology of influenza virus infections. Annu Rev Pathol 3:499–522

Ueland T, Holter JC, Holten AR, Muller KE, Lind A, Bekken GK, Dudman S, Aukrust P, Dyrhol-Riise AM, Heggelund L (2020) Distinct and early increase in circulating MMP-9 in COVID-19 patients with respiratory failure. J Inf 81:e41–e43

Ware LB, Matthay MA (2000) The acute respiratory distress syndrome. N Engl J Med 342:1334–1349

Xu T, Qiao J, Zhao L, Wang G, He G, Li K, Tian Y, Gao M, Wang J, Wang H, Dong C (2006) Acute respiratory distress syndrome induced by avian influenza A (H5N1) virus in mice. Am J Respir Crit Care Med 174:1011–1017

Zhou X, Zheng J, Ivan FX, Yin R, Ranganathan S, Chow VTK, Kwoh CK (2018) Computational analysis of the receptor binding specificity of novel influenza A/H7N9 viruses. BMC Genomics 19(Suppl. 2):88

Chapter 24
Therapeutic Repurposing Approach: New Opportunity for Developing Drugs Against COVID-19

Monu Yadav, Pratibha Dhakla, Rahul Rawat, Mini Dahiya, and Anil Kumar

Abstract The coronavirus disease 2019 (COVID-19) pandemic initiated by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has encouraged the repurposing of various drugs to treat the morbidity, mortality, and extent of the disease. Nowadays, the COVID-19 pandemic is a major health concern as it has already affected the whole world in all aspects. Drug repurposing is considered a new potential strategy as it is a cost-effective and less time-consuming process to establish a new indication for existing drugs. The present chapter has focused on the pathophysiology of COVID-19 and the reuse of the drugs based on pharmacological mechanisms. In the literature, various drugs like favipiravir, lopinavir, ritonavir, arbidol, chloroquine, hydroxychloroquine, interferons, etc. have been reported for repurposing purposes against COVID-19. Most of them are effective in in vitro and clinical studies. Drugs act mainly on viral entry, viral replication, angiotensin-converting enzyme-2 (ACE2), inflammatory mechanisms, etc. Based on viral pathogenesis and the mechanism of drugs using in silico, in vitro, and clinical studies,

M. Yadav (✉) · P. Dhakla
Department of Pharmacy, School of Medical & Allied Sciences, GD Goenka University, Gurgaon, Haryana, India

R. Rawat
Department of Pharmaceutical Sciences, Chaudhary Bansi Lal University, Bhiwani, Haryana, India

M. Dahiya
University Institute of Pharmaceutical Sciences (UIPS), UGC Centre of Advanced Studies (UGC-CAS), Panjab University, Chandigarh, Punjab, India

University Institute of Pharmaceutical Sciences (UIPS), UGC Centre of Advanced Studies (UGC-CAS), Panjab University, Chandigarh, Haryana, India

A. Kumar
University Institute of Pharmaceutical Sciences (UIPS), UGC Centre of Advanced Studies (UGC-CAS), Panjab University, Chandigarh, Punjab, India

University Institute of Pharmaceutical Sciences, UGC Centre of Advanced Studies (UGC-CAS), Panjab University, Chandigarh, Haryana, India
e-mail: aniluips@pu.ac.in

R. C. Sobti et al. (eds.), *Drug Repurposing for Emerging Infectious Diseases and Cancer*, https://doi.org/10.1007/978-981-19-5399-6_24

repurposing medicines might be considered an excellent opportunity to cure COVID-19.

Keywords Coronavirus disease · COVID-19 · Viral enzyme · RNA genome · Repurposing of drugs

24.1 Introduction

Coronavirus (also known as COVID-19) is a wide virus family that has been linked to a variety of diseases, extending from the common cold to more serious conditions including Middle East respiratory syndrome (MERS) and severe acute respiratory syndrome (SARS). A novel coronavirus (CoV) is a coronavirus strain that has never been previously discovered in humans. The global COVID-19 pandemic is a major public health problem (Parasher 2021; Saxena 2020). The novel coronavirus originated in Wuhan, China, and rapidly spread to the rest of the world (Chan et al. 2020). Coronavirus is enclosed in a positive-sense RNA virus sizing between 60 nm and 140 nm with spike-like projections on its outer surface that give it a crown-like look under the electron microscope. Seven coronaviruses can infect people all around the world, but the most prevalent illnesses are caused by four human coronaviruses: 229E, NL63, OC43, and HKU1 (Kaushik et al. 2020). Officials in Wuhan City, China, reported the first case of COVID-19 considered SARS-CoV-2, in December 2019, and it soon spread to become a pandemic. Several investigations into the cause of the outbreak in China are being conducted including studies into human cases with symptoms starting in late 2019 in and around Wuhan. The majority of the initial cases were connected to the Huanan seafood market, which dealt with the sale of dead seafood animals as well as the trade of living animals (Kumar et al., 2020). Polymerase chain reaction (PCR), whole-genome sequencing, and cell culture were used to evaluate the patients' broncho-alveolar lavage fluid for viral pneumonia. In the meantime, the Chinese government notified the World Health Organization (WHO) and stopped the Huanan seafood market on January 1, 2020. Early in January, SARS-CoV-2 was discovered. The number of cases started increasing drastically since then, even for those with no exposure to the seafood market, thus indicating its human-to-human transmission (Liu et al. 2020a). This turned out to be an epidemic, initially spreading to other countries like Thailand, South Korea, and Japan as there was a massive Chinese migration due to Chinese New Year's Eve. This virus was identified as -CoV on January 7th (Organization WH 2020). It had 96.2% homology to bat coronavirus, specifically the RaTG13 genome, and 79.5% homology to SARS coronavirus (Zheng 2020). The first fatality was reported on January 11th, and its genetic sequence was shared publicly on January 11–12, 2020. On January 12th, the World Health Organization (WHO) officially designated this CoV as 2019-novel coronavirus (2019-nCoV) (World Health Organization 2020). Since then, the number of incidents has risen significantly, even among those who have never been exposed to the fish market, demonstrating that the disease is spread from person to person (Liu et al. 2020a). Due to a massive Chinese exodus on Chinese New Year's Eve, this transformed into a pandemic that spread to other

nations like Thailand, South Korea, and Japan. On January 7th, this virus was discovered as -CoV (Organization WH 2020). It shared 96.2% of its genome with bat coronavirus, particularly the RaTG13 gene, and 79.5% with SARS coronavirus (Zheng 2020). On January 11th, the first death was reported, and its genomic code was made public on January 11–12, 2020. The WHO identified this CoV as 2019-novel coronavirus (2019-nCoV) on January 12th (World Health Organization 2020). COVID-19 was declared a Public Health Emergency of International Concern by WHO on February 1st. WHO later considered this the coronavirus illness 2019 on February 11, 2020. Since then, the number of cases has risen at an increasing rate. COVID-19 was subsequently recognized as a pandemic by WHO on March 11th (Anon n.d.; WHO-BJ 2020). SARS-CoV-2 is believed to have developed in bats, as per scientists. This is also how the Middle East respiratory syndrome (MERS) and severe acute respiratory syndrome (SARS) coronaviruses got their start. In 1965, researchers showed a coronavirus that produces the common cold in humans. Investigators eventually revealed several related human and animal viruses, which they named after their crown-like appearance. An adult human can be infected by seven different coronaviruses. SARS was first identified in southern China in 2002, and it soon spread to 28 nations. By July 2003, over 8000 people had been affected, with 774 of them dying. In 2004, there were just four more cases in a limited outbreak. Fever, headaches, and respiratory difficulties such as cough and breathlessness are all signs of this coronavirus. In 2012, MERS was first detected in Saudi Arabia. People who live in or travel to the Middle East were found to have approximately 2500 cases. This coronavirus is less infectious than SARS, but it is more lethal, having killed 858 individuals. This has the same respiratory problems as the common cold, but it could also lead to renal failure (Chen 2021).

24.2 COVID-19 Risk Factors

Governments made rules to fight with COVID-19 pandemic, especially social distancing, if someone is affected with COVID-19, they are advised to take more precautions, including self-isolation. It was found that obese patients had a sevenfold increased risk of having severe COVID-19, according to a French study. In the fight against COVID-19, promoting good meals to preserve nutritional well-being is more crucial (Simonnet et al. 2020). In a meta-analysis, cigarette smoking persons were found 1.5 times more than nonsmoking to have serious COVID-19 problems, as well as a higher death rate. In Italy, men are at higher risk than women, which may be owing to their higher smoking rates and subsequent co-morbidities (Alqahtani et al. 2020). A higher risk of death was linked to advanced age, cardiovascular disease, diabetes, chronic respiratory disease, hypertension, and cancer. A meta-analysis of eight trials involving 46,248 COVID-19 patients found that individuals with the most severe condition were more likely to have hypertension, pulmonary disease, and cardiovascular disease. According to a systematic analysis, people with diabetes are up to three times more likely to suffer serious COVID-19 symptoms, which can lead to mortality, and the condition appears to become worse for

individuals with uncontrolled diabetes (Roncon et al. 2020). Hypertension, cardiovascular disease, and cerebrovascular disorder all elevated the probability of severe COVID-19 according to a meta-analysis (Wang et al. 2020). Another report suggested that high blood pressure raised the chance of death from COVID-19 by 3.5 times (Dong et al. 2020a). COVID-19 was found to increase the risk of serious complications or death in people with COPD in a meta-analysis. According to a study conducted in the United Kingdom, people with respiratory disorders, such as asthma, had a higher risk of dying from COVID-19 (D'ascanio et al. 2021). In a meta-analysis, patients with COPD were at an increased risk of severe complications or death from COVID-19. A study in the United Kingdom suggested that the presence of respiratory diseases, including asthma, increased patients' risk of mortality from COVID-19 (D'ascanio et al. 2021).

24.3 Pathophysiology Targets for COVID

Researchers can find targets for new therapeutic drugs to prevent and reduce SARS-CoV-2 by understanding the disease's transmissibility and pathophysiology (Fig. 24.1). The SARS-CoV-2 virus is a single-stranded RNA-enveloped virus with four structural proteins: the S (spike), E (envelope), M (membrane), and N

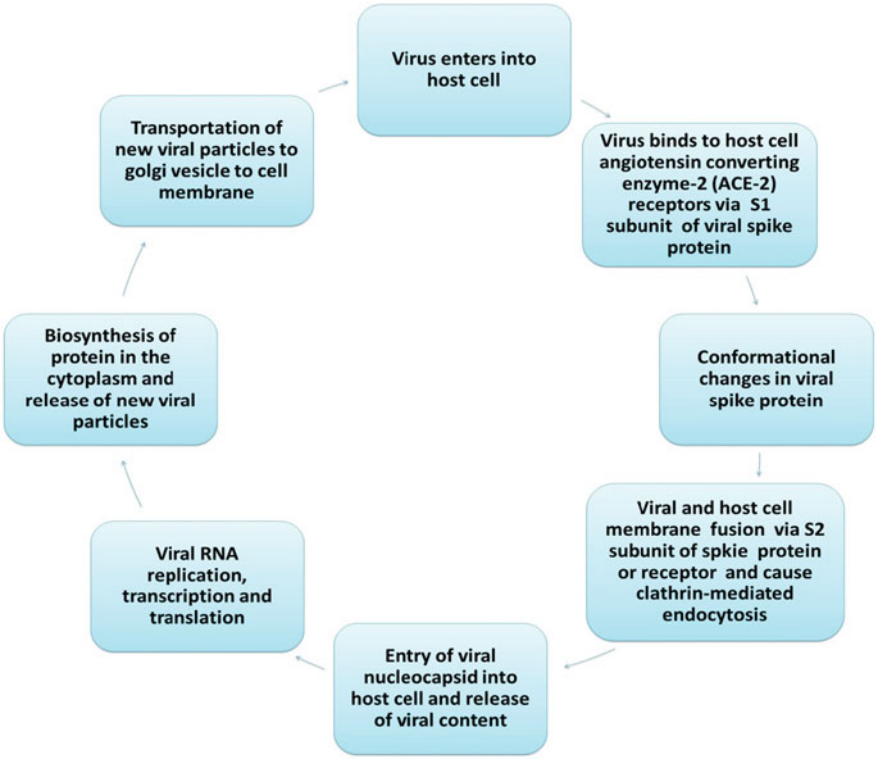


Fig. 24.1 Pathophysiology of COVID virus

(nucleocapsid). The N protein encodes the RNA genome, while the S, E, and M proteins collectively form the viral envelope (Parasher 2021). SARS-CoV-2 is anticipated to bind to nasal epithelial cells located respiratory tract after being inhaled via respiratory aerosols. ACE-2 receptors are bound by the spike-like viral structural (S) protein. SARS-CoV-2 uses its S-protein to attach the ACE-2 receptors on the surface of human cells, and two transmembrane serine proteases (TMPRSS2) activate the S-protein after this initial interaction, allowing its entry into the cell via clathrin-mediated endocytosis (Cao et al. 2020; Belouzard et al. 2012; Bayati et al. 2021). The S protein is cleaved by lysosomal proteases namely cathepsin L and P during endocytosis and the formation of endosomes, leading to the fusion of the viral envelope with endosomal membranes and the unbound the viral RNA into the cytoplasm of the cell. So, cathepsin inhibitors possess the property to inhibit the penetration of the virus into host cells (Shirato et al. 2018; Padmanabhan et al. 2020). The nucleocapsid penetrates the cytoplasm and delivers viral RNA once the viral envelope fuses with the endosomal membrane. The chemical composition of coronavirus RNA is comprised of a 5' methylated head and a 3' polyadenylated tail that permits the virus to bind to the host cell's free ribosomes. This initiates the translation process, which results in the production of a lengthy polypeptide chain. The RNA is transformed into two polyproteins, pp1a and pp1ab, that are then split by cytoplasmic proteolytic enzymes into 16 nonstructural proteins (NSP1 + NSP16), which together make up the viral replicase transcriptase complex (Fehr and Perlman 2015; Knoops et al. 2008). The NSP proteins transform the rough endoplasmic reticulum's membranes into the double vesicles wherein the virus multiplies and transcribes (Astuti 2020) Viral replication in double-membrane vesicles is a critical mechanism that permits viral RNA to avoid detection by cell signal transduction pathways and avoid the cell-mediated immunity initially during illness (Astuti 2020). The newly generated envelope glycoproteins are integrated into the endoplasmic reticulum, whereas the nucleocapsid is produced using RNA and a nucleocapsid protein combination (N). The viral particles then penetrate the endoplasmic reticulum and the intermediate segment of the Golgi apparatus (ERGIC). Eventually, the virus is released when the vesicles holding viral particles merge with the plasma membrane (Neerukonda and Katneni 2020). Because ACE-2 is widely expressed in the nasal mucosa, bronchi, lungs, heart, gullet, kidneys, stomach, bladder, and ileum, Severe acute respiratory can infect any of these internal tissues. Recently, studies have proposed that SARS-CoV-2 may be harmful to seminiferous tubules, leading to infertility issues in young individuals. The new virus particles are now available to infect neighboring epithelial cells and offer fresh infectious material for global spread through respiratory secretions.

24.4 Clinical Feature

The main symptoms of this illness differ from individual to individual, ranging from asymptomatic to acute breathing difficulties, septicemia, and multiorgan failure (dysfunction). [20] Fever (98.6%), fatigue (69.6%), dry cough, loose bowels,

Table 24.1 COVID-19 symptoms

Condition	Symptoms
Asymptomatic	• Nasal swab examination positive
Acute condition	• Lethargy, temperature, throat infection, hoarse throat • Nausea, vomiting, stomach ache
Moderate condition	• Pneumonia signs (consistent temperature and cough) without hypoxemia • On a high-resolution CT scan of the chest, there are severe lesions
Severe condition	• Pneumonia along with hypoxemia
Clinical condition	• Shock, coagulation problems, encephalopathy, heart problems, and acute renal failure are all symptoms of acute respiratory infections

soaring throat, headache, muscle pain, and breathlessness are some of the symptoms that people with the condition experience (Table 24.1). In some cases, conjunctivitis has been noted. Infection is spread through huge droplets produced by symptomatic sufferers' coughing or sneezing, although it could also occur in asymptomatic individuals and before symptoms appear. Its incubation time, which would be the interval between virus contact and symptom start, generally lasts 5–6 days, although it can last up to 14 days. Infected persons can be communicable and spread the infection to normal people in a population throughout this period, also referred to as the "pre-symptomatic" span. This virus can be categorized into three phases based on the cells that are most likely to be infected, which correlate to variable clinical phases of the infection.

24.4.1 Asymptomatic Phase (Stage 1)

SARS-CoV-2 attaches to nasal epithelial cells in the upper respiratory tract after being inhaled by respiratory aerosols. ACE-2, which is extensively expressed in human nose epithelia, is the major host receptor facilitating viral penetration into cells (Wan et al. 2021). Acute replication and proliferation of the virus occur, also as penetration of ciliated cells in the upper airways. The stage only lasts a few days, and the immunological reaction produced throughout this period is limited. The patients are highly susceptible, although having the lowest viral replication during this time, as well as the virus can be diagnosed using nasal swab analysis (Reyfman et al. 2019; Sims et al. 2005).

24.4.2 Stage 2: Upper Airway and Airway Response (in the Coming Days)

The virus spreads and migrates downward through the large airways from the nasal epithelium toward the upper airways, triggering a stronger innate immunity. Fever, fatigue, and hoarseness are all indications of the condition (Tang et al. 2005). Virus-infected epithelial cells release C-X-C motif chemokine ligand 10 (CXCL-10) and interferons (IFN- and IFN-) during this phase, resulting in a significant immune reaction. CXCL-10 is an interferon-responsive gene in this condition (Padmanabhan et al. 2020; Fehr and Perlman 2015; Knoops et al. 2008; Astuti 2020; Neerukonda and Katneni 2020; Wan et al. 2021; Reyfman et al. 2019; Sims et al. 2005; Tang et al. 2005; Hancock et al. 2018).

24.4.3 Stage 3: Hypoxia, Ground-Glass Infiltration, and Progression to ARDS

Consequently, approximately 20% of people with the disease may proceed to stage 3 of the illness and experience respiratory infiltrates, with many of these resulting in life-threatening conditions (Wu and McGoogan 2020). The virus subsequently infects alveolar type II epithelial cells through the human receptor ACE-2 in the lungs. In comparison to type I cells, SARS-CoV mainly attacks type II cells (Weinheimer and Becher 2012; Mossel et al. 2008). The contaminated alveolar units are usually found on the periphery and in the subpleural space. As SARS-CoV replicates in type II cells, it releases a huge number of viral proteins, causing the cells to apoptosis and die. As the released virus particle attacks type II cells in nearby cells, the consequence is most probably a self-replicating respiratory toxin. The problematic lung regions would certainly lose the majority of their type II cells, triggering a subsequent epithelium renewal process. Type II cells are usually the cells that produce type I cells (Qian et al. 2013; Mason 2020). Using the human receptor ACE-2, the virus invades and infects type 2 alveolar epithelial cells, where it begins replication to make additional viral nucleocapsids. Many cytokines and pathological indicators are generated by adware pneumocytes, including interleukins (IL-1, IL-6, IL-8, IL-120, IL-12), tumor necrosis factor (TNF), interferon (IFN), CXCL-10, monocyte chemoattractant protein-1 (MCP-1), and macrophage inflammatory protein-1 (MIP-1). This "cytokine flood" attracts neutrophils, CD4 helper T cells, and CD8 cytotoxic T cells, which ultimately become trapped in lung tissue. These cells defend against the virus, but they are also involved in inflammation and lung damage. The host cell dies, releasing more virus proteins that infect nearby type 2 alveolar epithelial cells. There is extensive alveolar damage as a result of the chronic injury induced by localized inflammatory cells and viral multiplication, which causes the loss of both type 1 and type 2 pneumocytes, resulting in acute respiratory infections (Mishra and Singh 2020; Chowdhury et al. 2020).

24.5 Therapeutic Approach for COVID-19

Because the SARS-CoV-2 E, M, and N proteins are essential for viral survival and replication, they could be used as therapeutic targets. Since these viral proteins are physically distinct from host proteins, medicines that target them may have minimal side effects. These structural proteins not only protect the viral genome but also suppress the host immune system, giving the virus significant benefits over the host (Bojkova et al. 2020; Borgio et al. 2020). The N protein inhibits RNA silencing and siRNA-mediated RNA interference. As a result, several siRNA-based treatments suppress viral replication by targeting viral E, M, and N protein translation. Because of intrinsic durability problems and the lack of dependable delivery techniques, siRNA-based medicines are currently not ready for human use. Because of its crucial role in viral RNA replication and transcription, the N protein is a multipurpose RNA-binding protein that is considered a potential therapeutic candidate. The N protein is a multifunctional protein; it covers the virus RNA genome in a ribonucleoprotein structure called a nucleocapsid to defend it. Interestingly, the N protein seems to be very immunogenic, making it an ideal candidate for a vaccine (McBride et al. 2014; Tan and Yin 2004). The E protein's capacity to act as an ion channel is inhibited by hexamethylene amiloride. The drug inhibitor, PJ34, targets a particular ribonucleotide-binding site in the N protein's N-terminal region. It's important to note that most such inhibitors are designed to fight the SARS virus; but due to changes in the SARS-CoV-2 virus, these might not be quite as helpful in fighting the ongoing COVID-19 virus (Pervushin et al. 2009; Lin et al. 2014). Recently, WHO has certified eight vaccinations (Table 24.2).

Table 24.2 Vaccine approved by WHO for the treatment of COVID-19 (https://COVID19.trackvaccines.org/agency/who/)

Name of vaccine	Form of vaccine	Number of clinical trials	Approved in several countries
Moderna mRNA-1273	RNA	32	77
Pfizer/BioNTech BNT162b2	RNA	45	10
Janssen (Johnson & Johnson) Ad26.COV2.S	Nonreplicated viral vector	16	78
Oxford/AstraZeneca AZD1222	Nonreplicated viral vector	49	125
Serum Institute of India Covishield (Oxford/AstraZeneca formulation)	Nonreplicated viral vector	02	46
Bharat Biotech Covaxin	Inactivated	07	10
Sinopharm (Beijing) BBIBP-CorV (Vero Cells)	Inactivated	17	68
Sinovac CoronaVac	Inactivated	26	43

24.6 Repurposing of the Drugs to Cure COVID-19

24.6.1 Repurposed Drugs That Act on Virus-Related Targets

The viruses enter the host cell in two ways: i) by endocytosis, in which the virus enters into the cell along with the endosomes, and ii) by infusion method, the SARS-CoV-2 utilizes the spike protein "S" found on the virus surface to obtain access into the host cells. The protein–protein interaction between both the spike protein subunits and the active site of the ACE-2 receptor may be considered to find a therapy target (Wrapp et al. 2020). So, antivirals by constantly changing the active site of the spike protein can be effective for coronavirus treatment. As a result, the SARS-CoV-2 spike proteins target the ACE-2 receptor better than the SARS virus that has been previously investigated (Albini et al. 2020). The receptor-binding domain (RBD) of the spike protein, in particular, is a known for having antibody-mediated binding disruption. Several antibodies that have shown to be capable of disrupting this binding are still in the preclinical development stages (Chen and Hotez 2020; Tai et al. 2020). Another approach is to use recombinant human ACE-2, which is usually found on the cell surface, to activate and defeat the ACE-2 receptor. As a result, delivering an oversupply of soluble ACE-2 inactivates the virus by interacting with SARS-CoV-2 competitively (Basit et al. 2021; Ameratunga et al. 2020). Arbidol (umifenovir) seems to be a more potential repurposed medicine that inhibits viral fusion and penetration by inhibiting the S protein/ACE2 interaction. It is now approved in Russia and China for the prophylaxis and treatment of influenza and shows in vitro viral efficacy against SARS-CoV-2, while not being FDA-approved (Wrapp et al. 2020). Aside from the ACE-2 receptor, the cellular serine protease TMPRSS2 is also involved in enabling virus penetration into host cells. Camostat Mesylate, a therapeutically proven pharmacological inhibitor of TMPRSS2, can also decrease inflammation in human lung cell lines (Hoffmann et al. 2020; Rahman et al. 2020). The virus is absorbed into the cells via a pH and receptor-dependent endocytosis when the spike protein fuses with the ACE-2 receptor (Glebov 2020; Chu et al. 2006). Another technique for identifying possible therapeutic candidates against SARS-CoV-2 might be to target endocytosis. AP-2-associated protein kinase 1 (AAK1) controls clathrin-mediated endocytosis (Uitdehaag et al. 2019). The Janus kinase inhibitor Baricitinib is found as a potential candidate medicine for SARS-CoV-2 depending on library analysis (Cantini et al. 2021). Ouabain, a clathrin-mediated inhibitor, is being investigated in therapeutic studies for SARS-CoV-2 positive cases (Sisk and Frieman 2018). Chloroquine as well as its derivative, hydroxychloroquine, has recently attracted attention as a possible therapy for SARS-CoV-2 infection. These compounds are mostly used for their antiplasmodium activity. Chloroquine is derived from the Cinchona plant, which is a potent coronavirus inhibitor. Many drug studies are being conducted to determine the role of chloroquine medicine in reducing SARS-C0V-2 virus development. Endosome-mediated viral penetration or late phases of viral replication are believed to be inhibited by hydroxychloroquine (Alia and Grant-Kels 2020; Alexander et al.

2020; Costanzo et al. 2020). As weak diprotic bases, chloroquine and hydroxychloroquine focus endosomes by raising the pH of the endosomal fluid. The acidic pH of endosomes is required for the virus's potential activity in proteolysis and post-translational modification of promising proteins. The reproduction and maturation of viruses are inhibited when the essential pH is disturbed. Additionally, these drugs prevent ACE2 from becoming glycosylated. Defective glycosylation makes the virus's binding and penetration into host cells even more difficult (Vincent et al. 2005; Al-Bari 2017). Because of their immunomodulatory and anti-inflammatory qualities, statins (hypolipidemic medicines) have pleiotropic effects that protect against acute lung injury. As a result, it is utilized as a repurposing medicine to treat COVID-19 patients. The use of statins has been linked to an increase in ACE2 expression (Tikoo et al. 2015a). Angiotensin II (Ang II), which causes cardiovascular disease and endothelial dysfunction, is cleaved by ACE2 to produce angiotensin (1–7), which counteracts Ang II's effects (Ferrario 2011). The ACE2/angiotensin-(1–7) and angiopoietin/Tie-2 signaling axes are both stimulated to assist reverse virus-induced endothelial dysfunction and preserve patient homeostasis. Atorvastatin and angiotensin II receptor blockers (ARBs) have been found to increase the activity of ACE2 (Tikoo et al. 2015a; Ferrario 2011). Immunomodulatory effects of statins have been suggested as a possible strategy for MERS coronaviruses (Ferrario 2011). Myeloid differentiation main reaction protein (MYD) 88 causes chronic respiratory problems that could lead to death. Statins have been found to block MYD88 signaling (Durán et al. 2020; Yuan 2015; Wösten-van Asperen et al. 2011). Statins also interact with protease inhibitors and thus are contraindicated when used concurrently (Chauvin and Drouot 2013).

24.6.2 Repurposed Drugs Act Through Inhibition of Viral Enzymes

Before packaging into virions, viral RNAs evolve into polypeptide chains that are broken into proteins. The cleavage of polypeptide chains is carried out by protease enzymes. HIV-1 is treated with protease inhibitors, which have also been demonstrated to be effective against SARS-CoV. The enzyme RNA-dependent RNA polymerase is required for SARS-CoV2 replication (RDRP). The therapeutic drug target polymerase for SARS-CoV2 and SARS-CoV has been discovered to be highly conserved. As a result, polymerase inhibitors may be effective against SARS-CoV2-71. Flavipiravir, lopinavir, and remdesivir, which were developed for other viral infections, are currently being tested in clinical studies for their efficacy in preventing the COVID-19 pandemic. Remdesivir is an adenosine analog that RNA-dependent RNA polymerases (RdRps) incorporate into viral RNA chains, causing premature transcription termination (Gordon et al. 2020; Cao and Deng 2020). Antiviral medicines such as ribavirin and guanosine are also available. Hepatitis C and respiratory syncytial viruses are among the diseases for which it is

approved. It even has antiviral properties against SARS and SARS-CoV2 (Graci and Cameron 2006; Chu et al. 2004). The viral RDRP enzyme is inhibited when the intracellular GTP supply is low. It also disrupts the capping of mRNA. Favipiravir and ribaviorin, for example, are guanine analogs that have been approved for the treatment of many viral infections (Fan et al. 2020). Lopinavir and ritonavir, for example, are protease inhibitors that target SARS-3C–like CoV-2's protease (3CLpro). 3CLpro, the major coronavirus protease, seems to control the conversation of the polypeptide into NSPs (Bhatnagar et al. 2020). Prulifloxacin, tegobuvir, bictegravir, and nelfinavir were discovered via high-throughput screening for drugs against 3CLpro (Gordon et al. 2020; Cao and Deng 2020; Graci and Cameron 2006; Liu et al. 2020b; De Clercq 2006; Barnard et al. 2006).

24.6.3 Repurposed Drugs Targeting the Virus Uptake Pathways

The host immune response depends on a well-coordinated cytokine response. Some SARS-CoV-2–infected individuals have been found to have a hyper-inflammatory response, presumably due to a dysregulated cytokine response. When compared to non-ICU patients, COVID-19 patients in the ICU had higher levels of cytokines in their plasma, suggesting that cytokine dysregulation is involved in the severe type of COVID-19 disease (Channappanavar and Perlman 2020; Novel Coronavirus 2020). In addition, compared to ICU naive patients, SARS-CoV-2–infected patients admitted to the ICU have higher amounts of GM-CSF and IL6+CD4+T cells (Boettcher et al. 2007). The preceding information suggests that inhibiting an overactive inflammatory response may help to lower the severity of COVID-19 disease. The pharmacological potential of corticosteroids in decreasing systemic inflammation is well documented. Their use in COVID-19 patients, however, is still questionable and requires further research. While current worldwide consensus and the WHO advise against the use of corticosteroids, Chinese guidelines do recommend short-term treatment with low-to-moderate dosages of corticosteroids in COVID-19 ARDS. Dexamethasone has recently been discovered to be useful in lowering mortality in severe patients (Zha et al. 2020; Liu et al. 2020c). It has been shown that CD4+T cells are triggered to create GM-CSF and other cytokines after SARS-CoV-2 infection, resulting in the activation of CD14+CD16+ monocytes with elevated interleukin 6 expression (IL-6). This finding suggests that by inhibiting the IL-6 receptor, might be able to minimize the immunological stress induced by SARS-CoV-2 (Conti et al. 2020; Jawhara 2020). The convalescent plasma treatment is another significant advancement in COVID-19 treatment. With infection rates rising and no specific treatment available, convalescent plasma (CP) therapy has been advocated as a primary treatment option. The plasma taken from a disease-free donor is used to establish humoral immunity toward SARS-CoV-2–infected patients in this therapy. Human antibodies against infection are obtained from the plasma of

the donor patient (Shi et al. 2020). However, huge human trials are needed to properly assess and analyze CP as a means of COVID-19 treatment. Rheumatoid arthritis and inflammatory disorder are treated with tocilizumab. This medicine does not work against the virus; instead, it reduces the host's cytokine response. It works by preventing IL-6-induced signaling (Ferrey et al. 2020). In China, the first instance of COVID-19 was discovered, accompanied with chest discomfort and multiple myeloma. The patient was given tocilizumab, as well as arbidol and moxifloxacin, as treatment. However, another patient in the United States remained critically ill despite treatment with tocilizumab and hydroxychloroquine (Mihai et al. 2020). Tocilizumab has been evaluated as a promising chemical in chronic autoimmune illnesses in severe COVID-19 instances, according to a few investigations (Phadke and Saunik 2020).

24.6.4 Repurposed Drugs Act Through Host Targets Such as Antiviral Immunity

Antiviral interferon (IFN) is activated when the host's immune cells recognize viral pathogen-associated molecular patterns (PAMPs) (Xie et al. 2018). Interferons are thought to be effective antiviral medicines. Interferons that have been activated stimulate numerous interferon-activated genes that encode proteins which have a significant antiviral effect. Type I (IFN, IFN, IFN, and IFN) and type II (IFN, IFN, IFN, and IFN) IFNs are the most common (IFNs). Pegylated IFN-2a and IFN-2b are two kinds of pegylated IFN-2 (Li et al. 2021). The host interferon receptor (IFNAR1) targets pegylated interferon alfa-2b, which boosts the immune response against the virus. The influenza B virus, parainfluenza virus, and coronavirus have all been found to be susceptible to recombinant human IFN-2b (Wang et al. 2014). In a study conducted in Saudi Arabia, researchers discovered that IFN-2b combined with ribavirin had a possible effect against the coronavirus in rhesus macaques (Khalid et al. 2015). IFN, in combination with ribavirin, has been observed to affect COVID-19 patients in China (Bersanelli 2020). On COVID-19 patients, lopinavir and ritonavir in conjunction with IFN-1b had a prospective effect (Hung et al. 2020).

24.6.5 Other Repurposed Drugs for the Treatment of COVID

Recently, nitazoxanide (an antiprotozoal agent) has been effective against many viruses, including coronaviruses, by intrusive host-regulated pathways involved in viral replication (Rossignol 2016; Yavuz and Ünal 2020). In another study, nafamostat (an anticoagulant drug) has also been shown to be potent against Vero E6 cells infected with SARS-CoV2 by inhibiting the protease. Ivermectin (an anthelmintic drug) has also been observed to have efficiency against some

viral infections. Moreover, it is also believed that it can act via blocking membrane fusion by decreasing the secretion of cathepsin B. Still, more pieces of evidence need to be proven to show their effects against COVID-19 infection. Cepharanthine, an alkaloid, showed a therapeutic effect on leukopenia. It inhibits GX-P2V infection and reduces the viral RNA production in the pangolin coronavirus GX-P2V model (Fan et al. 2020). Auranofin (gold-containing triethyl phosphine coated agent) is an antiarthritic drug. It has found inhibitors against bacterial, parasitic, and viral infections. Recently it has also been observed that the significant effect of Auranofin in Huh 7 cells infected with SARS- CoV2 by the inhibition of redox enzymes and the production of endoplasmic reticulum stress (Rothan and Kumar 2019). Janus kinase inhibitors (JAK) have antiviral, anti-inflammatory, and immunomodulatory properties (Mehta et al. 2020). Pan-JAK inhibitor (TD-0903), JAK1/2 inhibitor (baricitinib, ruxolitinib), and JAK 1/3 inhibitor (tofacitinib) are recently being tested in clinical trials for the management of COVID-19. In a study, methylprednisolone was observed to reduce the death rates of COVID-19 patients (Brotherton et al. 2020). In Table 24.3, various drugs have been studied against COVID-19/SARS-CoV2 infection.

24.7 Conclusion and Future Perspective

COVID-19 is pandemic condition which is affecting the whole world in all aspects. After so many trials, eight vaccines namely Pfizer/BioNTech BNT162b2, Janssen (Johnson & Johnson) Ad26.COV2.S, Oxford/AstraZeneca AZD1222, Serum Institute of India Covishield (Oxford/AstraZeneca formulation), Bharat Biotech Covaxin, Sinopharm (Beijing) BBIBP-CorV (Vero Cells), and Sinovac CoronaVac have been approved by the WHO for the treatment of COVID-19. As it is a pandemic condition, so a large number of populations are suffering from COVID-19, which increases the demand for more drugs. Repurposing existing drugs for new indications is an effective and economic strategy. Several clinical trials have been done against COVID-19 on existing drugs such as antimalarials, antivirals, ARBs, ACEIs, statins, and monoclonal antibodies approved by the FDA for the management of other disorders. Interestingly, many drugs have shown a potent effect on COVID-19 and are used to decrease the mortality of patients with COVID-19. However, controversial results have been reported with the use of hydroxychloroquine and chloroquine; hence, WHO has stopped the SOLIDARITY trials on these drugs. Moreover, in-silico studies could be the primary tool to test the existing drugs of various categories against COVID-19 before going for in-vivo, in-vitro, and clinical trial studies to save money and time.

Table 24.3 List of potential pharmacological agents that may be used against COVID-19/SARS-CoV2 infection

Class of drugs	Drug	Target	Mechanism of action	Clinical trials	Reference
Drugs acting on viral replication	Remdesivir	RdRP	Blocks viral replication	NCT04292730 NCT04292899 NCT04401579	Holshue et al. (2020)
	Favipiravir	RdRP	Inhibits viral replication	NCT04358549 NCT04303299 NCT04346628	Dong et al. (2020b)
	Ribavirin	Inosine monophosphate dehydrogenase, RdRP	Inhibits viral replication	IRCT20200324046850N2 NCT04276688 NCT04392427	Preston et al. (1999)
	Elbasvir	RdRP, papain-like proteinase, and helicase (Based on molecular docking study)	Inhibits viral replication (probable)	None	Balasubramaniam and Reis (2020)
	Cepharanthine	Viral RNA (reducing plasma membrane fluidity in HIV), RDRP, main protease	Inhibit viral permeation and replication	None	Ruan et al. (2021)
	IDX-184	RdRP, GTP binding (Based on molecular docking study)	Inhibit viral replication (probable)	None	Elfiky (2021)
	Sofosbuvir			IRCT20100228003449N29IRCT20130812014333N145 IRCT20200128046294N2	Elfiky (2021)

		RdRP (Based on molecular docking study)	Inhibit viral replication (probable)		
Drugs acting on viral entry	Lopinavir-ritonavir	Protease	Block virus permeability	NCT04372628 NCT04276688 NCT04330690	Choy et al. (2020)
	Darunavir	Protease	Inhibits viral entry	NCT04252274 NCT04303299 NCT04425382	De Meyer et al. (2020)
	Arbidol	Hemagglutinin fusion machinery, spike glycoprotein	Inhibits viral entry	NCT04286503 NCT0426059 NCT04255017	Vankadari (2020)
	Angiotensin Receptor Blockers	Angiotensin-converting enzyme	Inhibits viral entry	NCT04335123 NCT04428268 NCT04312009	Wan et al. (2020)
	Nafamostat	Prevents membrane fusion (MERS-CoV)	Inhibits viral entry	NCT04352400 NCT04418128 jRCTs031200026	Jang and Rhee (2020)
	Chloroquine and hydroxychloroquine	Change in endosomal pH; glycosylation of the host receptor for the virus, angiotensin-converting enzyme 2	Inhibits viral entry as well as post entry	NCT04382625 NCT04355026 NCT04303299	Yao et al. (2020)
	Prulifloxacin	Proteases (Based on molecular docking study)	Blocks the active sites or interrupt the dimer	None	Li et al. (2020)

(continued)

Table 24.3 (continued)

Class of drugs	Drug	Target	Mechanism of action	Clinical trials	Reference
			formation of viral protein (probable)		
	Tegobuvir	Proteases (Based on molecular docking study)	Blocks the active sites or interrupt the dimer formation of viral protein (probable)	None	Li et al. (2020)
	Nelfinavir	Proteases (on the basis of molecular docking study)	Blocks the active sites or interrupt the dimer formation of viral protein (probable)	None	Li et al. (2020)
	Bictegravir	Proteases (on the basis of molecular docking study)	Blocks the active sites or interrupt the dimer formation of viral protein (probable)	None	Li et al. (2020)
Drugs acting on cytokine release	Azithromycin	Not conclusive (alteration in endosomal pH), cytokines	Inhibits viral replication and IL-6 production	NCT04381962 NCT04332107 NCT04381962	Sargiacomo et al. (2020)

	Doxycycline	Cytokines	Inhibits viral replication and IL-6 production	NCT04371952 NCT04433078 IRCT20200418047121N1	Sargiacomo et al. (2020)
	Tocilizumab	IL-6 receptor	Inhibits IL-6 release	NCT04356937 NCT04445272 NCT04403685	Xu et al. (2021)
	Auranofin	Viral RNA	Blocking viral RNA and Cytokines	None	Rothan et al. (2020)
	Ruxolitinib	Janus-kinase 1/2	Blocking cytokine storm	NCT04414098 NCT04338958 NCT04362137	Marinho et al. (2020)
	Baricitinib	Janus-kinase 1/2	Blocking cytokine storm	NCT04421027 NCT04358614 NCT04373044	Ullah Khan and Htar (2020)
	Dexamethasone	Inflammatory cells	Preventing release of cytokines	NCT04325061 NCT04395105 NCT04347980	Shah et al. (2020)
Miscellaneous	Ivermectin	Nuclear transport blocking response (probable)	Reduced viral RNA	IRCT20200408046987N1 NCT04390022 NCT04381884	Thomas et al. (2003)
	Pegylated IFNα-2b	B cells via host interferon receptor, IFNAR1 signaling	Increased immune response towards viral infections	NCT04349410 NCT04273581 NCT04251871	Wösten-Van Asperen et al. (2011)
	Statins	ACE (Angiotensin converting	Improve endothelial dysfunction	NCT04380402 IRCT20190727044343N2	Anastasiou et al. (2020), Tikoo et al. (2015b)

(continued)

Table 24.3 (continued)

Class of drugs	Drug	Target	Mechanism of action	Clinical trials	Reference
		enzyme 2) (Probable)			
	Nitazoxanide	Exactly not known	Exactly not known	IRCT20190727044343N2 NCT04359680 NCT04406246	Anastasiou et al. (2020)

References

Al-Bari MA (2017) Targeting endosomal acidification by chloroquine analogs as a promising strategy for the treatment of emerging viral diseases. Pharmacol Res Perspect 5(1):293. https://doi.org/10.1002/prp2.293

Albini A, Di Guardo G, Noonan DM, Lombardo M (2020) The SARS-CoV-2 receptor, ACE-2, is expressed on many different cell types: implications for ACE-inhibitor-and angiotensin II receptor blocker-based cardiovascular therapies. Internal Emerg Med 15(8):1581–1582. https://doi.org/10.1007/s11739-020-02406-z

Alexander P, Debono V, Mammen M (2020) COVID-19 coronavirus research has overall low methodological quality thus far: case in point for chloroquine/hydroxychloroquine. J Clin Epiemiol 123:120–126. Available from: https://www.sciencedirect.com/science/article/pii/S0895435620303711

Alia E, Grant-Kels JM (2020) Does hydroxychloroquine combat COVID-19? A timeline of evidence. J Am Acad Dermatol 83(1):e33. Available from: https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Does+hydroxychloroquine+combat+COVID-19%3F+A+timeline+of+evidence.+Journal+of+the+American+Academy+of+Dermatology.+&btnG=

Alqahtani JS, Oyelade T, Aldhahir AM, Alghamdi SM, Almehmadi M, Alqahtani AS et al (2020) Prevalence, severity and mortality associated with COPD and smoking in patients with COVID-19: a rapid systematic review and meta-analysis. PLoS ONE 15. Public Library of Science

Ameratunga R, Lehnert K et al (2020) Inhaled modified angiotensin converting enzyme 2 (ACE2) as a decoy to mitigate SARS-CoV-2 infection. NZ Med J 133(1515):112–118. Available from: https://search.proquest.com/openview/a20e7a9ab761c6312fcb2714d87ce972/1?pq-origsite=gscholar&cbl=1056335

Anastasiou IA, Eleftheriadou I, Tentolouris A, Tsilingiris D, Tentolouris N (2020) In vitro data of current therapies for SARS-CoV-2. Curr Med Chem 27(27):4542–4548. Available from: https://www.ingentaconnect.com/content/ben/cmc/2020/00000027/00000027/art00009

Anon (n.d.). https://www.who.int/director-general/speeches/detail/who... - Google Scholar [Internet]. [cited 2021 Nov 21]. Available from: https://scholar.google.com/scholar?lookup=0&q=https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-COVID-19%2D%2D-11-march-2020.+Accessed+20+Dec+2020&hl=en&as_sdt=0,5

Astuti I (2020) Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2): an overview of viral structure and host response. Diab Metab Syndr: Clin Res Rev 14(4):407–412. Available from: https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=An+overview+of+viral+structure+and+host+response.+Diabetes+%26+Metabolic+Syndrome%3A+Clinical+Research+%26+Reviews.+2020%3B14%284%29%3A407-12&btnG=

Balasubramaniam M, Reis RJS (2020) Computational target-based drug repurposing of elbasvir, an antiviral drug predicted to bind multiple SARS-CoV-2 proteins. ChemRxiv. Available from https://www.ncbi.nlm.nih.gov/pmc/articles/pmc7263767/

Barnard DL, Day CW, Bailey K, Heiner M, Montgomery R, Lauridsen L et al (2006) Evaluation of immunomodulators, interferons and known in vitro SARS-CoV inhibitors for inhibition of SARS-CoV replication in BALB/c mice. Antivir Chem Chemother 17(5):275–284

Basit A, Ali T, Rehman SU (2021) Truncated human angiotensin converting enzyme 2; a potential inhibitor of SARS-CoV-2 spike glycoprotein and potent COVID-19 therapeutic agent. J Biomol Struct Dyn 39(10):3605–3614

Bayati A, Kumar R, Francis V, McPherson PS (2021) SARS-CoV-2 infects cells after viral entry via clathrin-mediated endocytosis. J Biol Chem 296. American Society for Biochemistry and Molecular Biology Inc.

Belouzard S, Millet J, Licitra B, Viruses GW (2012) Mechanisms of coronavirus cell entry mediated by the viral spike protein. Viruses 4(6):1011–1033. Available from: https://www.mdpi.com/1999-4915/4/6/1011

Bersanelli M (2020) Controversies about COVID-19 and anticancer treatment with immune checkpoint inhibitors. Immunotherapy 12(5):269–273

Bhatnagar T, Murhekar M et al (2020) Lopinavir/ritonavir combination therapy amongst symptomatic coronavirus disease 2019 patients in India: protocol for restricted public health emergency use. Indian J Med Res 151(2–3):184. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/pmc7288773/

Boettcher IC, Steinberg T, Matiasek K, Greene CE, Hartmann K, Fischer A (2007) Use of anti-coronavirus antibody testing of cerebrospinal fluid for diagnosis of feline infectious peritonitis involving the central nervous system in cats. Am Vet Med Assoc 230(2):199–205. https://doi.org/10.2460/javma.230.2.199

Bojkova D, Klann K, Koch B, Widera M, Nature DK (2020) Proteomics of SARS-CoV-2-infected host cells reveals therapy targets. Nature 583(7816):469–472. Available from https://www.nature.com/articles/s41586-020-2332-7?LinkSource=PassleApp

Borgio J, Alsuwat H, et al. WAO-A of medical, 2020 undefined. State-of-the-art tools unveil potent drug targets amongst clinically approved drugs to inhibit helicase in SARS-CoV-2. Arch Med Sci 2020;16(1). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/pmc7212215/

Brotherton H, Usuf E, Nadjm B, Forrest K, Bojang K, Samateh AL, Bittaye M, Roberts CA, d'Alessandro U, Roca A (2020) Dexamethasone for COVID-19: data needed from randomised clinical trials in Africa. Lancet Global Health 8(9):e1125–e1126. Available from: https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Dexamethasone+for+COVID-19%3A+data+needed+from+randomised+clinical+trials+in+Africa.+Lancet+Glob+Health.+2020&btnG=

Cantini F, Niccoli L, Matarrese D et al (2021) Baricitinib therapy in COVID-19: a pilot study on safety and clinical impact. J Infect 81(2):318–356. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/pmc7177073/

Cao Y, Deng Q (2020) Remdesivir for severe acute respiratory syndrome coronavirus 2 causing COVID-19: an evaluation of the evidence. Travel Med Infect Dis 35:101647. Available from: https://www.sciencedirect.com/science/article/pii/S1477893920301162

Cao Z, Wu Y, Faucon E, Sabatier J-M (2020) SARS-CoV-2 & COVID-19: key-roles of the 'renin-angiotensin' system/vitamin d impacting drug and vaccine developments. Infect Disord Drug Targets 20(3):348–349

Chan JFW, Yuan S, Kok KH, To KKW, Chu H, Yang J et al (2020) A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet 395(10223):514–523

Channappanavar R, Perlman S (2020) Evaluation of activation and inflammatory activity of myeloid cells during pathogenic human coronavirus infection. Methods Mol Biol 2099:195–204

Chauvin B, Drouot S (2013) Drug–drug interactions between HMG-CoA reductase inhibitors (statins) and antiviral protease inhibitors. Clin Pharmacokinet 52(10):815–831. Available from: https://link.springer.com/article/10.1007%252Fs40262-013-0075-4

Chen M (2021) 2021-肝动脉灌注化疗治疗肝细胞癌中国专家 共识. Chinese J Digest Surg 20:754–759

Chen W, Hotez P (2020) Potential for developing a SARS-CoV receptor-binding domain (RBD) recombinant protein as a heterologous human vaccine against coronavirus infectious disease. Taylor Fr 16(6):1239–1242. https://doi.org/10.1080/21645515.2020.1740560

Chowdhury M, Hossain N, Kashem MA, Shahid MA, Alam A (2020) Immune response in COVID-19: a review. J Infect Public Health 13(11):1619–1629. Available from: https://www.sciencedirect.com/science/article/pii/S1876034120305670

Choy K, Wong A, Kaewpreedee P, Sia S (2020) Remdesivir, lopinavir, emetine, and homoharringtonine inhibit SARS-CoV-2 replication in vitro. Antiviral Res 178:104786. Available from: https://www.sciencedirect.com/science/article/pii/S016635422030200X

Chu CM, Cheng CC, Hung FN, Wong MML, Chan H, Chan S et al (2004) Role of lopinavir/ritonavir in the treatment of SARS: initial virological and clinical findings. Thorax 59:252–256. Available from https://thorax.bmj.com/content/59/3/252.short

Chu VC, McElroy LJ, Chu V, Bauman BE, Whittaker GR (2006) The Avian coronavirus infectious bronchitis virus undergoes direct low-pH-dependent fusion activation during entry into host cells. J Virol 80(7):3180–3188

Conti P, Ronconi G, Caraffa AL, Gallenga CE, Ross R, Frydas I, Kritas SK (2020) Induction of pro-inflammatory cytokines (IL-1 and IL-6) and lung inflammation by Coronavirus-19 (COVI-19 or SARS-CoV-2): anti-inflammatory strategies. J Biol Regul Homeost Agents 34(2): 327–331. Available from: https://www.researchgate.net/profile/Pio-Conti/publication/339984438_Induction_of_pro-inflammatory_cytokines_IL-1_and_IL-6_and_lung_inflammation_by_COVID-19_anti-inflammatory_strategies/links/5ea1607d92851c87d1ad2e3c/Induction-of-pro-inflammatory-cytokines-IL-1-and-IL-6-and-lung-inflammation-by-COVID-19-anti-inflammatory-strategies.pdf

Costanzo M, De Giglio MA, Roviello GN (2020) SARS-CoV-2: recent reports on antiviral therapies based on lopinavir/ritonavir, darunavir/umifenovir, hydroxychloroquine, remdesivir, favipiravir and other drugs for the treatment of the new coronavirus. Curr Med Chem 27(27): 4526–4541. Available from: https://www.ingentaconnect.com/content/ben/cmc/2020/00000027/00000027/art00008

D'ascanio M, Innammorato M, Pasquariello L, Pizzirusso D, Guerrieri G, Castelli S et al (2021) Age is not the only risk factor in COVID-19: the role of comorbidities and of long staying in residential care homes. BMC Geriatr 21(1)

De Clercq E (2006) Potential antivirals and antiviral strategies against SARS coronavirus infections. Expert Rev Anti Infect Ther 4(2):291–302

De Meyer S, Bojkova D, Cinatl J, Van Damme E, Buyck C, Van Loock M, Woodfall B, Ciesek S (2020) Lack of antiviral activity of darunavir against SARS-CoV-2. Int J Infect Dis 97:7–10. Available from: https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Lack+of+antiviral+activity+of+darunavir+against+SARS-CoV-2.+Int+J+Infect+Dis.+2020%3B97%3A7–10.+&btnG=

Dong C, Zhang J, Wu J, Sun X, Xue H, Shao J et al (2020a) Association of hypertension with the severity and fatality of SARS-CoV-2 infection: a meta-analysis. Epidemiol Infect 148. Available from: https://www.cambridge.org/core/journals/epidemiology-and-infection/article/association-of-hypertension-with-the-severity-and-fatality-of-sarscov2-infection-a-metaanalysis/4116FAD7D866737099F976E7E7FAEB15

Dong L, Hu S, Gao J (2020b) Discovering drugs to treat coronavirus disease 2019 (COVID-19). Drug Disc Ther 14(1):58–60. Available from: https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Discovering+drugs+to+treat+coronavirus+disease+2019+%28COVID-19%29.+Drug+Discov+Ther&btnG=

Durán N, Fávaro WJ, Wagner P, Fávaro J (2020) Immunomodulators acting on COVID-19: actual knowledge and perspectives. J Appl Microb 3(2):37–44. Available from: https://www.preprints.org/manuscript/202007.0090

Elfiky AA (2021) Ribavirin, Remdesivir, Sofosbuvir, Galidesivir, and Tenofovir against SARS-CoV-2 RNA dependent RNA polymerase (RdRp): a molecular docking study. Life Sci 253: 117592. Available from: https://www.sciencedirect.com/science/article/pii/S0024320520303404

Fan HH, Wang LQ, Liu WL, An XP, Liu ZD, He XQ, Song LH, Tong YG (2020) Repurposing of clinically approved drugs for treatment of coronavirus disease 2019 in a 2019-novel coronavirus-related coronavirus model. Chinese Med J 133(09):1051–1056. Available from: https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=.+Repurposing+of+clinically+approved+drugs+for+treatment+of+coronavirus+disease+2019+in+a+2019-novel+coronavirus-related+coronavirus+model.+C&btnG=

Fehr A, Perlman S (2015) Coronaviruses: an overview of their replication and pathogenesis. Methods Mol Biol 1282:1–23. https://doi.org/10.1007/978-1-4939-2438-7_1.pdf

Ferrario CM (2011) ACE 2: more of Ang 1–7 or less Ang II? Curr Opin Nephrol Hypertension 20(1):1–6. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/pmc5826562/

Ferrey AJ, Choi G, Hanna RM, Chang Y, Tantisattamo E, Ivaturi K et al (2020) A case of novel coronavirus disease 19 in a chronic hemodialysis patient presenting with gastroenteritis and developing severe pulmonary disease. Am J Nephrol 51(5):337–342

Glebov OO (2020) Understanding SARS-CoV-2 endocytosis for COVID-19 drug repurposing. Wiley Online Libr 287(17):3664–3671. https://doi.org/10.1111/febs.15369

Gordon C, Tchesnokov E, Feng J (2020) The antiviral compound remdesivir potently inhibits RNA-dependent RNA polymerase from Middle East respiratory syndrome coronavirus. J Biol Chem 295(15):4773–4779. Available from: https://www.jbc.org/article/S0021-9258(17)48574-7/abstract

Graci JD, Cameron CE (2006) Mechanisms of action of ribavirin against distinct viruses. Rev Med Virol 16(1):37–48

Hancock AS, Stairiker CJ, Boesteanu AC, Monzón-Casanova E, Lukasiak S, Mueller YM et al (2018) Transcriptome analysis of infected and bystander Type 2 alveolar epithelial cells during influenza A Virus infection reveals in vivo Wnt pathway downregulation. J Virol 92(21): e01325-18

Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N (2020) SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 181(2): 271–280. Available from https://www.sciencedirect.com/science/article/pii/S0092867420302294

Holshue ML, DeBolt C, Lindquist S, Lofy KH, Wiesman J, Bruce H et al (2020) First case of 2019 Novel coronavirus in the United States. N Engl J Med 382(10):929–936

Hung I, Lung K, Tso E, Liu R et al (2020) Triple combination of interferon beta-1b, lopinavir–ritonavir, and ribavirin in the treatment of patients admitted to hospital with COVID-19: an open-label, randomised phase 2 trial. Lancet 395:1695–1704. Available from: https://www.sciencedirect.com/science/article/pii/S0140673620310424

Jang S, Rhee JY (2020) Three cases of treatment with nafamostat in elderly patients with COVID-19 pneumonia who need oxygen therapy. Int J Infect Dis 96:500–502. Available from: https://www.sciencedirect.com/science/article/pii/S1201971220303799

Jawhara S (2020) Could intravenous immunoglobulin collected from recovered coronavirus patients protect against COVID-19 and strengthen the immune system of new patients? Int J Mol Sci 21(7):2272. Available from: https://www.mdpi.com/674034

Kaushik A, Gupta S, Sood M (2020) COVID-19 in children: clinical approach and management-correspondence. Indian J Pediatr 87(11):970–972

Khalid M, Rabiah F, Khan B (2015) Ribavirin and interferon-α2b as primary and preventive treatment for Middle East respiratory syndrome coronavirus: a preliminary report of two cases. Antiviral Ther 20(1):87–91. https://doi.org/10.3851/IMP2792

Knoops K, Kikkert M, Van Den Worm SHE, Zevenhoven-Dobbe JC, Van Der Meer Y, Koster AJ et al (2008) SARS-coronavirus replication is supported by a reticulovesicular network of modified endoplasmic reticulum. PLoS Biol 6(9):1957–1974

Kumar D, Malviya R, Sharma PK (2020) Corona virus: a review of COVID-19. EJMO 4(1):8–25. Available from: https://www.ams-forschungsnetzwerk.at/downloadpub/Corona Virus A Review of COVID19-51418.pdf

Li J, Lehmann C, Chen X, Romerio F, Lu W (2021) Total chemical synthesis of human interferon alpha-2b via native chemical ligation. J Peptide Sci 21(7):554–560. Available from https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Total+chemical+synthesis+of+human+interferon+alpha-2b+via+native+chemical+ligation.+J+Pept+Sci.+2015%3B21%3A554–60.+&btnG=

Li Y, Zhang J, Wang N, Li H, Shi Y, Guo G et al (2020) Therapeutic drugs targeting 2019-nCoV main protease by high-throughput screening. BioRxiv. https://doi.org/10.1101/2020.01.28.922922v1.abstract

Lin SY, Liu CL, Chang YM, Zhao J, Perlman S, Hou MH (2014) Structural basis for the identification of the N-terminal domain of coronavirus nucleocapsid protein as an antiviral target. J Med Chem 57(6):2247–2257

Liu B, Li M, Zhou Z, Guan X (2020c) Can we use interleukin-6 (IL-6) blockade for coronavirus disease 2019 (COVID-19)-induced cytokine release syndrome (CRS)? J Autoimmunity 111: 102452. Available from: https://www.sciencedirect.com/science/article/pii/S0896841120300676

Liu S, Zheng Q, Wang Z (2020b) Potential covalent drugs targeting the main protease of the SARS-CoV-2 coronavirus. Bioinformatics 36(11):3295–3298. Available from: https://academic.oup.com/bioinformatics/article-abstract/36/11/3295/5814924

Liu YC, Kuo RL, Shih SR (2020a) COVID-19: the first documented coronavirus pandemic in history. Biomed J 43:328–333

Marinho EM, de Andrade Neto JB, Silva J, da Silva CR, Cavalcanti BC, Marinho ES, Júnior HV (2020) Virtual screening based on molecular docking of possible inhibitors of COVID-19 main protease. Microbial Pathogenesis 148:104365. Available from: https://www.sciencedirect.com/science/article/pii/S0882401020307312

Mason RJ (2020) Pathogenesis of COVID-19 from a cell biology perspective. Eur Respir J 55(4) Google Scholar [Internet]. [cited 2021 Nov 22]. Available from: https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Pathogenesis+of+COVID-19+from+a+cell+biology+perspective.+European+Respiratory+Journal.+2020%3B55%284%29%3A2000607&btnG=

McBride R, van Zyl M, Fielding BC (2014) The coronavirus nucleocapsid is a multifunctional protein. Viruses 6(8):2991–3018. Available from: https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=The+coronavirus+nucleocapsid+is+a+multifunctional+protein.+Viruses.+2014%3B6%288%29%3A2991-3018.&btnG=

Mehta P, Ciurtin C, Scully M et al (2020) JAK inhibitors in COVID-19: the need for vigilance regarding increased inherent thrombotic risk. Eur Respir Soc 56(3) Available from: https://erj.ersjournals.com/content/56/3/2001919.short

Mihai C, Dobrota R, Schröder M, Garaiman A, Jordan S, Becker MO, Maurer B, Distler O (2020) COVID-19 in a patient with systemic sclerosis treated with tocilizumab for SSc-ILD. Ann Rheum Dis 79(5):668–669. Available from: https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=COVID-19+in+a+patient+with+systemic+sclerosis+treated+with+tocilizumab+for+SSc-ILD.+Ann+Rheum+Dis.+2020%3B79%3A668–9.&btnG=

Mishra K, Singh A (2020) Hyperinflammation and immune response generation in COVID-19. Neuroimmunomodulation 27(2):80–86. Available from: https://www.karger.com/Article/Abstract/513198

Mossel E, Wang J, Jeffers S, Edeen K, Virology SW (2008) SARS-CoV replicates in primary human alveolar type II cell cultures but not in type I-like cells. Virology 372(1):127–135. Available from: https://www.sciencedirect.com/science/article/pii/S0042682207006411

Neerukonda S, Katneni U (2020) A review on SARS-CoV-2 virology, pathophysiology, animal models, and anti-viral interventions. Pathogens 9(6):426. Available from: https://www.mdpi.com/729390

Novel Coronavirus (2019-nCoV) infections trigger. 2020. WHO. [cited 2021 Nov 22]. Available from: https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=novel+coronavirus+%282019-nCoV%29+infections+trigger+an+exaggerated+cytokine+response+aggravating+lung+injury.+ChinaXiv.+2020.&btnG=

Organization WH. Coronavirus disease 2019 (COVID-19): situation report, 94. 2020 [cited 2021 Nov 22]; Available from: https://apps.who.int/iris/bitstream/handle/10665/331865/nCoVsitrep23Apr2020-eng.pdf

Padmanabhan P, Desikan R, Dixit NM (2020) Targeting TMPRSS2 and Cathepsin B/L together may be synergistic against SARSCoV- 2 infection. PLoS Comput Biol 16(12)

Parasher A (2021) COVID-19: Current understanding of its pathophysiology, clinical presentation and treatment. Postgraduate Med J 97:312–320. Available from: https://pmj.bmj.com/content/97/1147/312.abstract

Pervushin K, Tan E, Parthasarathy K, Lin X, Jiang FL, Yu D et al (2009) Structure and inhibition of the SARS coronavirus envelope protein ion channel. PLoS Pathog 5(7)

Phadke M, Saunik S (2020) COVID-19 treatment by repurposing drugs until the vaccine is in sight. Drug Dev Res 81(5):541–543

Preston SL, Drusano GL, Glue P, Nash J, Gupta SK, McNamara P (1999) Pharmacokinetics and absolute bioavailability of ribavirin in healthy volunteers as determined by stable-isotope methodology. Antimicrob Agents Chemother 43(10):2451–2456

Qian Z, Travanty EA, Oko L, Edeen K, Berglund A, Wang J et al (2013) Innate immune response of human alveolar type II cells infected with severe acute respiratory syndrome–coronavirus. Am J Respir Cell Mol Biol 48(6):742–748. https://doi.org/10.1165/rcmb.2012-0339OC

Rahman N, Basharat Z, Yousuf M, Castaldo G, Rastrelli L, Khan H et al (2020) Virtual screening of natural products against type II transmembrane serine protease (TMPRSS2), the priming agent of coronavirus 2 (SARS-CoV-2). Molecules 25(10):2271. Available from: https://www.mdpi.com/713882

Reyfman PA, Walter JM, Joshi N, Anekalla KR, McQuattie-Pimentel AC, Chiu S et al (2019) Single-cell transcriptomic analysis of human lung provides insights into the pathobiology of pulmonary fibrosis. Am J Respir Crit Care Med 199(12):1517–1536

Roncon L, Zuin M, Rigatelli G, Zuliani G (2020) Diabetic patients with COVID-19 infection are at higher risk of ICU admission and poor short-term outcome. J Clin Virol (127):104354

Rossignol JF (2016) Nitazoxanide, a new drug candidate for the treatment of Middle East respiratory syndrome coronavirus. J Infect Public Health 9(3):227–230. Available from: https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Nitazoxanide%2C+a+new+drug+candidate+for+the+treatment+of+Middle+East+respiratory+syndrome+coronavirus.+J+Infect+Public+Health.+&btnG=

Rothan HA, Kumar M (2019) Role of endoplasmic reticulum-associated proteins in flavivirus replication and assembly complexes. Pathogens 8(3):148. Available from: https://www.mdpi.com/532930

Rothan HA, Stone S, Natekar J, Kumari P, Arora K, Kumar M (2020) The FDA-approved gold drug auranofin inhibits novel coronavirus (SARS-COV-2) replication and attenuates inflammation in human cells. Virology 547:7–11. Available from: https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=The+FDA-+approved+gold+drug+auranofin+inhibits+novel+corona-+virus+%28SARSCOV-2%29+replication+and+attenuates+inflammation+in+human+cells.+Virology.+&btnG=

Ruan Z, Liu C, Guo Y, He Z, Huang X, Jia X et al (2021) SARS-CoV-2 and SARS-CoV: virtual screening of potential inhibitors targeting RNA-dependent RNA polymerase activity (NSP12). J Med Virol 93(1):389–400

Sargiacomo C, Sotgia F, Lisanti MP (2020) COVID-19 and chronological aging: senolytics and other anti-aging drugs for the treatment or prevention of corona virus infection? Aging (Albany NY) 12(8):6511. Available from https://www.ncbi.nlm.nih.gov/pmc/articles/pmc7202514/

Saxena A (2020) Drug targets for COVID-19 therapeutics: Ongoing global efforts. J Biosci 45. Springer

Shah B, Modi P, Sagar SR (2020) In silico studies on therapeutic agents for COVID-19: drug repurposing approach. Life Sci 252:117652. Available from: https://www.sciencedirect.com/science/article/pii/S0024320520304008

Shi H, Zhou C, He P, Huang S, Duan Y, Wang X, Lin K, Zhou C, Zhang X, Zha Y (2020) Successful treatment with plasma exchange followed by intravenous immunoglobulin in a critically ill patient with COVID-19. Int J Antimicrob Agents 56(2) Available from: https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Successful+treatment+with+plasma+exchange+followed+by+intravenous+immunoglobulin+in+a+critically+ill+patient+with+COVID-19.+International+journal+of+antimicrobial+agents.+&btnG=

Shirato K, Kawase M, Matsuyama S (2018) Wild-type human coronaviruses prefer cell-surface TMPRSS2 to endosomal cathepsins for cell entry. Virology (517):9–15

Simonnet A, Chetboun M, Poissy J, Raverdy V, Noulette J, Duhamel A et al (2020) High prevalence of obesity in Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) requiring invasive mechanical ventilation. Obesity 28(7):1195–1199

Sims AC, Baric RS, Yount B, Burkett SE, Collins PL, Pickles RJ (2005) Severe acute respiratory syndrome coronavirus infection of human ciliated airway epithelia: role of ciliated cells in viral spread in the conducting airways of the lungs. J Virol 79(24):15511–15524

Sisk J, Frieman M (2018) Coronavirus S protein-induced fusion is blocked prior to hemifusion by Abl kinase inhibitors. Virology 99(5):619

Tai W, He L, Zhang X, Pu J et al (2020) Characterization of the receptor-binding domain (RBD) of 2019 novel coronavirus: implication for development of RBD protein as a viral attachment inhibitor and vaccine. Cell Mol Immunol 17(6):613–620. Available from https://www.nature.com/articles/s41423-020-0400-4?report=reader

Tan FL, Yin JQ (2004) RNAi, a new therapeutic strategy against viral infection. Cell Res 14(6): 460–466. Available from: https://www.nature.com/articles/7290248

Tang NLS, Chan PKS, Wong CK, To KF, Wu AKL, Sung YM et al (2005) Early enhanced expression of interferon-inducible protein-10 (CXCL-10) and other chemokines predicts adverse outcome in severe acute respiratory syndrome. Clin Chem 51(12):2333–2340

Thomas H, Foster G, Platis D (2003) Mechanisms of action of interferon and nucleoside analogues. J Hepatol 39:93–98. Available from https://www.journal-of-hepatology.eu/article/S0168-8278(03)00207-1/abstract

Tikoo K, Patel G, Kumar S, Karpe PA, Sanghavi M, Malek V et al (2015b) Tissue specific up regulation of ACE2 in rabbit model of atherosclerosis by atorvastatin: role epigenetic histone modifications. Biochem Pharmacol 93(3):343–351

Tikoo K, Patel G, Kumar S et al (2015a) Tissue specific up regulation of ACE2 in rabbit model of atherosclerosis by atorvastatin: role of epigenetic histone modifications. Biochem Pharmacol 93(3):343–351. Available from: https://www.sciencedirect.com/science/article/pii/S0006295214006947

Uitdehaag JCM, Kooijman JJ, De Roos JADM, Prinsen MBW, Dylus J, Willemsen-Seegers N et al (2019) Combined cellular and biochemical profiling to identify predictive drug response biomarkers for kinase inhibitors approved for clinical use between 2013 and 2017 Selectivity and Response Biomarkers of Kinase Inhibitors. Mol Cancer Therap 18(2):470–481. Available from: https://mct.aacrjournals.org/content/18/2/470.abstract

Ullah Khan S, Htar TT. Deciphering the binding mechanism of Dexamethasone against SARS-CoV-2 main protease: Computational molecular modelling approach. 2020. Available from: https://chemrxiv.org/engage/chemrxiv/article-details/60c74cb1f96a00b33b287814

Vankadari N (2020) Arbidol: a potential antiviral drug for the treatment of SARS-CoV-2 by blocking trimerization of the spike glycoprotein. Int J Antimicrobial Agents 56(2):105998. Available from: https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=A+potential+antiviral+drug+for+the+treatment+of+SARS-CoV-2+by+blocking+trimerization+of+the+spike+glycopro-+tein.+Int+J+Antimicrob+Agents.+&btnG=

Vincent MJ, Bergeron E, Benjannet S, Erickson BR, Rollin PE, Ksiazek TG et al (2005) Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. Virol J 2(1):1–10

Wan Y, Shang J, Graham R, Baric R (2021) Receptor recognition by the novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS coronavirus. Am Soc Microbiol 94(7). https://doi.org/10.1128/JVI.00127-20

Wan Y, Shang J, Graham R, Baric RS, Fang L (2020) Receptor recognition by novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS coronavirus. J Virol 94(7):e00127-20. https://doi.org/10.1128/JVI.00127-20. Available from: https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=.+Receptor+rec-+ognition+by+novel+coronavirus+fromWuhan%3A+An+analysis+based+on+decade-long+structural+studies+of+SARS.+J+Virol.+&btnG=

Wang B, Li R, Lu Z, Huang Y (2020) Does comorbidity increase the risk of patients with COVID-19. Aging (Albany NY) 12(7):6049–6057

Wang H, Ma L, Jiang J et al (2014) Recombinant human interferon alpha 2b broad-spectrum anti-respiratory viruses pharmacodynamics study in vitro. Acta Pharmaceutica Sin 49(11): 1547–1553. Available from: https://europepmc.org/article/med/25757280

Weinheimer V, Becher A (2012) Influenza A viruses target type II pneumocytes in the human lung. J Infect Dis 206(11):1685–1694. Available from https://academic.oup.com/jid/article-abstract/206/11/1685/895991

WHO-BJ, 2020. Naming the coronavirus disease (COVID-19) and the virus that causes it. bjihs.emnuvens.com.br [Internet]. [cited 2021 Nov 21]; Available from: https://bjihs.emnuvens.com.br/bjihs/article/download/173/238

World Health Organization (2020) Declares novel coronavirus (2019-nCoV) sixth public health emergency of international concern. Euro Surveill: Eur Commun Dis Bullet. Available from: https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2020.25.5.200131e?crawler=true

Wösten-van Asperen RM, Lutter R, Specht PA, Moll GN, van Woensel JB, van der Loos CM, van Goor H, Kamilic J, Florquin S, Bos AP (2011) Acute respiratory distress syndrome leads to reduced ratio of ACE/ACE2 activities and is prevented by angiotensin-(1–7) or an angiotensin II receptor antagonist. J Pathol 225(4):618–627. https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Acute+respiratory+distress+syndrome+leads+to+reduced+ratio+of+ACE%2FACE2+activities+and+is+prevented+by+angiotensin-%281–7%29+or+an+angiotensin+II+receptor+antagonist.+J+Pathol.+2011%3B225%3A618–27&btnG=

Wösten-Van Asperen RM, Lutter R, Specht PA, Moll GN, Van Woensel JB, Van Der Loos CM et al (2011) Acute respiratory distress syndrome leads to reduced ratio of ACE/ACE2 activities and is prevented by angiotensin-(1-7) or an angiotensin II receptor antagonist. J Pathol 225(4): 618–627

Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, Graham BS, McLellan JS (2020) Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science 367(6483):1260–1263. Available from https://science.sciencemag.org/content/367/6483/1260.abstract

Wu Z, McGoogan JM (2020) Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. JAMA 323(13):1239–1242. Available from https://jamanetwork.com/journals/jama/article-abstract/2762130

Xie S, Chen X, Qiao S, Li R, Sun Y, Xia S et al (2018) Identification of the RNA Pseudoknot within the 3′ end of the porcine reproductive and respiratory syndrome virus genome as a pathogen-associated molecular pattern to activate antiviral signaling via RIG-I and Toll-like receptor 3. J Virol 92(12):e00097-18

Xu X, Han M, Li T, Sun W, Wang D, Fu B et al (2021) Effective treatment of severe COVID-19 patients with tocilizumab. Proc Natl Acad Sci. Available from: https://www.pnas.org/content/117/20/10970.short

Yao X, Ye F, Zhang M, Cui C et al (2020) In vitro antiviral activity and projection of optimized dosing design of hydroxychloroquine for the treatment of severe acute respiratory syndrome coronavirus 2 (SARS-COV-2). Clin Infect Dis 71(15):732–739. Available from: https://academic.oup.com/cid/article-abstract/71/15/732/5801998

Yavuz S, Ünal S (2020) Antiviral treatment of COVID-19. Turkish J Med Sci 50(9):611–619. Available from: https://journals.tubitak.gov.tr/medical/abstract.htm?id=27238

Yuan S (2015) Statins may decrease the fatality rate of middle east respiratory syndrome infection. MBio 6(4):e01120-15

Zheng J (2020) SARS-coV-2: an emerging coronavirus that causes a global threat. Int J Biol Sci 16(10):1678–1685

Zha L, Li S, Pan L, Tefsen B, Li Y, French N, Chen L, Yang G, Villanueva EV (2020) Corticosteroid treatment of patients with coronavirus disease 2019 (COVID-19). Med J Austr 212(9):416–420. Available from: https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Corticosteroid+treatment+of+patients+with+coronavirus+disease+2019+%28COVID-19%29.+Medical+Journal+of+Australia.+2020%3B212%289%29%3A416-20&btnG=

Chapter 25
Repurposing of Therapeutic Approaches for the Treatment of Vitiligo

Shiva Tushir, Monu Yadav, Anil Kumar, and Kajal Joshi

Abstract The present chapter focuses on discussing the redevelopment of old drugs for repurposing as vitiligo treatments. Vitiligo is a frequent disorder in which melanocytes are destroyed by the immune system, resulting in patches of depigmented white skin. Vitiligo is a frequent acquired illness with a prevalence of 1–2% worldwide. Patients may experience significant psychological distress as a result of their pigment loss like 10% depression, 7–9% accounts for dysthymia, 3.3% for anxiety and 3% for suicidal ideation of the population suffering from vitiligo. Vitiligo is classified on the basis of lesion distribution pattern, being either segmental (also called unilateral vitiligo) or non-segmental (bilateral and typically symmetrical) vitiligo. Drug repositioning (also known as repurposing, rediscovery, reprofiling, retasking, or rescue) is the process of discovering new uses for authorised and experimental medications. Current vitiligo treatment medications, such as immunomodulators (glucocorticoids) and calcineurin inhibitors focus on skin repigmentation as a phenotypic intervention (tacrolimus and pimecrolimus) However, many patients find these medications unsatisfactory because they are time-consuming expensive and have adverse reactions. Vitiligo therapy necessitates a multimodal approach that targets three different components at the same time. The first is to reduce oxidative stress and optimise the melanocyte microenvironment, followed by immunomodulatory and

S. Tushir (✉)
Department of Pharmacy, Panipat Institute of Engineering and Technology (PIET), Samalkha, Panipat, Haryana, India

M. Yadav
Department of Pharmacy, School of Medical & Allied Sciences, GD Goenka University, Gurgaon, Haryana, India

A. Kumar
University Institute of Pharmaceutical Sciences (UIPS), UGC Centre of Advanced Studies (UGC-CAS), Panjab University, Chandigarh, Punjab, India

University Institute of Pharmaceutical Sciences, UGC Centre of Advanced Studies (UGC-CAS), Panjab University, Chandigarh, Haryana, India

K. Joshi
Himachal Institute of Pharmaceutical Education & Research, Nadaun, Himachal Pradesh, India

R. C. Sobti et al. (eds.), *Drug Repurposing for Emerging Infectious Diseases and Cancer*, https://doi.org/10.1007/978-981-19-5399-6_25

immunosuppressive effects, and finally, melanocyte regeneration. Various methods are used for achieving each of these goals. A long list of suggested treatment has been compiled in this chapter. Treatment is based on the disease's stability and the amount of body surface area affected, and it must be tailored to the patient's needs. Abatacept is the best example it is a fusion protein and is now licensed by the FDA for the treatment of rheumatoid arthritis. Abatacept's efficacy in treating vitiligo has been tested in an open label pilot research.

Keywords Vitiligo · Repurposing approaches · Immunotherapy · Photochemotherapies

25.1 Introduction

Vitiligo is characterised by the loss of melanocytes in the skin. It is a frequent disorder in which melanocytes are destroyed by the immune system, resulting in patches of depigmented white skin (Gauthier et al. 2003). Melanin cells are destroyed by vitiligo. Melanin is the pigment that gives skin its colour. The skin develops white spots due to a lack of melanin in some areas. Many patients are concerned about the white patches that are characteristic of this illness. People with dark skin have more apparent spots (Braun-Falco et al. 2000). Vitiligo is a frequent acquired illness with a prevalence of 1–2% worldwide. The majority of cases start in childhood (Handa and Dogra 2003). Vitiligo in males and females are compared on an equal footing without regard to ethnicity or socioeconomic differences (Terézhalmy et al. 2013). Vitiligo is classified on the basis of lesion distribution pattern, being either segmental (also called unilateral vitiligo) or non-segmental (bilateral and typically symmetrical) vitiligo. In both segmental and non-segmental vitiligo, immune-based death of melanocytes occurs (Speeckaert and van-Geel 2017). Segmental vitiligo involves pigment loss in skin patches on only one side of the body; it is rare for stable lesion to begin expanding again. Non-segmental vitiligo is characterised by widespread, symmetrical pigment loss across the entire body, the lesions generally first seen on the hands, at the wrists body folds and orifices. Non-segmental comprise the focal, mucosal acrofacial, common and universal types (Hann et al. 2019; Alikhan and Hocker 2016). During childhood, segmental vitiligo appears unilaterally and settles after a time of activity. Before the introduction of new patches, some patients suffer itching. There are a number of therapies available, some of which can restore pigment but none of which can cure the illness (Whitton et al. 2015). Human skin colour is noteworthy for the variety of colours it contains, as well as fascinating for its science and biology. Vitiligo can appear at any age, although according to various studies, 50% of cases appear before the age of 20. Between 16% and 35% of vitiligo, patients have substantial psychological illness. Depression accounts for 10%, dysthymia for 7–9%, anxiety 3.3% and suicidal ideation for 3% of the population suffering from vitiligo (Alzolibani 2009). Vitiligo can also make it difficult to develop relationships, make people avoid particular social circumstances and make sexual relationships difficult. Vitiligo is sometimes confused with leprosy, which causes pigment loss as well, further

stigmatising individuals (Pahwa et al. 2013). Vitiligo is a multifactorial polygenetic illness that causes melanocytic loss in the skin and, in some cases, in the hair. The genitals may be implicated, posing a unique treatment difficulty. Vitiligo in the genital area can have a significant impact on a patient's sexuality (Hamzavi et al. 2015). Surprisingly, information on male genital vitiligo is lacking. Vitiligo with white patch affects the patients' self-esteem and is derived from the Latin word "vitium" which means "blemishing fault" and is a cause of stigma even to this day. It rarely affects the melanocytes in the eyes and membranous labyrinth of the inner ear (Khaitan and Sindhuja 2021). Before the introduction of new patches, some patients suffer itching. Vitiligo symptoms are more likely to appear first on body parts that are exposed to the sun the most, such as the face, lips, arms and feet. Visible lightening of regions of skin in vitiligo-affected locations, as vitiligo pigmentation can extend to the eyes, mucous membranes and facial hour. Vitiligo is a systemic condition with far-reaching consequences that extend beyond the skin, such as autoimmune illness, ocular abnormalities and neurological abnormalities (Ezzedine and Silverberg 2016).

25.2 Medical Treatment of Vitiligo

Halting diseases progressive (systemic steroids) such as methotrexate, minicycline, cortisteriods**,** calcineurin inhibitors**,** VitaminD3 analogues and cyclosporine are used to treat halting diseases progressive.

Combination therapy, such as physical and medical treatment, physical and surgical treatment, and surgical and medical treatment.

Physical therapy, such as phototherapy and laser therapy.

Surgical therapy for vitiligo: Surgical therapy for vitiligo is an important topic that was first reported in 1947 by Haxthasen and colleagues. Since then, surgical techniques have become more sophisticated and varied, with each method having unique advantages and disadvantages, such as tissue grafts, mini punch grafts, bluster grafts, cellular grafts, split thickness skin grafts, cultured grafts.

Photochemotherapy like steroids treatment, non-steroids immunosupressive agents, depigmentation therapies, micropigmentation.

Other vitiligo oral treatments include phenylalanine, antimalarials, khellin, levamirale and clafazimime.

25.3 Drug Repositioning

Drug repositioning (also known as repurposing, rediscovery, reprofiling, retasking or rescue) is the process of discovering new uses for authorised and experimental medications (Sotiropoulou et al. 2021). Drug repositioning has a lower risk of failure, shorter timeframes and smaller financial commitment as compared to

traditional drug development (Austin and Gadhia 2017). The present cost of bringing a novel chemical entity to market is projected to be in the region of US $2–3 billion, with a time frame of 10–17 years (Nagle et al. 2003). Supposedly cheaper and faster, repositioned medicine typically costs $300 million and takes 6.5 years to develop (Nosengo 2016). Sildenafil, which was originally created as a hypertensive medicine and is now marketed for other purposes like erectile dysfunction, is a good example of repositioning (Jourdan et al. 2020).

25.4 Repurposing of Approved Therapeutics for Vitiligo

At a molecular and genetic level, there has been substantial progress in understanding the aetiology of vitiligo, with a greater emphasis on the intracellular environment and the interplay of cytokines and signalling pathways (Katz and Harris 2021). Targeted immunotherapy is currently at the forefront of the R & D pipeline, followed by melanocyte regeneration and the decrease of microenvironmental oxidative stress (Giri et al. 2020).

25.4.1 Treatment Goals

- Keeping the illness stable for a longer period of time and avoiding recurrences.
- Developing effective medications with a low risk of side effects.
- Extensive repigmentation and aesthetic matching of the depigmented regions.
- Patient satisfaction is important.
- Melanocyte stress-antioxidants are currently one of the most important treatments in the treatment of vitiligo.
- Targeted immunotherapy interferes with the functioning and signalling of cytokines, facilitating the regeneration and repigmentation of melanocytes.

25.4.2 Disadvantage of Current Vitiligo Therapeutics

Vitiligo susceptibility loci have been identified in genome-wide association studies, revealing that it is a complex disease network mediated by immunomodulatory factors, apoptotic and melanogenic proteins (Spritz 2013). Despite the fact that available research can help us to understand the molecular process of vitiligo, developing anti-vitiligo drugs remains a difficult task (Wang et al. 2021). Current vitiligo treatment medications, such as immunomodulators (glucocorticoids) and calcineurin inhibitors, focus on skin repigmentation as a phenotypic intervention (tacrolimus and pimecrolimus). However, many patients find these medications

unsatisfactory because they are time-consuming, expensive and have adverse reactions (Valle et al. 2012).

25.4.3 Advantage Associated with Repurposing of Drugs

Thousands of molecules enter clinical trials each year, but about 70% of phase II therapeutic molecules fail, while phase III clinical trials have failure rates of more than 50% (Novac 2013). Failure of therapeutic molecules at a later stage costs companies money and delays the development and commercialisation of appropriate medication. To help pharmaceutical businesses deal with high attrition rates, repositioning or medication repurposing offers a variety of commercial options through low-risk, high-cost-effective tactics that help realise the potential of drugs across a wide range of applications (Sleigh and Barton 2010). Drug research and development can be greatly assisted by drug repurposing (Levin et al. 2020). The fact that existing knowledge of medication safety and bioavailability profiles is available strengthens the process and allows for lower failure rates and development costs, resulting in better treatment choices for diseases with unmet clinical requirements (Feldman et al. 2016). The life cycle of a drug molecule starts with the phase of drug research and development and ends with the stage of marketing and making the drug available to all patients (Begam and Kumar 2012).

25.4.4 Mechanism Target-Vitiligo

Vitiligo therapy necessitates a multimodal approach that targets three different components at the same time. The first is to reduce oxidative stress and optimise the melanocyte microenvironment, followed by immunomodulatory and immunosuppressive effects, and finally, melanocyte regeneration (Karagaiah et al. 2020). Treatment is based on the disease's stability and the amount of body surface area affected, and must be tailored to the patient's needs. The first step is to get the illness process under control. The focus is on stimulating melanocytes to produce patch repigmentation (Moretti et al. 2006). For example, bimatoprost was licenced by the FDA in 2008 for the treatment of eyelash hypotrichosis (Chanasumon et al. 2018). This treatment caused periocular hyperpigmentation, which was discovered to be a side effect. According to a detailed evaluation of eyelid biopsies, bimatoprost increased hyperpigmentation by enhancing melanogenesis without causing irritation. Bimatoprost may be used to treat vitiligo because it stimulates melanogenesis (Grimes 2016).

25.5 Available Therapy

Medical and surgical methods are the two primary categories of current therapy options. Topical, systemic and phototherapy are the three types of medical treatment (Colucci et al. 2012). According to the European Dermatology Forum's consensus guidelines, the first line of treatment for segmental vitiligo includes topical corticosteroids and topical calcineurin inhibitors, as well as triggering factor inhibition and cosmetic camouflage (Taieb et al. 2013). If repigmentation is not accomplished after the disease has been stabilised, surgical options can be considered. If stability isn't obtained, targeted phototherapy can be employed to slow down the development and induce pigmentation (Passeron 2017).

25.5.1 Topical Corticosteroids

Local immunomodulation and activation of melanocytes for pigment formation are the effects of corticosteroids in the afflicted skin (Bagherani 2012). They primarily function by binding to the glucocorticoid receptor pigment (GC receptor) and inhibiting the gene expression of a wide range of cytokines, including IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, GM-CSF and interferon. As a result, the activation of cytotoxic T cells is inhibited (Masuria et al. 1999). It has also been shown to suppress B cell responses to self-antigens (Bacigalupi et al. 2012). Class III and IV corticosteroids were found to be effective in treating non-segmental vitiligo in a meta-analysis of nonsurgical vitiligo treatments, with 40–56% of patients achieving >75% repigmentation (Njoo et al. 1998). Although clobetasol propionate and betamethasone valearate at various concentrations have been shown to be effective, long-term use has been linked to considerable local adverse effects (Hengge et al. 2006). On the other hand, mometasone has similar efficacy but has fewer side effects and a favourable safety profile in both adults and children (Boström et al. 2001). Fluticasone propionate combined with UVA produced greater benefits than fluticasone propionate alone in a comparison trial (Passeron and Ortonne 2006). Topical corticosteroids may be slightly more effective than calcineurin inhibitors in terms of efficacy, and they function best when used in combination rather than alone (Falabella and Barona 2009). A few well-known side effects of topical corticosteroids include atrophy, telangiectasia, hypopigmentation, straie, folliculitis, and acneiform outbreaks, especially with high-potency corticosteroids (Coondoo et al. 2014). Mometasone and fluticasone, on the other hand, can be used safely in extrafacial locations for short periods of time (Taieb et al. 2013). When a broad region is involved and strong steroids are administered for lengthy periods of time in children, systemic absorption and accepted suppression of the hypothalamic-pituitary-adrenal (HPA) axis may be a concern (Schäcke et al. 2002).

25.5.2 Topical Calcineurin Inhibitors

Topical calcineurin inhibitors like tacrolimus block IL-2 and IFN-, which have an immunomodulatory effect on cytotoxic T cells (Ricci et al. 2007), have also been proven to lower systemic oxidative stress (Ermertcan and Ozturkcan 2007), which leads to disease control and repigmentation in vitiligo (Lerche and Wulf 2010). Tacrolimus demonstrated superior repigmentation rates than placebo in a double-blind randomised controlled experiment, especially on the face and upper back, with pigment developing within the first 4 months of treatment (Cavalié et al. 2015). A double-blinded RCT found that tacrolimus 0.1% ointment was more effective than clobetasol 0.05% ointment (Lepe et al. 2003a, b).

25.5.3 Topical Vitamin D Analogues

Despite the fact that it has shown a moderate response in children, with 50–75% repigmentation (Silverberg et al. 2004), studies have demonstrated that when paired with NB-UVB, calcipotriol has no additional effect on repigmentation and may even delay the start of pigmentation. While a few studies have found that when NB-UVB and PUVAsol are combined, the response rates are higher, with a faster and more sustained response in fewer sessions (Kullavanijaya and Lim 2004; Nordal et al. 2011). In patients with unstable vitiligo, a combination of calcipotriol 0.005% and betamethasone dipropionate 0.05% produced a mild-to-moderate response (25–75% pigmentation) and was well tolerated (Binić and Stanimirović 2019).

25.5.4 Topical Prostaglandin Analogues

The use of topical prostaglandin analogues in vitiligo was suggested by the hyperpigmentation of periocular skin when treating glaucoma (Parsad et al. 2002). Prostaglandin analogues are more effective for treating periocular vitiligo and short-term diseases. Mild irritability and burning were the only side effects (Kanokrungsee et al. 2021).

25.5.5 Topical Antioxidants

Antioxidants can be used in conjunction with other treatments. A case study of 33 patients indicated that combining topical pseudocatalase/calcium with short-term NB-UVB therapy was extremely efficient in producing repigmentation on the face and dorsum of the hands (Karagaiah et al. 2020).

25.5.6 Phototherapy

Phototherapy, which is an immunomodulator and inducer of melanocyte growth, is the first line of treatment for vitiligo that affects more than 10% of the body surface area, with a special focus on children (Karagaiah et al. 2020).

25.5.7 PUVA-Psoralen Plus UVA-A

It promotes melanocyte growth by stimulating follicular melanocytes and releasing keratinocyte growth factors if a light source with broad band UVA (320–380 nm) and either oral or topical psoralen is given, and the dose is increased until the maximum acceptable dose is attained after identifying the minimum phototoxic dose (Bansal et al. 2013). Topical 8-methoxypsoralen is favoured over systemic psoralen because it has fewer negative effects (Ermis et al. 2001).

25.5.8 Narrow Band UVB

NB-UVB operates by stimulating the tyrosinase enzyme and boosting the production of HMB45 on the melanosome surface, with a peak emission at 311 nm (Cho et al. 2016) (Karagaiah et al. 2020). In the last decade, NB-UVB has replaced PUVA. According to study by Yones et al. a larger percentage of patients showed more than 50% repigmentation after 6 months of treatment with NB-UVB than those in the PUVA group (Yones et al. 2007a, b), 44% of patients in the PUVA group had good colour match, whereas all patients in the NBUVB group had excellent colour match (Bhatnagar et al. 2007). Erythema, itching and moderate burning are some of the side effects of NB-UVB, which usually go away after a few hours of treatment (Khanna and Khandpur 2019).

25.5.9 Other Photochemotherapies

Khellin is a vasodilator furanochrome (Quimby and Ammi visnaga Lam 1953) that can induce melanogenesis when exposed to UVA radiation (Nordlund and Ortonne 1992). It is taken orally at a dose of 50–100 mg/kg between 45 min and 1 h before UV exposure (Karagaiah et al. 2020). A repigmentation rate was reported to be equivalent to PUVA and had fewer side effects, but required longer treatment times and larger doses (Valkova et al. 2004). Topical khellin, on the other hand, has been proven to be ineffectual in delivering any further benefit when paired with either UVA or Monochromatic Excimer laser 308 nm (Felsten et al. 2011).

- L-Phenylalanine (L-Phe)

Felsten et al. (2011) recommend taking phenylalanine, an important amino acid for melanogenesis, orally 45 min before phototherapy to achieve good repigmentation rates of 50–100% (Felsten et al. 2011).

25.5.10 Lasers

25.5.10.1 Monochromatic Excimer Laser (MEL)

The FDA has approved the MEL 308 nm laser for the treatment of vitiligo (Chu 2015). Passeron and Ortonne (2006) suggest that MEL may provide better clinical outcomes than NB-UVB and is more effective when used in conjunction with topical hydrocortisone and tacrolimus.

25.5.10.2 Helium Neonspiepr146 Laser

The helium neon (632.8-nm) (HeNe) laser has been used in the treatment of head and neck segmental vitiligo (Wu et al. 2008).

25.6 Systemic Treatment

In patients who do not respond to topical medicines or NB-UVB, systemic corticosteroids (CS) are used as a last resort (Taieb et al. 2013). In comparison to PUVA, oral pulse steroids achieved the most repigmentation when used as an adjuvant to phototherapy (El Mofty et al. 2016).

25.7 Surgical Methods

The outer root sheath of the hair follicle serves as a melanocyte reservoir, which is critical for the efficacy of medical therapy (Nishimura 2011). The goal of surgical treatments is to reintroduce harvested melanocytes into depigmented vitiliginous lesions. Tissue grafting and cellular grafting are two types of surgical techniques (Stoner and Wood 2000).

25.7.1 Cellular Grafts

The goal of cellular grafts is to take viable tissue from a pigmented normal site using various methods like punch grafting, blister roof grafting, split thickness skin grafts or STSG and separate the epidermis cells into a suspension before transferring them to de-epithelialised recipient vitiliginous skin. Melanocyte-only transplantation, keratinocyte and melanocyte transplantation, and follicular epidermal cell suspension transplantation are some of the techniques used. This procedure has been shown to be simple, safe, minimally invasive, and effective, with less donor scarring (Khunger et al. 2009; Majid and Imran 2012; Karagaiah et al. 2020).

25.8 Emerging Treatments by Drug Repurposing

25.8.1 Minocycline

Because of its inhibitory action on generation of free radicals and cytokine production, interference with protein synthesis, potent antiapoptotic properties and modulation of matrix metalloproteinases, minocycline, an antibiotic with immunomodulatory and anti-inflammatory properties (Parsad and Kanwar 2010), has recently been tried in active vitiligo patients. Some adverse effects may be observed, and the drawback of using medications on a daily basis is that it may impair compliance (Miquelin et al. 2019).

25.8.2 Methotrexate

Methotrexate is an antimetabolite and antifolate medication (Hannoodee and Mittal 2021) that has been used for many years in the treatment of autoimmune illnesses and has been found to be safe when used for lengthy periods of time (Koźmiński et al. 2020). It reduces the number of T cells that produce TNF-α, which may aid with vitiligo (AlGhamdi and Khurrum 2013). A prospective randomised open-label study found that oral methotrexate 10 mg weekly dose was comparable to corticosteroid oral mini pulse (total weekly dose of 5 mg dexamethasone) after 6 months of treatment, and was well tolerated. Lesions that were exposed to the sun responded the best, but lesions on the palms, soles and mucosa did not respond well to methotrexate. Methotrexate is known to produce myelosuppression and hepatotoxicity; therefore, blood parameters must be regularly watched (Singh et al. 2015).

25.8.3 Cyclosporine

By blocking NFAT, a transcription factor required for the transcription of genes producing interleukin 2, cyclosporine, an oral calcineurin inhibitor, interferes with interleukin 2 syntheses (IL 2). Inhibiting IL 2, a critical cytokine that mediates lymphocyte inflow could be a treatment option for vitiligo patients (Junjappa et al. 2018). A recent study found that treating 18 individuals with oral cyclosporine (3 mg/kg/day) in two divided doses for 3 months resulted in a statistically significant reduction in Vitiligo Area Scoring Index (VASI) score (Karagaiah et al. 2020). Thus, cyclosporine was capable of producing repigmentation as well as slowing disease progression, most likely due to its direct effect on melanogenesis (Ibrahim et al. 2021).

25.8.4 JAK-STAT Inhibitors

Oral JAK 1/3 inhibitor tofacitinib 5 mg twice daily was reported to result in complete re-pigmentation of depigmented lesions on the face and hands after 5 months of treatment in a case with widespread progressive vitiligo (Khondker 2020). In another case series of 10 patients, 5 reacted to oral tofacitinib with some repigmentation, and all of them had received either enough sun exposure or low dosage of NBUVB (Liu et al. 2017). In 11 patients with vitiligo on the face, the combination of topical 2% tofacitinib and NBUVB was tested for 2–4 months, and the VASI score was reduced by 70% on average (Ciechanowicz et al. 2019). Tofacitinib is mostly metabolised in the liver by CYP3A4, with a small contribution from CYP2C19. Tofacitinib pharmacokinetics can be affected by CYP3A4 inhibitors (ketoconazole) or strong CYP2C19 inhibitors (fluconazole), which reduce metabolism and lead to higher tofacitinib levels (Dowty et al. 2014). To avoid dosage-dependent side effects, such circumstances necessitate a dose decrease. Other than arthralgia, weight gain, slight increase in lipids and liver enzymes, and dose-dependent drop in blood counts, oral tofacitinib is well tolerated in most patients (Bae et al. 2020).

25.8.5 Ruxolitinib

In a 20-week, open-label pilot study, 11 individuals with vitiligo and a maximum of 10% body surface area (BSA) involvement did well with topical ruxolitinib 1.5% cream applied twice daily. The best results were reported in cases of facial vitiligo. On average, the VASI score was reduced by 76% (Rothstein et al. 2017). Eight individuals with vitiligo were treated for 32 weeks with 1.5% topical ruxolitinib cream with optional NBUVB in an open-label trial, which was completed by five participants. The average improvement in vitiligo of the face was 92%. Because

low-dose light therapy is required to stimulate the melanocytes, concomitant NBUVB and enough sun exposure result in good repigmentation (Joshipura et al. 2018). One randomised controlled trial investigated the therapeutic potential of ruxolitinib cream in patients with vitiligo and reported efficacy and safety up to 52 weeks. Treatment with ruxolitinib cream was associated with substantial repigmentation of vitiligo lesions, and all doses were well tolerated (Rosmarin et al. 2020).

25.8.6 STAT Inhibitors

The first person to respond to oral simvastatin was a 55-year-old man who was being treated for uncontrolled hypertension and long-standing vitiligo. Simvastatin and atorvastatin are two types of statins. Trials with topical atorvastatin are being done in vitiligo to circumvent the possible hazards of high-dose systemic statins and NBUVB (Zar et al. 2019; Verma et al. 2021).

25.8.7 Alpha-Melanocyte-Stimulating Hormone (MSH)

MSH is a synthetic analogue of the naturally occurring hormone alpha-melanocyte-stimulating hormone (MSH) that has a higher affinity for the melanocortin 1 receptor (MC1R) and a longer half-life and can stimulate melanogenesis and enhance eumelanin transfer inside the melanosome (Minder 2010). It may also restore cytokine equilibrium by acting on inflammatory cells that express the MC1R receptor (neutrophils and lymphocytes) (Rouzaud et al. 2005). To investigate the safety and efficacy of afamelanotide subcutaneous implants in generalised vitiligo, 28 patients were randomly assigned to combination therapy (Afamelanotide plus NB-UVB) and 27 to monotherapy with NBUVB in a randomised comparative multicentre trial. The combination therapy group received 16 mg of afamelanotide subcutaneous implants monthly after receiving NB-UVB for 1 month, while the other group received NB-UVB monotherapy. In 72% of patients, afamelanotide combination therapy was found to be superior than NB-UVB monotherapy, and when paired with other treatment choices, more repigmentation was attained (Lim et al. 2015a, b).

25.8.8 UVA1 Lasers

Because of its deeper penetration and immunomodulatory capabilities, UVA1 represents a new potent tool in the phototherapeutic arsenal (Kemény et al. 2019). During oxidative phosphorylation in the mitochondria, the UVA-1 laser primarily

mediates the creation of reactive oxygen species, which causes damage to DNA, proteins, lipids and cellular organelles. This may suppress immunological responses while promoting melanogenesis. Transient irritation and erythema were the only side effects (Karagaiah et al. 2020).

25.8.9 Photodymanic Therapy

It generates singlet oxygen and other active oxides under 635-nm laser irradiation, which can damage cell membranes, mitochondria and DNA, cause necrosis and apoptosis, and then destroy sick cells. Some researchers have discovered that PDT is effective by stimulating local specific immunity in recent years. PDT caused epidermal keratinocytes and Langerhans cells to die, according to one study. However, no clinical investigations on the therapeutic mechanism of PDT in vitiligo have been conducted (Serrano et al. 2009; Rahimi et al. 2021); 5-aminolevulinic acid ALA is successful in treating vitiligo when the medication concentration is 1.5%, the application time is 3 h, the irradiation dose is 80 mw/cm^2 and the irradiation time is 20 min. As a result, ALA-PDT is safe and effective in treating vitiligo, with just minor side effects, and may provide a novel treatment option in the future (Zhang et al. 2018).

25.8.10 Oral Antioxidants

In a double-blind, placebo-controlled trial, oral ginkgo biloba as a monotherapy significantly reduced vitiligo progression compared to placebo (Szczurko et al. 2011). Oral polypodium leucotomos has shown a considerable improvement in the repigmentation rates of head and neck lesions when paired with narrow band UVB (Pacifico et al. 2021).

25.8.11 Topical Immunosuppressants

In selected cases (Gawkrodger et al. 2008), topical immunosuppressants can be used as steroid substitutes.

25.8.12 Basic Fibroblast Growth Factor

In vitro investigations have revealed that b-FGF activates melanogenesis and migration of perilesional melanocytes in depigmented macules and that it is mitogenic to

melanocytes (Singh 2011). The goal of a multicentre phase IV double-blind randomised control trial on bFGF-related deca peptide was to see if it was effective in repigmenting stable non-segmental vitiligo macules on sun-protected areas, as well as to evaluate the safety and efficacy of topical deca peptide in vehicles and in combination with NB-UVB. In the absence of sun exposure, deca peptide alone was found to be less effective in repigmenting vitiligo. In the patients, it was well tolerated (Ramaiah et al. 2015).

25.9 Targeted Immunotherapy

Many studies have highlighted the importance of cytokine and signalling molecule imbalances in the pathogenesis of vitiligo and its link to increased cytokines such as TNF-, INF-, IL-1, IL-2, IL-6, IL-8 and IL-17 (Singh et al. 2019). Newer compounds targeting the specific immune pathway are being developed as the need of the hour to tackle the problem of a lack of safe and effective medications in treating vitiligo (Narayanan 2015). With the efficacy of rituximab (a murine/human monoclonal antibody to CD20) in autoimmune illnesses in mind, a pilot study was conducted in which five patients with active vitiligo received 1 g of rituximab in a single intravenous infusion and were observed for 6 months. Three patients showed significant clinical and histological improvement, whereas one patient showed only minor improvement. One patient showed no improvement. These findings point to the need for more clinical studies of human monoclonal antibodies to CD20 in the treatment of vitiligo (Ruiz-Argüelles et al. 2013). Recently, researchers have explored the role of low-dose cytokines, growth factors and neuropeptides in treating vitiligo. Neovir is a sodium oxo-dihydro-acridinyl-acetate (ODHAA)-based intramuscular immunomodulatory drug used to treat multiple sclerosis, immunodeficiencies and oncological disorders (Agarwal et al. 2020). In an experimental study, 60 patients with active non-segmental vitiligo were treated with 10 doses of ODHAA every 48 h. The findings were outstanding, with high effectiveness in attaining long-term non-segmental vitiligo stability (Gianfaldoni et al. 2018). Abatacept is a fusion protein that is connected to the extracellular domain of CTLA-4, an immunological checkpoint regulator, via the Fc region of the immunoglobulin IgG1 and is now licenced by the FDA for the treatment of rheumatoid arthritis. Abatacept's efficacy in treating vitiligo has been tested in an open-label pilot study (Moreland et al. 2006).

25.10 Future Scope

Preclinical trials of PD-1 ligand (PD-L1, a PD-1 agonist) for the treatment of psoriasis and inflammatory bowel disease are presently used its involvement in immunological homeostasis; it may become a therapeutic alternative in the future

(Vanella et al. 2021). IL15 works through the JAK STAT signalling pathways and has recently been linked to melanocyte death caused by oxidative stress. Targeting IL15–JAK STAT interactions, according to researchers, could be a possibility to investigate in the future (Shenoy et al. 2014). In an attempt to investigate the effect of miR-155 on CD8+ T cell proliferation, Treg cell proliferation and melanocyte proliferation, a recent study found that miR-155 agonist can greatly increase Treg cell expression, limiting CD8+ T cell expansion and encouraging melanocyte proliferation. As a result, activation of mi-RNA could be a potential therapy option for vitiligo (Lv et al. 2019). HLA-A2, A30, A31, B13, B27, B46, B56, B60, Cw4, Cw6, DR4, DR5, DR7, DR53 and DQ3 are among the HLA-associated genes implicated in the vitiligo causes in diverse populations (Karagaiah et al. 2020). Other MHC region genes linked to generalised vitiligo include low molecular weight polypeptide-2 and -7 (LMP2 and LMP7) and transporter associated with antigen processing protein-1 (TAP-1) (Jin et al. 2011). As a result, these foci could be a unique target for gene therapy in many populations. Catalase deficiency in vitiligo patients may be caused by mutations in or near the CAT gene, which codes for the enzyme catalase (Casp et al. 2002). In the future, these genes could be used as medicinal targets.

25.11 Reported Clinical Trials

Various drugs have been studied by conducting clinical study on patients of vitiligo. Some of them are shown in the Table 25.1.

25.12 Future Prospects and Conclusion

Medication repurposing is a groundbreaking new strategy for harnessing the potential of a single medicinal molecule to treat a wide range of ailments while also repurposing it for new therapeutic purposes. Compared to the previous method, this method is less expensive and time-consuming. Repositioning medicine is considered a commercially desirable approach because preclinical and clinical development data for the drug (phase I and phase II studies) are already available. Drug repositioning is typically beneficial for rare diseases because of the benefits of tax exemptions, quicker approval, grants and monetary funding (orphan medications). The results of drug repurposing have proved beneficial in the treatment of autoimmune disorders like vitiligo and other rare diseases that are not prioritised by industry due to minimal financial return.

Table 25.1 Reported clinical trials of drug repurposing for vitiligo treatment

Study design & year	Sample size	Interventions	Result
Nguyen et al. (2018) Interventional, bicentric, prospective, randomised single-blind trial	30 patients	**Group A**: Atorvastatin (40 mg/d for 1 month followed by 80 mg/d for 5 months) along with NB-UVB phototherapy **Group B**: NB-UVB alone phototherapy twice a week for 6 months	NB-UVB phototherapy alone can effectively decrease spreading of active vitiligo. Atorvastatin did not add any benefit to NB-UVB treatment for repigmentation or halting disease progression
Vanderweil et al. (2017) Randomised, double-blind, placebo-controlled, phase II clinical trial	15 patients with vitiligo affecting 3–50% of their body surface area	40 mg of simvastatin daily for the first month and 80 mg for the remaining 5 months of the study period, or placebo	The treatment group experienced an average worsening of disease
Razmi et al. (2018)	30 patients	ECS was applied to 1 lesion and	Significantly superior
Prospective, observer-blinded, intra-patient, active-controlled, randomised clinical trial		ECS + FCS was applied to the anatomically based paired lesion of the same patient. No adjuvant treatment was given	Repigmentation outcome with epidermal and follicular cell suspension was observed in treatment-resistant acral vitiligo and non-segmental vitiligo
Lim et al. (2015a, b) Randomised, multicentric clinical trial	55 patients ($n = 28$) combination therapy vs NB-UVB monotherapy ($n = 27$).	Narrowband UVB monotherapy vs combined NB-UVB phototherapy and afamelanotide	Afamelanotide implant and NB-UVB phototherapy resulted in clinically apparent, statistically significant superior and faster repigmentation compared with NB-UVB monotherapy
Redondo et al. (2015) Randomised, double-blind, intra-individually placebo-controlled clinical trial	24 individuals	Cultured epidermal cell suspension (CES) and amniotic membrane (AM)–cultured epidermal cell grafting (CEG)	Both CES and AM-CEG treatments at the 3 and 6 month evaluations appeared to have a greater repigmentation effect compared with the control intervention
Craiglow and King (2015) Therapeutic trial	1	Treatment with oral tofacitinib citrate (Xeljanz) was initiated at a dosage of 5 mg every other day. After 3 weeks, the dosage was Increased to 5 mg/d	After 2 months of therapy, partial repigmentation of the face and upper extremities was evident.

(continued)

Table 25.1 (continued)

Study design & year	Sample size	Interventions	Result
Bae et al. (2017) Open-label trial	14 patients	Treated twice a week using a gain-switched 311-nm TSL (Pallas;	Marked response using a 311-nm TSL to treat
		Laseroptek). The treatment dose started at 300 mJ/cm^2 and was increased by 50 mJ/cm^2 in each subsequent session until post-treatment erythema occurred. Subsequently, the dose was kept constant at the minimal erythemal dose. Topical tacrolimus ointment, 0.1%, was applied concurrently to all lesions throughout the treatment period. Sun protection was recommended	Patients with non-segmental vitiligo on the face and neck
Grimes et al. (2013) Randomised clinical trial	56 patients	Efficacy and safety of afamelanotide and NB-UVB phototherapy compared with NB-UVB monotherapy	Developed repigmentation based on the scientific premise of combining a melanocyte agonist (afamelanotide) with NB-UVB phototherapy in the treatment of non-segmental vitiligo
Yones et al. (2007a, b) Double-blind randomised study	56 patients	Efficacy of oral psoralen–UVA (PUVA) with that of narrowband– B (NB-UVB) phototherapy in patients with non-segmental vitiligo-twice-weekly therapy with PUVA or NB-UVB	NB-UVB therapy is superior to oral PUVA therapy in the treatment of non-segmental vitiligo
Nordal et al. (2011) Randomised double-blind trial	40 patients	Half-side with tacrolimus ointment (0.1%) and half-side with placebo ointment	Combination of NB-UVB and tacrolimus ointment (0.1%) is more effective than UV treatment alone
Hamzavi et al. (2004) Prospective, randomised,	22	Treatment with NB-UVB was given 3 times a week to half of the body on all patients	Patients treated with NB-UVB achieve approximately 42.9% repigmentation after

(continued)

Table 25.1 (continued)

Study design & year	Sample size	Interventions	Result
controlled, bilateral left- right comparison trial		for either 60 treatments or 6 months. The contralateral side served as a no-treatment control	6 months of treatment, with the greatest response being achieved over the trunk and nonacral portions of the extremities
Mulekar 2004Long-term follow-up study	50 patients	Autologous, non-cultured melanocyte-keratinocyte cell transplantation	Patients with segmental and focal vitiligo can experience a prolonged disease-free period, which may extend through the rest of their lives
Travis et al. (2003) Therapeutic trial	3	Topical 0.1% tacrolimus ointment was begun for the face and eyelids in each of these patients	All 3 patients have maintained repigmentation after 6–9 months of follow-up, even with discontinuation of treatment
Lepe et al. (2003a, b) Randomised double-blind trial	20 children	Treatment with topical tacrolimus and clobetasol for a 2-month period	Tacrolimus proved almost as effective as clobetasol propionate to restore skin colour in lesions of vitiligo in children
Westerhof et al. (1999) Prospective, randomised, controlled, left- right comparison study	135	FP alone and a combination of FP and UVA (FP group) or with UVA alone and a combination of FP and	Combination treatment with FP and UVA is much more effective in
		UVA (UVA group). Fluticasone propionate cream was applied once daily at about bedtime, and UVA (10 J/cm^2) exposure was twice a week	Reaching complete repigmentation than are FP and UVA used alone

References

Agarwal K, Podder I, Kassir M, Vojvodic A, Schwartz RA, Wollina U, Valle Y, Lotti T, Rokni GR, Grabbe S, Goldust M (2020) Therapeutic options in vitiligo with special emphasis on immunomodulators: a comprehensive update with review of literature. Dermatol Ther 33(2):e13215

AlGhamdi K, Khurrum H (2013) Methotrexate for the treatment of generalized vitiligo. Saudi Pharm J 21(4):423

Alikhan A, Hocker T (2016) General dermatology. Rev Dermatol E-Book 59

Alzolibani A (2009) Genetic epidemiology and heritability of vitiligo in the Qassim region of Saudi Arabia. Acta Dermatovenerol Alp Panonica Adriat 18(3):119–125
Austin BA, Gadhia AD. New therapeutic uses for existing drugs. Rare diseases epidemiology: update overview. 2017:233–47
Bacigalupi RM, Postolova A, Davis RS (2012) Evidence-based, non-surgical treatments for vitiligo. Am J Clin Dermatol 13(4):217–237
Bae JM, Lee JH, Jung YS, Kim GM (2017) The efficacy of a gain-switched 311-nm titanium: sapphire laser in the treatment of vitiligo: a pilot study of 14 patients. JAMA Dermatol 153(10): 1055–1056. https://doi.org/10.1001/jamadermatol.2017.2175
Bae SH, Chang SY, Kim SH (2020) Slower elimination of tofacitinib in acute renal failure rat models: contribution of hepatic metabolism and renal excretion. Pharmaceutics 12(8):714
Bagherani N (2012) Role of corticosteroids in treatment of vitiligo. State of the art of therapeutic endocrinology. INTECH, pp 187–224
Bansal S, Sahoo B, Garg V (2013) Psoralen–narrowband UVB phototherapy for the treatment of vitiligo in comparison to narrowband UVB alone. Photodermatol Photoimmunol Photomed 29(6):311–317
Begam BF, Kumar JS (2012) A study on cheminformatics and its applications on modern drug discovery. Procedia Eng 38:1264–1275
Bhatnagar A, Kanwar AJ, Parsad D, De D (2007) Comparison of systemic PUVA and NB- UVB in the treatment of vitiligo: an open prospective study. J Eur Acad Dermatol Venereol 21(5): 638–642
Binić I, Stanimirović A. Pharmacological therapy of vitiligo. In: hypopigmentation 2019. CRC Press, pp. 49–67.
Boström Å, Lindman H, Swartling C, Berne B, Bergh J (2001) Potent corticosteroid cream (mometasone furoate) significantly reduces acute radiation dermatitis: results from a double-blind, randomized study. Radiother Oncol 59(3):257–265
Braun-Falco O, Plewig G, Wolff HH, Burgdorf WH. Disorders of melanin pigmentation. In: dermatology, 2000. Springer, Berlin, Heidelberg, pp. 1013–1042
Casp CB, She JX, Mccormack WT (2002) Genetic association of the catalase gene (CAT) with vitiligo susceptibility. Pigment Cell Res 15(1):62–66
Cavalié M, Ezzedine K, Fontas E, Montaudié H, Castela E, Bahadoran P, Taïeb A, Lacour JP, Passeron T (2015) Maintenance therapy of adult vitiligo with 0.1% tacrolimus ointment: a randomized, double blind, placebo–controlled study. J Investig Dermatol 135(4):970–974
Chanasumon N, Sriphojanart T, Suchonwanit P (2018) Therapeutic potential of bimatoprost for the treatment of eyebrow hypotrichosis. Drug Des Dev Ther 12:365
Cho SE, Kim YM, Kang KH, Kim SC, Park JK, Seo YK (2016) Pigmentation effect of electromagnetic fields at various intensities to melanocytes. Tissue Eng Regen Med 13(5):560–567
Chu EC (2015) Treatment of vitiligo: medical treatment, phototherapy and surgical treatment. Hong Kong J Dermatol Venereol 23:113–117
Ciechanowicz P, Rakowska A, Sikora M, Rudnicka L (2019) JAK-inhibitors in dermatology: current evidence and future applications. J Dermatol Treat 30(7):648–658
Colucci R, Lotti T, Moretti S (2012) Vitiligo: an update on current pharmacotherapy and future directions. Expert Opin Pharmacother 13(13):1885–1899
Coondoo A, Phiske M, Verma S, Lahiri K (2014) Side-effects of topical steroids: a long overdue revisit. Indian Dermatol Online J 5(4):416
Craiglow BG, King BA (2015) Tofacitinib citrate for the treatment of vitiligo: a pathogenesis-directed therapy. JAMA Dermatol 151(10):1110–1112. https://doi.org/10.1001/jamadermatol.2015.1520
Dowty ME, Lin J, Ryder TF, Wang W, Walker GS, Vaz A, Chan GL, Krishnaswami S, Prakash C (2014) The pharmacokinetics, metabolism, and clearance mechanisms of tofacitinib, a janus kinase inhibitor, in humans. Drug Metab Dispos 42(4):759–773

El Mofty M, Essmat S, Youssef R, Sobeih S, Mahgoub D, Ossama S, Saad A, El Tawdy A, Mashaly HM, Saney I, Helal R (2016) The role of systemic steroids and phototherapy in the treatment of stable vitiligo: a randomized controlled trial. Dermatol Ther 29(6):406–412

Ermertcan AT, Ozturkcan S (2007) Topical calcineurin inhibitors, pimecrolimus and tacrolimus. Anti-Inflamm Anti-Allergy Agents Med Chem (Formerly Curr Med Chem-Anti-Inflamm Anti Allergy Agents) 6(3):237–243

Ermis O, Alpsoy E, Cetin L, Yilmaz E (2001) Is the efficacy of psoralen plus ultraviolet a therapy for vitiligo enhanced by concurrent topical calcipotriol? A placebo-controlled double-blind study. Br J Dermatol 145(3):472–475

Ezzedine K, Silverberg N (2016) A practical approach to the diagnosis and treatment of vitiligo in children. Pediatrics 138(1)

Falabella R, Barona MI (2009) Update on skin repigmentation therapies in vitiligo. Pigment Cell Melanoma Res 22(1):42–65

Feldman SR, Goffe B, Rice G, Mitchell M, Kaur M, Robertson D, Sierka D, Bourret JA, Evans TS, Gottlieb A (2016) The challenge of managing psoriasis: unmet medical needs and stakeholder perspectives. Am Health Drug Benefits 9(9):504

Felsten LM, Alikhan A, Petronic-Rosic V (2011) Vitiligo: a comprehensive overview: Part II: Treatment options and approach to treatment. J Am Acad Dermatol 65(3):493–514

Gauthier Y, Andre MC, Taïeb A (2003) A critical appraisal of vitiligo etiologic theories. Is melanocyte loss a melanocytorrhagy? Pigment Cell Res 16(4):322–332

Gawkrodger DJ, Ormerod AD, Shaw L, Mauri-Sole I, Whitton ME, Watts MJ, Anstey AV, Ingham J, Young K (2008) Guideline for the diagnosis and management of vitiligo. Br J Dermatol 159(5):1051–1076

Gianfaldoni S, Tchernev G, Wollina U, Lotti J, Rovesti M, Satolli F, França K, Lotti T (2018) Vitiligo in children: what's new in treatment? Open access Maced. J Med Sci 6:221

Giri PS, Dwivedi M, Begum R (2020) Decreased suppression of CD8+ and CD4+ T cells by peripheral regulatory T cells in generalized vitiligo due to reduced NFATC1 and FOXP3 proteins. Exp Dermatol 29(8):759–775

Grimes PE (2016) Bimatoprost 0.03% solution for the treatment of nonfacial vitiligo. J Drugs Dermatol 15(6):703–710

Grimes PE, Hamzavi I, Lebwohl M, Ortonne JP, Lim HW (2013) The efficacy of Afamelanotide and narrowband UV-B phototherapy for Repigmentation of vitiligo. JAMA Dermatol 149(1): 68–73. https://doi.org/10.1001/2013.jamadermatol.386

Hamzavi I, Jain H, McLean D, Shapiro J, Zeng H, Lui H (2004) Parametric modeling of narrow-band UV-B phototherapy for vitiligo using a novel quantitative tool: the vitiligo area scoring index. Arch Dermatol 140(6):677–683. https://doi.org/10.1001/archderm.140.6.677

Hamzavi I, Mahmoud BH, Isedeh PN (2015) Handbook of vitiligo: basic science and clinical management. JP Medical Ltd

Handa S, Dogra S (2003) Epidemiology of childhood vitiligo: a study of 625 patients from North India. Pediatr Dermatol 20(3):207–210

Hann SK, Yu HS, Lan CC, Wu CS, Gauthier Y, Benzekri L, Taïeb A. Segmental Vitiligo. InVitiligo 2019. Springer, Champions, pp. 53-71

Hannoodee M, Mittal M (2021) Methotrexate. In: StatPearls [Internet]. StatPearls Publishing

Hengge UR, Ruzicka T, Schwartz RA, Cork MJ (2006) Adverse effects of topical glucocorticosteroids. J Am Acad Dermatol 54(1):1–5

Ibrahim HM, El-din Abd El-Aziz E, Nada SA, Hegazy EM, Hassan MH (2021) Macrophage migration inhibitory factor in vitiligo: pathogenesis and potential therapeutic aspects. Asian J Biochem Genet Mol Biol 8(2):8–24

Jin Y, Birlea SA, Fain PR, Gowan K, Riccardi SL, Holland PJ, Bennett DC, Herbstman DM, Wallace MR, McCormack WT, Kemp EH (2011) Genome-wide analysis identifies a quantitative trait locus in the MHC class II region associated with generalized vitiligo age of onset. J Investig Dermatol 131(6):1308–1312

Joshipura D, Alomran A, Zancanaro P, Rosmarin D (2018) Treatment of vitiligo with the topical Janus kinase inhibitor ruxolitinib: a 32-week open-label extension study with optional narrow-band ultraviolet B. J Am Acad Dermatol 78(6):1205–1207

Jourdan JP, Bureau R, Rochais C, Dallemagne P (2020) Drug repositioning: a brief overview. J Pharm Pharmacol 72(9):1145–1151

Junjappa RP, Patil P, Bhattarai KR, Kim HR, Chae HJ (2018) IRE1α implications in endoplasmic reticulum stress-mediated development and pathogenesis of autoimmune diseases. Front Immunol 9:1289

Kanokrungsee S, Pruettivorawongse D, Rajatanavin N (2021) Clinical outcomes of topical bimatoprost for nonsegmental facial vitiligo: a preliminary study. J Cosmet Dermatol 20(3): 812–818

Karagaiah P, Valle Y, Sigova J, Zerbinati N, Vojvodic P, Parsad D, Schwartz RA, Grabbe S, Goldust M, Lotti T (2020) Emerging drugs for the treatment of vitiligo. Expert Opin Emerg Drugs 25(1):7–24

Katz EL, Harris JE (2021) Translational research in vitiligo. Front Immunol 12:175

Kemény L, Varga E, Novak Z (2019) Advances in phototherapy for psoriasis and atopic dermatitis. Expert Rev Clin Immunol 15(11):1205–1214

Khaitan BK, Sindhuja T (2021) Autoimmunity in vitiligo: therapeutic implications and opportunities. Autoimmun Rev 102932:102932

Khanna U, Khandpur S (2019) What is new in narrow-band ultraviolet-B therapy for vitiligo? Indian Dermatol Online J 10(3):234

Khondker L (2020) Role of Tofacitinib in the treatment of vitiligo. Gulf J Dermatol Venereol 21(1)

Khunger N, Kathuria SD, Ramesh V (2009) Tissue grafts in vitiligo surgery–past, present, and future. Indian J Dermatol 54(2):150

Koźmiński P, Halik PK, Chesori R, Gniazdowska E (2020) Overview of dual-acting drug methotrexate in different neurological diseases, autoimmune pathologies and cancers. Int J Mol Sci 21(10):3483

Kullavanijaya P, Lim HW (2004) Topical calcipotriene and narrowband ultraviolet B in the treatment of vitiligo. Photodermatol Photoimmunol Photomed 20(5):248–251

Lepe V, Moncada B, Castanedo-Cazares JP, Torres-Alvarez MB, Ortiz CA, Torres- Rubalcava AB (2003a) A double-blind randomized trial of 0.1% tacrolimus vs 0.05% clobetasol for the treatment of childhood vitiligo. Arch Dermatol 139(5):581–585

Lepe V, Moncada B, Castanedo-Cazares JP, Torres-Alvarez MB, Ortiz CA, Torres- Rubalcava AB (2003b) A double-blind randomized trial of 0.1% tacrolimus vs 0.05% Clobetasol for the treatment of childhood vitiligo. Arch Dermatol 139(5):581–585. https://doi.org/10.1001/archderm.139.5.581

Lerche CM, Wulf HC (2010) Photocarcinogenicity of selected topically applied dermatological drugs: calcineurin inhibitors, corticosteroids, and vitamin D analogs. Dermatol Rep 2(2):13

Levin JM, Oprea TI, Davidovich S, Clozel T, Overington JP, Vanhaelen Q, Cantor CR, Bischof E, Zhavoronkov A (2020) Artificial intelligence, drug repurposing and peer review. Nat Biotechnol 38(10):1127–1131

Lim HW, Grimes PE, Agbai O et al (2015a) Afamelanotide and narrowband UV-B phototherapy for the treatment of vitiligo: a randomized multicenter trial. JAMA Dermatol 151(1):42–50. https://doi.org/10.1001/jamadermatol.2014.1875

Lim HW, Grimes PE, Agbai O, Hamzavi I, Henderson M, Haddican M, Linkner RV, Lebwohl M (2015b) Afamelanotide and narrowband UV-B phototherapy for the treatment of vitiligo: a randomized multicenter trial. JAMA Dermatol 151(1):42–50

Liu LY, Strassner JP, Refat MA, Harris JE, King BA (2017) Repigmentation in vitiligo using the Janus kinase inhibitor tofacitinib may require concomitant light exposure. J Am Acad Dermatol 77(4):675–682

Lv M, Li Z, Liu J, Lin F, Zhang Q, Li Z, Wang Y, Wang K, Xu Y (2019) MicroRNA-155 inhibits the proliferation of CD8+ T cells via upregulating regulatory T cells in vitiligo. Mol Med Rep 20(4):3617–3624

Majid I, Imran S (2012) Ultrathin split-thickness skin grafting followed by narrowband UVB therapy for stable vitiligo: an effective and cosmetically satisfying treatment option. Indian J Dermatol Venereol Leprol 78(2):159

Masuria BL, Batra A, Kothiwala RK, Khuller R, Singhi MK (1999) Topical mometasone furoate for the treatment of childhood vltiligo. Indian J Dermatol Venereol Leprol 65(5):219–221

Minder EI (2010) Afamelanotide, an agonistic analog of α-melanocyte-stimulating hormone, in dermal phototoxicity of erythropoietic protoporphyria. Expert Opin Investig Drugs 19(12): 1591–1602

Miquelin GM, Colferai MM, Steiner D (2019) Comparative study on the use of systemic minocycline versus systemic corticosteroid therapy in the treatment of active vitiligo. Surg Cosmet Dermatol 11(2):132–137

Moreland L, Bate G, Kirkpatrick P (2006) Abatacept. Nat Rev Drug Discov 5:3

Moretti S, Amato L, Bellandi S, Fabbri P (2006) Focus on vitiligo: a generalized skin disorder. Eur J Inflamm 4(1):21–30

Mulekar SV (2004) Long-term follow-up study of segmental and focal vitiligo treated by autologous, noncultured melanocyte-keratinocyte cell transplantation. Arch Dermatol 140(10): 1211–1215. https://doi.org/10.1001/archderm.140.10.1211

Nagle T, Berg C, Nassr R, Pang K (2003) The further evolution of biotech. Nat Rev Drug Discov 2(1):75–79

Narayanan R (2015) Druggable vitiligo genome: a fast track approach to take the genome wide association to the clinic. MOJ Proteom Bioinform 2(3):102–110

Nguyen S, Chuah SY, Fontas E et al (2018) Atorvastatin in combination with narrowband UV-B in adult patients with active vitiligo: a randomized clinical trial. JAMADermatol 154(6):725–726. https://doi.org/10.1001/jamadermatol.2017.6401

Nishimura EK (2011) Melanocyte stem cells: a melanocyte reservoir in hair follicles for hair and skin pigmentation. Pigment Cell Melanoma Res 24(3):401–410

Njoo MD, Spuls PI, Bos JT, Westerhof W, Bossuyt PM (1998) Nonsurgical repigmentation therapies in vitiligo: meta-analysis of the literature. Arch Dermatol 134(12):1532–1540

Nordal EJ, Guleng GE, Rönnevig JR (2011) Treatment of vitiligo with narrowband-UVB (TL01) combined with tacrolimus ointment (0.1%) vs. placebo ointment, a randomized right/left double-blind comparative study. J Eur Acad Dermatol Venereol 25(12):1440–1443. https://doi.org/10.1111/j.1468-3083.2011.04002.x

Nordlund JJ, Ortonne JP (1992) Vitiligo and depigmentation. Curr Probl Dermatol 4(1):5–30

Nosengo N (2016) New tricks for old drugs: faced with skyrocketing costs for developing new drugs, researchers are looking at ways to repurpose older ones--and even some that failed in initial trials. Nature 534(7607):314–317

Novac N (2013) Challenges and opportunities of drug repositioning. Trends Pharmacol Sci 34(5): 267–272

Pacifico A, Damiani G, Iacovelli P, Conic RR, Young Dermatologists Italian Network (YDIN), Gonzalez S, Morrone A (2021) NB-UVB plus oral Polypodium leucotomos extract display higher efficacy than NB-UVB alone in patients with vitiligo. Dermatol Ther 34(2):e14776

Pahwa P, Mehta M, Khaitan B, Sharma V, Ramam M (2013) The psychosocial impact of vitiligo in Indian patients. Indian J Dermatol Venereol Leprol 79(5):679

Parsad D, Kanwar A (2010) Oral minocycline in the treatment of vitiligo–a preliminary study. Dermatol Ther 23(3):305–307

Parsad D, Pandhi R, Dogra S, Kumar B (2002) Topical prostaglandin analog (PGE2) in vitiligo–a preliminary study. Int J Dermatol 41(12):942–945

Passeron T (2017) Medical and maintenance treatments for vitiligo. Dermatol Clin 35(2):163–170

Passeron T, Ortonne JP (2006) Use of the 308-nm excimer laser for psoriasis and vitiligo. Clin Dermatol 24(1):33–42

Quimby MW, Ammi visnaga Lam. (1953) A Medicinal Plant. Econ Bot 7:89–92

Rahimi H, Zeinali R, Tehranchinia Z (2021) Photodynamic therapy of vitiligo: a pilot study. Photodiagn Photodyn Ther 36:102439

Ramaiah A, Kar HK, Garg VK, Bajaj N, Gupta L (2015) A double blind randomized phase IV clinical trial of basic fibroblast growth factor related Deca-peptide in vitiligo. Pigment Disord 3: S3-004

Razmi TM, Kumar R, Rani S, Kumaran SM, Tanwar S, Parsad D (2018) Combination of follicular and epidermal cell suspension as a novel surgical approach in difficult- to-treat vitiligo: a randomized clinical trial. JAMA Dermatol 154(3):301–308. https://doi.org/10.1001/jamadermatol.2017.5795

Redondo P, Gímenez de Azcarate A, Núñez-Córdoba JM et al (2015) Efficacy of autologous melanocyte transplantation on amniotic membrane in patients with stable leukoderma: a randomized clinical trial. JAMA Dermatol 151(8):897–899. https://doi.org/10.1001/jamadermatol.2015.0299

Ricci G, Dondi A, Patrizi A (2007) Role of topical calcineurin inhibitors on atopic dermatitis of children. Curr Med Chem 14(14):1579–1591

Rosmarin D, Pandya AG, Lebwohl M, Grimes P, Hamzavi I, Gottlieb AB, Butler K, Kuo F, Sun K, Ji T, Howell MD (2020) Ruxolitinib cream for treatment of vitiligo: a randomised, controlled, phase 2 trial. Lancet 396(10244):110–120

Rothstein B, Joshipura D, Saraiya A, Abdat R, Ashkar H, Turkowski Y, Sheth V, Huang V, Au SC, Kachuk C, Dumont N (2017) Treatment of vitiligo with the topical Janus kinase inhibitor ruxolitinib. J Am Acad Dermatol 76(6):1054–1060

Rouzaud F, Kadekaro AL, Abdel-Malek ZA, Hearing VJ (2005) MC1R and the response of melanocytes to ultraviolet radiation. Mutat Res/Fundam Mol Mech Mutagen 571(1–2):133–152

Ruiz-Argüelles A, García-Carrasco M, Jimenez-Brito G, Sánchez-Sosa S, Pérez- Romano B, Garcés-Eisele J, Camacho-Alarcón C, Reyes-Núñez V, Sandoval-Cruz M, Mendoza-Pinto C, López-Colombo A (2013) Treatment of vitiligo with a chimeric monoclonal antibody to CD 20: a pilot study. Clin Exp Immunol 174(2):229–236

Schäcke H, Döcke WD, Asadullah K (2002) Mechanisms involved in the side effects of glucocorticoids. Pharmacol Ther 96(1):23–43

Serrano G, Lorente M, Reyes M, Millán F, Lloret A, Melendez J, Navarro M (2009) Photodynamic therapy with low-strength ALA, repeated applications and short contact periods (40–60 minutes) in acne, photoaging and vitiligo. J Drugs Dermatol 8(6):562–568

Shenoy AR, Kirschnek S, Häcker G (2014) IL-15 regulates Bcl-2 family members Bim and Mcl-1 through JAK/STAT and PI3K/AKT pathways in T cells. Eur J Immunol 44(8):2500–2507

Silverberg NB, Lin P, Travis L, Farley-Li J, Mancini AJ, Wagner AM, Chamlin SL, Paller AS (2004) Tacrolimus ointment promotes repigmentation of vitiligo in children: a review of 57 cases. J Am Acad Dermatol 51(5):760–766

Singh NS (2011) A systemic review on vitiligo. Int J Pharmacol Biol Sci 5(1):63–84

Singh H, Kumaran MS, Bains A, Parsad D (2015) A randomized comparative study of oral corticosteroid minipulse and low-dose oral methotrexate in the treatment of unstable vitiligo. Dermatology 231(3):286–290

Singh M, Kotnis A, Jadeja SD, Mondal A, Mansuri MS, Begum R (2019) Cytokines: the yin and yang of vitiligo pathogenesis. Expert Rev Clin Immunol 15(2):177–188

Sleigh SH, Barton CL (2010) Repurposing strategies for therapeutics. Pharm Med 24(3):151–159

Sotiropoulou G, Zingkou E, Pampalakis G (2021) Redirecting drug repositioning to discover innovative cosmeceuticals. Exp Dermatol 30(5):628–644

Speeckaert R, van Geel N (2017) Vitiligo: an update on pathophysiology and treatment options. Am J Clin Dermatol 18(6):733–744

Spritz RA (2013) Modern vitiligo genetics sheds new light on an ancient disease. J Dermatol 40(5): 310–318

Stoner ML, Wood FM (2000) The treatment of hypopigmented lesions with cultured epithelial autograft. J Burn Care Rehabil 21(1):50–54

Szczurko O, Shear N, Taddio A, Boon H. Ginkgo biloba for the treatment of vitilgo vulgaris: an open label pilot clinical trial. BMC Complement Altern Med 2011;11(1):1–9

Taieb AV, Alomar A, Böhm M, Dell'Anna ML, De Pase A, Eleftheriadou V, Ezzedine K, Gauthier Y, Gawkrodger DJ, Jouary T, Leone G (2013) Guidelines for the management of vitiligo: the European dermatology forum consensus. Br J Dermatol 168(1):5–19

Terézhalmy GT, Huber MA, Jones AC (2013) Physical evaluation in dental practice. John Wiley & Sons

Travis LB, Weinberg JM, Silverberg NB (2003) Successful treatment of vitiligo with 0.1% tacrolimus ointment. Arch Dermatol 139(5):571–574. https://doi.org/10.1001/archderm.139.5.571

Valkova S, Trashlieva M, Christova P (2004) Treatment of vitiligo with local khellin and UVA: comparison with systemic PUVA. Clin Exp Dermatol: Exp Dermatol 29(2):180–184

Valle Y, Lotti TM, Hercogova J, Schwartz RA, Korobko IV (2012) Multidisciplinary approach to R & D in vitiligo, a neglected skin disease. Dermatol Ther 25:S1–S9

Vanderweil SG, Amano S, Ko WC et al (2017) A double-blind, placebo-controlled, phase-II clinical trial to evaluate oral simvastatin as a treatment for vitiligo. J Am Acad Dermatol 76(1):150–151.e3

Vanella V, Festino L, Vitale MG, Alfano B, Ascierto PA (2021) Emerging PD-1/PD-L1 antagonists for the treatment of malignant melanoma. Expert Opin Emerg Drugs 1-4:79

Verma D, Hussain K, Namiq KS, Firoz A, Bouchama M, Raza M, Haris M, Khan S. Vitiligo: The association with metabolic syndrome and the role of simvastatin as an Immunomodulator. Cureus 2021;13(3)

Wang J, Luo L, Ding Q, Wu Z, Peng Y, Li J, Wang X, Li W, Liu G, Zhang B, Tang Y (2021) Development of a multi-target strategy for the treatment of vitiligo via machine learning and network analysis methods. Front Pharmacol 12

Westerhof W, Nieuweboer-Krobotova L, Mulder PGH, Glazenburg EJ (1999) Left-right comparison study of the combination of fluticasone propionate and UV-A vs either fluticasone propionate or UV-A alone for the long-term treatment of vitiligo. Arch Dermatol 135(9):1061–1066. https://doi.org/10.1001/archderm.135.9.1061

Whitton ME, Pinart M, Batchelor J, Leonardi-Bee J, Gonzalez U, Jiyad Z, Eleftheriadou V, Ezzedine K (2015) Interventions for vitiligo. Cochrane Database Syst Rev 2

Wu CS, Hu SC, Lan CC, Chen GS, Chuo WH, Yu HS (2008) Low-energy helium-neon laser therapy induces repigmentation and improves the abnormalities of cutaneous microcirculation in segmental-type vitiligo lesions. Kaohsiung J Med Sci 24(4):180–189

Yones SS, Palmer RA, Garibaldinos TM, Hawk JL (2007a) Randomized double-blind trial of treatment of vitiligo: efficacy of psoralen–UV-A therapy vs narrowband–UV-B therapy. Arch Dermatol 143(5):578–584

Yones SS, Palmer RA, Garibaldinos TM, Hawk JLM (2007b) Randomized double-blind trial of treatment of vitiligo: efficacy of psoralen–UV-A therapy vs narrowband– UV-B therapy. Arch Dermatol 143(5):578–584. https://doi.org/10.1001/archderm.143.5.578

Zar AR, Malik A, Mahmood A, Naseer QA, Yumei L (2019) Pathogenesis and the emerging therapy of vitiligo. Arch Clin Biomed Res 3(6):361–373

Zhang Y, Lin H, Guo X, Zou X (2018) A case series pilot study on the combination of 5-aminolevulinic acid and photodynamic therapy (ALA-PDT) for treatment of vitiligo. An Bras Dermatol 93:539–545

Chapter 26
Emerging Infections and Their Management

Pramil Tiwari and Prity Rani Deshwal

Abstract Emerging infectious diseases (EIDs) are infections with increasing incidence and spread with a threat to increase in the near future. New EIDs have evolved at an alarming rate in recent years, and a global pandemic has erupted to emphasize the necessity of managing such infections. The origin of EIDs has always been a mystery to researchers. Many interconnections between human and animal hosts, further the factors like socioeconomic, environmental, and ecological factors are linked with the emergence of the infections. Past emergence of these infections has signified the association of evolutionary pathogenesis with the occurrence of EIDs and it also indicated the importance of understanding variations in the pathogenesis of EIDs. In recent decades, the most challenging in combating emerging and re-emerging infections has been the development of universal and reliable methods of early identification. Several classes of molecules have been scrutinized as prospective biomarkers or candidates with diagnostic potential. The databases and hotspots of EIDs are identified with promising roles in the pursuit of early identification. However, a strategic management and organization perspective is the need of the hour for controlling the emergence of such infections. The risk assessment and surveillance systems are vital epidemiological methods for keeping a track of a population's health, guiding priority-setting, planning, and evaluating the public health policy and strategies. On the other hand, repurposing of drugs becomes a novel approach for the management of such emerging infections.

Abbreviations

AMR	Antimicrobial resistance
APTT	Activated Partial Thromboplastin Time
CCHF	Crimean Congo Hemorrhagic Fever

P. Tiwari (✉) · P. R. Deshwal
Department of Pharmacy Practice, National Institute of Pharmaceutical Education, Mohali, Punjab, India
e-mail: ptiwari@niper.ac.in

R. C. Sobti et al. (eds.), *Drug Repurposing for Emerging Infectious Diseases and Cancer*, https://doi.org/10.1007/978-981-19-5399-6_26

CDC	Centers for Disease Control and Prevention
CFP-10	10-kDa culture filtrate protein
CRKP	Carbapenem-resistant *Klebsiella pneumoniae*
DNA	Deoxyribonucleic acid
EID	Emerging infectious diseases
ESAT-6	6-kDa early secretory antigenic
ESBL	Extended spectrum beta-lactamase
EU	European union
GIDEON	Global Infectious Diseases and Epidemiology Network
HAI	Hospital-acquired illnesses
IDDO	Infectious Diseases Data Observatory
IND	Investigational new drug
JC virus	John Cunningham Virus
MERS-CoV	Middle East respiratory syndrome coronavirus
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSSA	Methicillin-sensitive *Staphylococcus aureus*
NLR	Neutrophil: lymphocyte ratio
ProMed	Program for Monitoring Emerging Diseases
RNA	Ribonucleic acid
RSV	Respiratory syncytial virus
SARS-CoV	Severe acute respiratory syndrome coronavirus
SEB	Staphylococcus enterotoxin B
SIR	Susceptible-Infected-Recovered Model
TMPRSS2	Type 2 transmembrane serine protease
VRE	Vancomycin-resistant Enterococci
WHO	World Health Organization
XDR-PA	Extensively drug-resistant, *Pseudomonas aeruginosa*

26.1 Emerging Infections

Emerging infectious diseases (EIDs) are illnesses brought on by pathogenic organisms that are becoming more prevalent among people. These include pathogens that are now gradually changing, dispersing to new geographical regions, going unnoticed, or being former infections that are reappearing as a result of flaws in public health procedures. Infectious diseases whose prevalence has increased over the last 20 years or which appear to be on the rise are the emerging infections (Woolhouse 2002; Woolhouse and Gowtage-Sequeria 2005; National Institute of Allergy and Infectious Diseases (NIAID) 2018). Therefore, the broad category of EIDs consists of (i) new diseases brought on by novel pathogens or novel strains of recognized pathogens, (ii) well-known diseases that have spread to new regions or host populations, and (iii) well-known diseases whose incidence has increased as a result of novel transmission mechanisms, environmental changes, or unusual

virulence mutations (National Institute of Allergy and Infectious Diseases (NIAID) 2018; Anderson et al. 2004; Shope and Oaks 1992).

The National Institute of Allergy and Infectious Diseases categorized emerging infectious diseases into three groups based on the pathogen's priority list in consideration of risk to public health and national security. The list is revised on a regular basis in collaboration with its government partners, including the U.S. Department of Homeland Security, which establishes threat assessments, and the Centers for Disease Control and Prevention (CDC), which is responsible for disease surveillance. The description of categories and a list of emerging pathogens in their respective categories are represented in Table 26.1.

Table 26.1 Emerging infectious diseases/pathogens category *(*National Institute of Allergy and Infectious Diseases (NIAID) 2018*)*

Category	Description	Pathogens
A	• Pose the highest risk to national security and public health • Can be easily disseminated or transmitted from person to person • Result in high mortality rates and have the potential for major public health impact • Might cause public panic	*Bacillus anthracis* (anthrax), *clostridium botulinum* toxin (botulism), *Yersinia pestis* (plague), Variola major (smallpox) and other related pox viruses, *Francisella tularensis* (tularemia), viral hemorrhagic fevers (dengue, Ebola and Marburg viruses)
B	• Second highest priority organisms/ biological agents • They are moderately easy to disseminate • Result in moderate morbidity rates and low mortality rates • Require specific enhancements for diagnostic capacity and enhanced disease surveillance	*Burkholderia pseudomallei*, *Coxiella burnetii*, Brucella species, *Burkholderia mallei*, *chlamydia psittaci*, ricin toxin, epsilon toxin, staphylococcus enterotoxin B (SEB), typhus fever, food- and water-borne pathogens, bacteria, viruses, protozoa, fungi and mosquito-borne viruses
C	• Third highest priority and include emerging pathogens that could be engineered for mass dissemination in the future • Ease of production and dissemination • Potential for high morbidity and mortality rates and major health impact	Nipah and Hendra viruses, additional hantaviruses, Tickborne hemorrhagic fever viruses, Tickborne encephalitis complex flaviviruses, Tickborne encephalitis viruses, tuberculosis, including drug-resistant TB, influenza virus, rabies virus, prions, severe acute respiratory syndrome associated coronavirus (SARS-CoV), MERS-CoV, and other highly pathogenic human coronaviruses
Other	• Additional emerging infectious diseases/pathogens	Acanthamebiasis, Anaplasmosis, Australian bat lyssavirus, Bartonella henselae, BK virus, Bordetella pertussis, Borrelia mayonii, Borrelia miyamotoi, Ehrlichiosis enterovirus 68, enterovirus 71, hepatitis C, hepatitis E, human herpesvirus 6, human herpesvirus 8, JC virus, leptospirosis, Mucormycosis, poliovirus, Rubeola (measles)

26.2 Origin of Emerging Infections

The major portion of emerging infection occurrences are induced by zoonoses, primarily originate in wildlife (such as Ebola and Marburg virus) and are on the rise (Petersen et al. 2018). Several reviews have indicated interconnections between human and animal hosts (Palmer et al. 1998; Chomel 1998; Daszak et al. 2000). Approximately 60% of identified human infections and near to 75% of "emerging" human pathogens are generally caused by zoonotic infectious agents (Taylor et al. 2001; Kilpatrick and Randolph 2012). The spread of zoonotic pathogens to humans through direct contact or food, water or environment has the potential to cause a major public health threat problem. Majorly, transmissions into human hosts are as a result of various ecological, demographical, and socioeconomic factors.

Two unique zoonotic disease emerging pathways have been recognized. Despite their beginnings as zoonoses, certain viruses appear to have evolved to human pathogens after migrating from animals to humans and becoming mostly or entirely human infections. Others require ongoing reintroduction from animal hosts and have never established themselves as self-sustaining epidemics in the human population (Cleaveland et al. 2007).

Bacteria or rickettsia are responsible for a substantial number of emerging infectious disease (EID) cases, indicating a huge number of drug-resistant microorganisms. However, EID origins are also strongly linked to socioeconomic, environmental, and ecological factors and could be used to highlight areas where new EIDs are most likely to emerge as "hotspots" of an emerging disease (Woolhouse and Gowtage-Sequeria 2005; Shope and Oaks 1992; Cleaveland et al. 2007).

Understanding the development of new infectious illnesses has been aided by the population genetics and molecular epidemiology of RNA virus. The five major processes of evolutionary change—genetic mutation, recombination, natural selection, and migration—interact to form the genetic structure of populations and are also essential to comprehending the evolution of RNA viruses, despite the fact that their relative strengths differ from those found in DNA-based species (Moya et al. 2004; Antia et al. 2003). To understand the cross-species infection scientists are extracting virus mostly from a chronically infected patient and characterizing it before using it to discover cell cultures that are tolerant for that particular infection in humans, pigs, and deer (Shukla et al. 2011).

The five intergrading stages explain how a pathogen that exclusively infects an animal may be transformed into a pathogen that exclusively infects a human. A microorganism seen in animals but not in human under natural context is considered to be at the first stage of infection. Stage two of transmission involves animal pathogen that has been transferred from animals to humans (primary infection) but not between humans (secondary infection). To reach the third stage, animal pathogens must only be capable of transmitting themselves between humans for a few cycles before it became extinct in the human population. The fourth stage is of Sylvatic cycle which is a natural cycle of transmission from animals to humans, and that also traverses extensive periods of transmission between humans without the

assistance of animal hosts. Fourth stage is further classified in three substages; 4a, 4b, and 4c. Sylvatic cycle is far more significant than direct transmission from human to human in Stage 4a. Sylvatic and direct transmission are essential in stage 4b. Human-to-human transmission is the most widespread in stage 4c. The last stage, stage 5 includes pathogens found solely in humans (Petersen et al. 2018; Wolfe et al. 2007).

26.3 ESKAPE Pathogen

It has emerged that pathogens have an inherent ability to withstand antimicrobial medications that have been administered over long periods of time. Resistance to antimicrobials often arises when bacteria and other pathogens develop resistance to commonly used treatments. Resistance to drugs has been related to human life and travel, animals and food commerce, wild animal movement and transportation, as well as water and wind flow, since ecosystems have no boundaries. Furthermore, EIDs are becoming untreatable and unmanageable as the existing treatment armament shrinks, owing primarily to AMR (Asokan and Kasimanickam 2013). Further, every year, over 700,000 deaths are associated with AMR (Silva et al. 2020). Antibiotic-resistant bacteria are predicted to cause over 33,000 deaths in the European Union (EU) by the year 2050 (Gajdács and Albericio 2019; EARS-Net ECS 2019).

ESKAPE pathogens, which stand for *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and Enterobacter species, are accountable for the most dangerous and potentially fatal hospital-acquired infections.

Opportunistic bacteria such as ***Enterococcus faecium***, which are responsible for serious infectious illness. It is a Gram-positive bacterium that is widely known for causing urinary tract infections, wound infections, endocarditis, and nosocomial bacteremia, all of which can lead to sepsis (Higuita and Huycke 2014). Enterococci have the ability to develop resistance to erythromycin, vancomycin (vancomycin-resistant enterococci; VRE), aminoglycosides, glycopeptides, gentamicin, tetracycline, and streptomycin (Kayser 2003; Cetinkaya et al. 2000).

The study of population genetics as well as genomics revealed that *E. faecium* comprises two distinct subpopulations. The first subpopulation is made up of commensals that live in the gastrointestinal tract and are often not causative for clinical infections. The second subpopulation consists of hospital-associated *E. faecium* lineages. These lineages are the ones that are responsible for nosocomial epidemics and opportunistic infections in inpatients (Zhou et al. 2020).

Gene acquisition and gene loss in *E. faecium*, aided by plasmid transfer, homologous recombination and mediated by insertion sequence elements, both certainly contributed to the transformation of *E. faecium* as a hospital-adapted pathogen (Leavis et al. 2007).

Staphylococcus aureus is a Gram-positive bacterium which causes the skin, bone, soft tissue, joints, or even infections linked with indwelling catheters or prosthetic devices. Skin infections are the most prevalent kind of infection caused by *S. aureus* (Tong et al. 2015). Methicillin, which spawned a novel superbug *S. aureus* that is resistant to methicillin. The methicillin-resistant *S. aureus* (MRSA) has long been known as a pathogen in healthcare facilities, it has worse clinical outcomes than methicillin-sensitive *S. aureus* (MSSA) (Ippolito et al. 2010). MRSA is known to commonly be the cause of severe skin and soft tissue infections that are purulent. Sepsis can also be raised on by infections in the blood that are driven on by *S. aureus* (Creech et al. 2015).

Predisposing factors for the establishment of MRSA include the use of antibiotics without including a prescribed medication, a lack of knowledge, the use of antibiotics prior to hospitalization, and long-term inpatient care (Ansari et al. 2013). *S. aureus* can become resistant either by horizontal transfer of resistance genes from mobile genetic elements like plasmids, transposons, and the staphylococcal cassette chromosome, or by mutations in chromosomal genes. Horizontally acquired resistance may result from any of the following mechanisms: (i) enzymatic drug modification and inactivation, (ii) enzymatic modification of the drug binding site, (iii) drug efflux, (iv) bypass mechanisms involving the acquisition of a novel drug-resistant target, or (v) displacement of the drug to protect the target. Further, acquisition of resistance by mutation can result in (i) modification of the drug target that hinders the inhibitor from binding, (ii) de-repression of chromosomally encoded multidrug resistance efflux pumps, and (iii) multiple stepwise mutations that alter the structure and/or composition of the cell wall and/or membrane, thereby reducing drug access to its target (Foster 2017).

Klebsiella pneumoniae is becoming increasingly resistant to the medications penicillin and ampicillin as it employs beta lactamases. In addition, the bacteria is a strain of extended-spectrum beta-lactamase, also known as ESBL, and it exhibits an increasing level of multidrug resistance to a broad spectrum of antibiotics—for example, cephalosporin or ceftazidime (Viale et al. 2013).

Carbapenems are normally reserved for usage as a last option in the treatment of multidrug-resistant infections in hospital patients. Unfortunately, several studies have identified that carbapenem-resistant *Klebsiella pneumoniae* (CRKP) infection is resistant to practically most of existing antimicrobials, is associated with significant death rates, and is recognized as one of the most serious antibiotic resistance concerns by the CDC and the World Health Organization (WHO) (Band et al. 2018; Centers for Disease Control and Prevention (CDC) 2019; World Health Organization 2017).

Acinetobacter baumannii, which is Gram-negative pathogen, usually causes infections in critically ill in-patients and accounts about a quarter of infections that occur in ICUs across the world (Fournies and Richet 2013). The resistance mechanisms in *A. baumannii* include enzymatic drug degradation, target changes, multidrug efflux pumps, and permeability defects (Gordon and Wareham 2010; Lin and Lan 2014).

Pseudomonas aeruginosa has become a prime driver of healthcare-associated infections (Rosenthal et al. 2014). It is most commonly found in hospitalized patients, and it has rapidly evolved resistance to drugs like ciprofloxacin and levofloxacin. *P. aeruginosa* builds a "shield" on surfaces by developing a biofilm, making it particularly difficult to eradicate, especially in cystic fibrosis patients (Lister et al. 2009). The recent advent of extensively drug-resistant *P. aeruginosa* (XDR-PA), which is characterized by strains that are exclusively sensitive to one or two types of antipseudomonal drugs, has become a serious concern because of the absence of a viable antibiotic therapy (Lister et al. 2009; Falagas et al. 2008). One of the mechanisms of resistance to antibiotics in its arsenal is the presence of several chromosomal determinants, in addition to the intricate regulatory networks that are engaged in both intrinsic and adaptive resistance (Horcajada et al. 2019).

Enterobacter spp. is largely responsible for numerous of severe nosocomial infections. These infections reflect a wide range of multidrug resistance due to the presence of plasmid-encoded ESBLs, *Klebsiella pneumoniae* carbapenemases and metallo—lactamase **(Navidinia** 2016**)**. Factors predisposing to such an infection include long-term antibiotic usage, immunocompromised conditions, such as malignancy, the exposure of invasive medical devices, recent hospitalization, or invasive surgery (Ramirez and Giron 2021).

26.4 Variations in the Pathogenesis of EIDs

Over the past decades, human lives have seen pandemics across several geographical locations of the globe. The understanding of the pathogenesis of the different emerging infectious diseases can help to plan effective preventive or therapeutic measures at an early stage against these pandemics (Jacobsen et al. 2016).

The genesis of infectious diseases in the past has indicated a relationship between evolutionary etiology and the incidence of these EIDs. For example, in the re-emergence of dengue fever, which has been exacerbated by alarming rises in a dangerous and formerly rare variant of the disease, hemorrhagic fever, a very lethal type. It arose from dengue viruses evolving to avoid high insight, as demonstrated in increasing viral virulence and human immunopathogenesis due to antibody-dependent intensification of viral infection (Morens 1994).

However, in case of cholera outbreaks environmental strains appear to serve as repositories for human virulence genes. These undergo gene transfer events that produce new strains with additional virulent gene combinations (Faruque and Mekalanos 2012; Davidson et al. 2012).

To enter the host cell, coronavirus utilizes the type 2 transmembrane serine protease (TMPRSS2) and the ACE2 receptor protein, which allows it to fuse and endocytose with the host cell after entering through one of the mucous membrane entrance points (Fehr and Perlman 2015; Chen and Guo 2020). Translated viral proteins are made from the RNA that has been devoid of its coating. New RNA is synthesized for new virions with the aid of RNA-dependent RNA polymerase. Once

the cell is lysed, plenty of other additional virions are released into the patient's system. Inflammatory cytokines are released in great quantities as a result of the infection, creating a "cytokine storm" (Schreiber et al. 2022). When a growing infection affects immunity, it can lead to an immunopathological cytokine storm that can be fatal. During cytokine storms, the levels of IL-6, IL-1, IL-8, TNF, and/or IP-10 are frequently increased. This phenomenon is also observed in a variety of newly developing infectious disorders (Yang et al. 2001; Yeh et al. 2006; Meftahi et al. 2020). Anti-IL6R and/or anti-IL1 antibodies are indicated for the treatment of COVID-19 cytokine storm. In addition, coronavirus infections have been associated to T helper cell type 17 (Th17) responses, which have been connected to immunosuppressive medication (Wong et al. 2017; Buckley et al. 2020).

The understanding of individual pathogenesis adds to the clarity on the emerging infections; and, this helps in taking effective preventive measures against epidemics.

To understand this heterogeneity in pathogenesis, the immunopathogenesis of major emerging viral illnesses may be categorized into four groups: (i) insufficient immunity with disseminated viremia (e.g., West Nile virus, Ebola); (ii) pneumocytotropism with/without subsequent hyperinflammation (e.g., SARS-CoV-1/2, Swine flu); (iii) increased immunopathology (e.g., Avian flu); and (iv) antibody-dependent (Yang and Yang 2021).

26.5 Identification

In recent decades, the most important challenge has been the development of universal and reliable methods for the early identification of emerging infectious diseases. The occurrence or origin of such disease usually emerges in the local region initially; however, the global economy and migration increase the risk of these infectious diseases the worldwide.

There has been a significant social and economic impact from the COVID-19 pandemic, the most recent infectious illness outbreak. The World Health Organization estimates that about 690 million people are undernourished, with tens of millions at risk of sliding into extreme poverty as a result (ILO, FAO, IFAD and WHO 2020).

Emerging infectious diseases are among the most significant risks to civilization since they pose such a threat to human life across the planet. Fortunately, it is expected that early detection and identification of such diseases considerably reduces mortality. With the use of markers, databases and hotspots which have the potential for early identification of emerging infectious diseases, the scientists may be able to detect novel infectious illnesses before they dispersed to the population at large. This section encompasses the use of markers, databases and the hotspots for earlier identifications of EIDs.

26.5.1 Markers

Biomarkers are defined as "any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease" (Meftahi et al. 2020). When it refers to infectious diseases, the initial steps toward disease control must proceed with the characterization and identification of host and pathogen predictors of protection or progression to disease in subjects who have been exposed to the pathogen. The research of this special concern, which brings together a mass of vital information on specified diseases and gives a glimpse into reliable methods for developing highly specific and sensitive biomarkers to determine the presence as well as the severity of infectious infection is the need of the hour (Jacobs and Wong 2016).

The platelet and lymphocyte ratios act as a marker in Crimean Congo Hemorrhagic Fever, predicting the requirement of blood transfusion and risk of mortality. Similarly, thrombocytopenia, prolonged activated partial thromboplastin time (APTT), melena, and somnolence were also reported to perform well in identifying patients at risk of mortality in Crimean-Congo hemorrhagic fever patients (Eren et al. 2016; Çevik et al. 2008).

Antibodies, metabolic activity markers, cytokines, and mycobacterial antigens have all been discovered as biomarkers of tuberculosis. Antigenic biomarkers are notably important in the establishment of novel tuberculosis diagnostics. One of most investigated antigens are CFP-10 (10-kDa culture filtrate protein), Ag85A, ESAT-6 (6-kDa early secretory antigenic target) CFP-7, Ag85B, and PPE18. A few of these antigens have been introduced as biomarkers for assessing crucial pathogen and host responses to infection (Rodríguez-Hernández et al. 2020).

Currently, research is more oriented on identifying single metabolites and more complex metabolomic signatures that are associated with severity of illness and clinical outcome in patients with systemic infections. Oxidative stress metabolomic indicators, steroid hormone and amino acid pathways, and nutritional markers are a few examples of these pathways (Zurfluh et al. 2018). During sepsis, peripheral blood neutrophilia, which includes demarginating and increased marrow recruitment, may cause tissue damage and organ failure due to inflammatory processes, when combined with systemic neutrophil activation (Rosales 2018).

In patients with infections, the neutrophil:lymphocyte ratio (NLR) and other leucocyte ratios have been described as relevant biomarkers. This reflects an underlying immunological dysfunction, at least in the peripheral blood. The NLR has potential to predict infections including, the malignant pertussis, postoperative sepsis after percutaneous nephrolithotomy (Sen et al. 2016), febrile urinary tract infection (Yang and Yang 2021), diabetes-related osteomyelitis or amputation (Yapıcı et al. 2017), variety in debridements of Fournier's gangrene (Kahramanca et al. 2014), and in-hospital outcomes in infective endocarditis (Turak et al. 2013).

26.5.2 Databases

Surveillance systems that track illnesses, infections, and clinical outcomes have historically been a pillar of infectious disease control (Thacker and Stroup 1994).

Traditional surveillance systems are known for their high time delays and limitations of spatial resolution; hence, more efficient, local, and timely systems are required. For emerging and reemerging illnesses, such as pandemic influenza, severe acute respiratory syndrome, Middle East respiratory syndrome, Zika, Ebola, and drug-resistant pathogens, monitoring and forecasting are of great relevance (Woolhouse et al. 2015).

More information is being produced by biological research on infectious diseases which are connected to the data end of the continuum and cannot be presented in conventional literature (Zou et al. 2015).

Biological databases are in charge of disseminating the knowledge generated to the scientific community. The primary data is extracted from the published literature in most biological databases, which serves as a minor literature resource (Leonelli and Ankeny 2012).

Research on biological databases passes through a lot of stages, including administration and curation of biological data, as well as quality assurance, integration, and data mining. Not only contemporary biological databases include data, and they often fit perfectly with powerful querying and data analysis capabilities. There is also enough information on infectious diseases available on the web that might be useful for scientific study of these diseases (Swetha and Anbarasu 2016).

On the other hand, the datasets like HealthMap were created through the introduction of an approach such as ResistanceOpen which is a new online platform for monitoring of bacterial drug resistance, based on timely scanning, aggregation, analysis, and dissemination of local and regional online resistance index reports are an example of the extension of prior efforts to track infectious disease outbreaks globally by curating and analyzing a variety of online data sources. The majority of the data in HealthMap is presented in the form of a map, with each point denoting a different epidemic. The information may be sorted in a number of different ways, including by disease, region, source, species, and date (Brownstein et al. 2008).

The infectious diseases databases, as well as the **WHO and UN datasets**, both of which provide public access to their webpages, give a wide diversity of the information that has been gathered. **GIDEON** (Global Infectious Illnesses and Epidemiology Network), which is particularly helpful for tropical and infectious diseases, epidemiology, microbiology, and antimicrobial chemotherapy; the **Global Burden of Disease**, for which data was gathered and released from 1970 up until 2016, encompasses 333 different diseases and injuries and contains information on both communicable and noncommunicable diseases; Observatories such as the **Infectious Diseases Data Observatory (IDDO),** which provide the methods, governance, and infrastructure to translate data into evidence that improves outcomes for patients worldwide. The **ProMED (Program for Monitoring Emerging Disease)**, collects data through media reports, official reports, online summaries, and other local

observers. The databases important in view of data availability on the infectious diseases have been enlisted in the Table 26.2 (WHO n.d.; ProMED (Program for Monitoring Emerging Disease) n.d.; Global Infectious Diseases and Epidemiology Network n.d.; Observatories like Infectious Diseases Data Observatory (IDDO) n.d.; Bollyky et al. 2019).

26.5.3 Hotspots

In general, hotspots have been defined as places of increased incidence or prevalence, increased transmission performance or risk, or increased likelihood of disease development (Hotez 2014; Lessler et al. 2017).

To be more precise, the term "burden hotspot" is used to describe areas with a high prevalence or incidence of disease, while "transmission hotspot" or the "risk hotspot" designates areas with a high transmission efficiency or a greater risk of disease acquisition, and "emergence hotspot" designates areas with a higher probability of disease occurrence or reappearance (Lessler et al. 2017).

According to 2017 reports, the global distribution of the EID risk index is more concentrated in tropical regions of globe. Zones having a higher likelihood of EID occurrence are uniformly dispersed across the continents, with no major landmass predicted to be free of EID-prone areas. Outside of the tropics, locations with dense populations, such as cities in Europe, the United States, Asia, and Latin America, remain at the top of the danger index. More widespread areas of predicted EID prevalence are found in tropical regions of North America, Asia, Central Africa, and South America (Allen et al. 2017).

Along with revealing the regions with potential infectious disease threats, hotspots also uncovered the specific factors that make a region predisposed to disease emergence.

Additionally, the bulk of the estimated geographic risk range is based on observed data of MERS, Marburg Virus Disease, and Rift Valley Fever which have been identified to exist in arid nations spanning the Middle East and Eastern regions of Africa. Although it is anticipated that Lassa fever and Ebola virus disease would be common across Western and Central Africa, in especially in the moist rainforests of those regions (Jagadesh et al. 2020).

26.6 Management of EIDs

26.6.1 Surveillance

Infectious disease surveillance is a vital epidemiological method for keeping track of a population's health (Murray and Cohen 2017). Disease surveillance data primarily (i) serves as an early alert system for oncoming emergence and spread that could

Table 26.2 List of databases that represent the data on the outbreak of infectious diseases

#	Database	Description of database	Link to access
1	Global burden of disease	• The mortality, morbidity, incidence, and prevalence are frequently used to estimate the burden of disease	http://ghdx.healthdata.org/gbd-2016/data-input-sources
2	Nature study	• The original study, 'Global Trends in Emerging Infectious Diseases,' published by Jones et al. (2008), has been revised by Allen et al. (2017), 'Global Hotspots and Correlates of Emerging Zoonotic Diseases'	https://www.nature.com/articles/nature06536 https://www.nature.com/articles/s41467-017-00923-8
3	Global infectious diseases and epidemiology network (GIDEON)	• Interactive infectious disease diagnostic tool. Diagnosis, epidemiology, treatment, and microbiology are covered	https://www.gideononline.com/
4	HealthMap	• Boston Children's hospital launched HealthMap in 2006 to enable real-time surveillance of infectious disease epidemics • The software makes advantage of publicly accessible, informal web data sources such as ProMED, WHO, OIE, FAO, Google news, and EuroSurveillance	https://www.healthmap.org/en/
5	Program for monitoring emerging disease (ProMED)	• ProMED is a database of infectious disease reports that has been archived • It is an International Society for Infectious Diseases initiative that monitors infectious disease outbreaks and acute toxicity exposures	https://promedmail.org/
6	WHO/UN	• Datasets for particular infectious diseases of diseases, such as HIV/AIDS, TB, malaria, neglected tropical diseases, cholera, influenza, meningitis, and sexually transmitted infections, are accessible	https://www.who.int/data/gho/data/themes/mortality-and-global-health-estimates https://www.euro.who.int/en/data-and-evidence/databases
7	Health protection research unit - healthcare associated infections and antimicrobial resistance	• This database supports the National Institute for health and care Research's health protection research unit in healthcare associated	https://www.hra.nhs.uk/planning-and-improving-research/application-summaries/research-summaries/

(continued)

Table 26.2 (continued)

#	Database	Description of database	Link to access
		infections and antimicrobial resistance (AMR) • This is a partnership between Imperial College London and Public Health England	database-for-infectious-disease-surveillance-models/
8	PathoPhenoDB	• Created by King Abdullah University of Science and Technology academics in partnership with the University of Cambridge in the United Kingdom • It can aid in the diagnosis and treatment of infectious diseases, as well as the research of the molecular processes underlying pathogen-host interactions	NA
9	Infectious disease biomarker database	• It is a community annotation database that uses collaborative web 2.0 features to link infectious diseases or pathogens to protein, gene, or carbohydrate biomarkers via search tools	http://biomarker.cdc.go.kr/
10	Point prevalence survey database (HAI-net)	• The HAI-net point prevalence survey (PPS) of healthcare-associated infections (HAIs) and antimicrobial usage online database provides European reference data on HAIs and antimicrobial use in acute care hospitals throughout Europe	https://www.ecdc.europa.eu/en/healthcare-associated-infections-acute-care-hospitals/surveillance-disease-data/database
11	Interactive database systems	• The surveillance resource center organizes and makes accessible to members of the public health surveillance community recommendations generated by the CDC and its partners for enhancing surveillance practices	https://www.cdc.gov/surveillancepractice/data.html
12	Infectious diseases data observatory (IDDO)	• It is a multidisciplinary, scientifically independent coalition of worldwide infectious disease and emerging infection communities	https://www.iddo.org/

become public health emergencies, (ii) involves the monitoring and analysis of the impact of an intervention, and (iii) supervises and reinforces the epidemiology of health problems, guiding priority-setting and planning and evaluating public health policy and strategies (WHO 2021). It entails the continuous systematic gathering, analysis, and interpretations of the data, as well as the timely dissemination of the whole data to those responsible for disease prevention and control (Thacker and Berkelman 1988).

Traditional methods of disease surveillance involve the use of disease-specific surveillance systems to monitor specific pathogens, diseases, or syndromes in a population of interest (Balajee et al. 2021); on the other hand, syndromic surveillance systems involve the real-time or near real-time collection, analysis, characterization, and propagation of health-related data for the purpose of identifying potential health threats as early as possible (Morgan et al. 2021); a real-time or near real-time manual or automatic collection and analysis of metadata is performed by an event-based surveillance system. This information comes from a wide variety of text sources and is written in a number of languages. The goal is to identify potential or confirmed health hazards (Balajee et al. 2021). However, the appearance of SARS-CoV shed light on the shortcomings of conventional systems of disease surveillance in virtually every nation.

Therefore, future surveillance systems should be based on integrated technologies. A solid surveillance foundation is required to keep a methodical, consistent, and statistically sound sight on the population; laboratory confirmation must be scaled accurately for different diseases and risks in these surveillance systems; a digitized system with specific health identifiers used to link individual-level data and privacy controls in place; and surveillance programs must employ standardized case definitions and common data components, and the community, state, and federal health authorities, regional bodies, and WHO must have sufficient access. Lastly, disease surveillance must be appropriately funded (Morgan et al. 2021).

26.6.2 Risk Assessment

Assessment of factors that defines the risk of occurrence of infectious disease has the potential to significantly reduce the impact of EIDs on human lives especially in view of emergence.

The World Health Organization has classified the risk factors of emerging infectious diseases into, **distal risk factors** which include extreme poverty, inequality, economic stagnation, environmental fragility, ethnic rivalry, resource competitiveness, climate change, political instability, proliferation of armaments, and seismic hazard**; intermediate risk factors** which include armed conflict, psychological and physical stress, abusive relationships, natural disasters, displacement, breakdown of government services, food insecurity/shortage, access to/utilization of health services; and **proximate risk factors** involving overcrowding, inadequate shelter, insufficient nutrient intake, insufficient vaccination coverage, violence, high

exposure to disease vectors, poor water, sanitation, hygiene conditions and lack of and/or delay in treatment (World Health Organization and Regional Office for South-East Asia New Delhi 2005).

The unavailability of data, which is critical for risk assessment and decision-making, has become a major challenge for international health authorities responding to EID outbreaks in the Asia-Pacific zone (McCloskey et al. 2014).

These challenges are amplified when seeking to develop preparations in advance of an epidemic, when epidemiological data relevant to a specific nation may not exist or be easily accessible to academics, policymakers, and response experts (Moss et al. 2016). Furthermore, pandemic risk estimates based on earlier than usual, insufficient information should be interpreted cautiously, because the recognition of highly contagious individuals and severe cases is more probable, and because surveillance resources are more readily available in high-income populations, where pathogen transmission characteristics may be atypical. Regardless of these challenges, assessing possible epidemic risks is critical for making timely decisions on effective preparedness and response methods (Cauchemez et al. 2013).

Rapidly developed transmissibility and severity estimates are critical for anticipating the magnitude and time course of a pandemic when considering the threat of emerging infectious disease (Morgan et al. 2021).

Transmissibility is generally evaluated through the use of the early growth rate of case numbers, which is independent of the proportion of cases reported (White et al. 2009). The fundamental reproduction number of an infectious disease is a key statistic that is important for the overall assessment of transmissibility (R_0). This is the average number of secondary cases that would be created by a primary case in a community that is vulnerable to the disease. It is characterized as "incidence rate." It offers an overall estimation of an infection's propensity for transmission throughout a population and is based not only on the transmission coefficient but also on the average length of infectiousness. The higher the value of R_0, the higher the percentage of the population that must be vaccinated in order to eliminate a disease (Miller 2003). However, early estimations of this epidemiological construct are frequently given disproportionate weight when evaluating the likelihood for a pandemic (Fisman et al. 2014).

In light of recent developments in risk assessment, the utilization of mathematical modeling as a method for supporting risk assessment and decision-making in regards to planning and reaction during an EID epidemic is one that might be considered (Woolhouse 2011; Keeling and Rohani 2011).

The development of a differential equation model of a disease, such as the typical deterministic susceptible-infected-recovered (SIR) model, is one of the most popular modeling techniques for assessing treatments in dynamic systems (Keeling and Rohani 2011; Gilbert et al. 2014; Vynnycky and White 2010). Additionally, the basic reproduction number, R_0—the typical number of secondary infections that would follow a primary infection in an uninfected host population—can also be determined from the differential equation model using a closed-form solution (Gilbert et al. 2014).

Depending on a disease's R_0 score, there are three possible outcomes for its potential spread or decline: (i) If R_0 is lower than 1, each infection that already exists results in less than one new infection. In this instance, the illness will deteriorate and ultimately vanish. (ii) There won't be an outbreak if R_0 is equal to 1, but the disease will still persist. (iii) If R_0 exceeds 1, the number of cases may increase exponentially, leading to an epidemic or perhaps a pandemic (Chen 2020).

26.6.3 Repurposing of Drugs

Drug repurposing is a newly popular drug development strategy that entails researching novel molecular mechanisms and intervention targets to give existing drugs a new indication (Strittmatter 2014; Saul and Einav 2020).

Innovative methods for target validation, such as gene editing by CRISPER/Cas9, and new experimental models have facilitated the identification of new antiviral drugs and the elucidation of molecular mechanisms underlying viral disease (Grammer and Lipsky 2017; Meganck and Baric 2021). The repurposed drugs for emerging infections are enlisted in the following Table 26.3.

Remdesivir is an antiviral medication with a broad range of activity that has shown encouraging signs of success in the treatment of Middle East respiratory syndrome and severe acute respiratory syndrome. It is the repurposed medication that has received the maximum attention in relation to the ongoing COVID-19 pandemic. The strategy used for it is investigating the drug molecules that block virus-cell fusion to prevent virus entry (Morgan et al. 2021).

The timeline for the repurposed medicine "remdesivir" is presented in Table 26.4 and extends from 2009 to February of 2020. In the year 2020, it was repurposed as a therapy for the COVID-19 infection. This table is compiled using information that is freely available in open domain (Mercorelli et al. 2018).

Table 26.3 Repurposed drugs for management of emerging infections

S. no.	Drug name	Original indication	New indication
1	Favipiravir	Influenza	COVID-19
2	Remdesivir	Broad-spectrum antiviral	COVID-19
3	Sarilumab	Rheumatoid arthritis	COVID-19
4	Danoprevir/ritonavir	Danoprevir-Hep C Ritonavir-HIV	COVID-19
5	Dexamethasone	Immunosuppressants	COVID-19-associated ARDS
6	Hydroxychloroquine	Malaria	COVID-19
7	Methylprednisolone	Immunosuppressant	COVID-19
8	Tocilizumab	Cytokine release syndrome	COVID-19 & severe pneumonitis
9	Interferon B1 b	Multiple sclerosis	MERS

Table 26.4 Timeline of the repurposed drug: Remdesivir

S. no.	Time (month/year)	Event
1	2009	• Gilead's remdesivir is an investigational new drug (IND) • Remdesivir was developed as a result of study into hepatitis C (HCV) and respiratory syncytial virus that began in 2009
2	2013	• antiviral profiling • Remdesivir has the potential to have broad-spectrum antiviral action
3	2014	• Remdesivir has been identified as having the potential to be active against emerging viruses such as Ebola. • the collaboration received a National Institutes of Health (NIH) funding in 2014 to assist the development of novel drugs against coronaviruses, among other studies.
4	July 2015	• Gilead files IND application for Remdesivir.
5	August 2015	• Gilead launched phase 1 trials in healthy volunteers to assess the safety and pharmacokinetics of remdesivir
6	2016	• the NIH launched a study of remdesivir in Ebola survivors • the NIH carried out studies of remdesivir in a non-human primate model of MERS infection
7	2018	• NIH began a study of remdesivir and other investigational treatments in patients with Ebola disease in the Democratic Republic of the Congo
8	Jan 2020	• Remdesivir was provided to the China CDC to test the compound against isolates of the virus that causes COVID-19 through their independent antiviral assays
9	Feb 2020	• supporting multiple clinical trials by Gilead to evaluate the safety and efficacy of remdesivir as a potential treatment for COVID-19

However, during the current COVID-19 epidemic, a multitude of benefits and potential drawbacks of adopting the repurposing strategy for the management of newly developing infectious diseases have been brought to the forefront. The use of the repurposing technique has a number of potential benefits, including: a low cost with smaller time investment; a higher probability of crossing preclinical trials and directly entering phase 2 clinical trials; the potential for combination strategies with the possibility of reducing resistance associated with monotherapy; formulations and manufacturing channels that are already established for the large-scale production, which eliminates the need for additional costs associated with the further launch (Sultana et al. 2020).

On the other hand, repurposing of drugs faces challenges/pitfalls like target identification in case of drugs that show polypharmacology, misidentification of toxic drugs as active as a result of high dose employment during screening and issues in exploration of medicinal chemistry in order to build more powerful analogs without sacrificing their potential for further applications (Swathi et al. 2021; Mercorelli et al. 2018).

References

Allen T, Murray KA, Zambrana-Torrelio C, Morse SS, Rondinini C, Di Marco M et al (2017) Global hotspots and correlates of emerging zoonotic diseases. Nat Commun 8(1):1–10

Anderson PK, Cunningham AA, Patel NG, Morales FJ, Epstein PR, Daszak P (2004) Emerging infectious diseases of plants: pathogen pollution, climate change and agrotechnology drivers. Trends Ecol Evol 19(10):535–544

Ansari MA, Khan HM, Khan AA, Pal R, Cameotra SS (2013) Antibacterial potential of Al2O3 nanoparticles against multidrug resistance strains of Staphylococcus aureus isolated from skin exudates. J Nanopart Res 15(10):1–12

Antia R, Regoes RR, Koella JC, Bergstrom CT (2003) The role of evolution in the emergence of infectious diseases. Nature 426(6967):658–661

Asokan G, Kasimanickam R (2013) Emerging infectious diseases, antimicrobial resistance and millennium development goals: resolving the challenges through one health. Cent Asian J glob. Health 2(2)

Balajee SA, Salyer SJ, Greene-Cramer B, Sadek M, Mounts AW (2021) The practice of event-based surveillance: concept and methods. Glob Secur Health Sci Policy 6(1):1–9

Band VI, Satola SW, Burd EM, Farley MM, Jacob JT, Weiss DS (2018) Carbapenem-resistant Klebsiella pneumoniae exhibiting clinically undetected colistin heteroresistance leads to treatment failure in a murine model of infection. MBio 9(2):e02448–e02417

Bollyky TJ, Templin T, Cohen M, Schoder D, Dieleman JL, Wigley S (2019) The relationships between democratic experience, adult health, and cause-specific mortality in 170 countries between 1980 and 2016: an observational analysis. Lancet 393(10181):1628–1640

Brownstein JS, Freifeld CC, Reis BY, Mandl KD (2008) Surveillance sans Frontieres: internet-based emerging infectious disease intelligence and the HealthMap project. PLoS Med 5(7):e151

Buckley LF, Wohlford GF, Ting C, Alahmed A, Van Tassell BW, Abbate A et al (2020) Role for anti-cytokine therapies in severe coronavirus disease 2019. Crit Care Explor 2(8):e0178

Cauchemez S, Van Kerkhove M, Riley S, Donnelly C, Fraser C, Ferguson N (2013) Transmission scenarios for Middle East respiratory syndrome coronavirus (MERS-CoV) and how to tell them apart. Eur Secur 18(24):20503

Centers for Disease Control and Prevention (CDC). Antibiotic Resistance Threats in the United States. (2019 AR Threats Report) [Internet]. U.S. Department of Health and Human Services, CDC; 2019. Available from: www.cdc.gov/DrugResistance/Biggest-Threats.html

Cetinkaya Y, Falk P, Mayhall CG (2000) Vancomycin-resistant enterococci. Clin Microbiol Rev 13(4):686–707

Çevik MA, Erbay A, Bodur H, Gülderen E, Baştuğ A, Kubar A et al (2008) Clinical and laboratory features of Crimean-Congo hemorrhagic fever: predictors of fatality. Int J Infect Dis 12(4): 374–379

Chen J (2020) Pathogenicity and transmissibility of 2019-nCoV—a quick overview and comparison with other emerging viruses. Microbes Infect 22(2):69–71

Chen YLQ, Guo D (2020) Emerging coronaviruses: genome structure, replication and pathogenesis. J Med Virol 92(4):1

Chomel BB (1998) New emerging zoonoses: a challenge and an opportunity for the veterinary profession. Comp Immunol Microbiol Infect Dis 21(1):1–14

Cleaveland S, Haydon D, Taylor L (2007) Overviews of pathogen emergence: which pathogens emerge, when and why? Wildl Emerg Zoonotic Dis Biol Circumst Consequences Cross-Species Transm:85–111

Creech CB, Al-Zubeidi DN, Fritz SA (2015) Prevention of recurrent staphylococcal skin infections. Infect Dis Clin 29(3):429–464

Daszak P, Cunningham AA, Hyatt AD (2000) Emerging infectious diseases of wildlife—threats to biodiversity and human health. Science 287(5452):443–449

Davidson JE, Lockhart A, Amos L, Stirnadel-Farrant HA, Mooser V, Sollberger M et al (2012) Plasma lipoprotein-associated phospholipase A2 activity in Alzheimer's disease, amnestic mild

cognitive impairment, and cognitively healthy elderly subjects: a cross-sectional study. Alzheimers Res Ther 4(6):51
EARS-Net ECS (2019) EARSS Report. Available Online https.www.Ecdc.Eur.Euenpublications-Datasurveillance-Antimicrob-Resist-Eur-2019. Accessed 13 Dec 2021
Eren SH, Zengin S, Büyüktuna SA, Gözel MG (2016) Clinical severity in forecasting platelet to lymphocyte ratio in Crimean–Congo hemorrhagic fever patients. J Med Microbiol 65(10): 1100–1104
Falagas ME, Rafailidis PI, Matthaiou DK, Virtzili S, Nikita D, Michalopoulos A (2008) Pandrug-resistant Klebsiella pneumoniae, Pseudomonas aeruginosa and Acinetobacter baumannii infections: characteristics and outcome in a series of 28 patients. Int J Antimicrob Agents 32(5): 450–454
Faruque SM, Mekalanos JJ (2012) Phage-bacterial interactions in the evolution of toxigenic vibrio cholerae. Virulence 3(7):556–565
Fehr AR, Perlman S (2015) Coronaviruses: an overview of their replication and pathogenesis. Coronaviruses:1–23
Fisman DN, Leung GM, Lipsitch M (2014) Nuanced risk assessment for emerging infectious diseases. Lancet 383(9913):189–190
Foster TJ (2017) Antibiotic resistance in Staphylococcus aureus. Current status and future prospects. FEMS Microbiol Rev 41(3):430–449
Fournies P, Richet H (2013) The epidemiology and control of Acinetobacter baumannii. Health Care Facil Clin Inf Dis 42:692–609
Gajdács M, Albericio F (2019) Antibiotic resistance: from the bench to patients. Antibiotics 8(3): 129
Gilbert JA, Meyers LA, Galvani AP, Townsend JP (2014) Probabilistic uncertainty analysis of epidemiological modeling to guide public health intervention policy. Epidemics 6:37–45
Global Infectious Diseases and Epidemiology Network (n.d.) [Internet]. Available from: https://www.gideononline.com/
Gordon NC, Wareham DW (2010) Multidrug-resistant Acinetobacter baumannii: mechanisms of virulence and resistance. Int J Antimicrob Agents 35(3):219–226
Grammer AC, Lipsky PE (2017) Drug repositioning strategies for the identification of novel therapies for rheumatic autoimmune inflammatory diseases. Rheum Dis Clin 43(3):467–480
Higuita NIA, Huycke MM (2014) Enterococcal disease, epidemiology, and implications for treatment. In: Enterococci commensals lead causes drug resist infect [Internet]. Massachusetts Eye and Ear Infirmary, Boston
Horcajada JP, Montero M, Oliver A, Sorlí L, Luque S, Gómez-Zorrilla S et al (2019) Epidemiology and treatment of multidrug-resistant and extensively drug-resistant Pseudomonas aeruginosa infections. Clin Microbiol Rev 32(4):e00031–e00019
Hotez PJ (2014) Ten global "hotspots" for the neglected tropical diseases. PLoS Negl Trop Dis 8(5): e2496
Joint statement by ILO, FAO, IFAD and WHO. Impact of COVID-19 on people's livelihoods, their health and our food systems [internet]. WHO 2020. Available from: https://www.who.int/news/item/13-10-2020-impact-of-covid-19-on-people's-livelihoods-their-health-and-our-food-systems
Ippolito G, Leone S, Lauria FN, Nicastri E, Wenzel RP (2010) Methicillin-resistant Staphylococcus aureus: the superbug. Int J Infect Dis 14:S7–S11
Jacobs L, Wong HR (2016) Emerging infection and sepsis biomarkers: will they change current therapies? Expert Rev Anti-Infect Ther 14(10):929–941
Jacobsen KH, Aguirre AA, Bailey CL, Baranova AV, Crooks AT, Croitoru A et al (2016) Lessons from the Ebola outbreak: action items for emerging infectious disease preparedness and response. EcoHealth 13(1):200–212
Jagadesh S, Combe M, Nacher M, Gozlan R (2020) In search for the hotspots of disease X: a biogeographic approach to mapping the predictive risk of WHO's blueprint priority diseases. Int J Infect Dis 101:220

Kahramanca Ş, Kaya O, Özgehan G, İrem B, Dural İ, Küçükpınar T et al (2014) Are neutrophil-lymphocyte ratio and platelet-lymphocyte ratio as effective as Fournier's gangrene severity index for predicting the number of debridements in Fourner's gangrene? Turk J Trauma Emerg Surg 20(2):107–112
Kayser F (2003) Safety aspects of enterococci from the medical point of view. Int J Food Microbiol 88(2–3):255–262
Keeling MJ, Rohani P (2011) Modeling infectious diseases in humans and animals. Princeton University Press
Kilpatrick AM, Randolph SE (2012) Drivers, dynamics, and control of emerging vector-borne zoonotic diseases. Lancet 380(9857):1946–1955
Leavis HL, Willems RJL, van Wamel WJB, Schuren FH, Caspers MPM, Bonten MJM (2007) Insertion sequence–driven diversification creates a globally dispersed emerging multiresistant subspecies of *E. faecium*. PLoS Pathog 3(1):e7
Leonelli S, Ankeny RA (2012) Re-thinking organisms: the impact of databases on model organism biology. Stud Hist Philos Sci Part C Stud Hist Philos Biol Biomed Sci 43(1):29–36
Lessler J, Azman AS, McKay HS, Moore SM (2017) What is a hotspot anyway? Am J Trop Med Hyg 96(6):1270
Lin MF, Lan CY (2014) Antimicrobial resistance in Acinetobacter baumannii: from bench to bedside. World J Clin Cases WJCC 2(12):787
Lister PD, Wolter DJ, Hanson ND (2009) Antibacterial-resistant Pseudomonas aeruginosa: clinical impact and complex regulation of chromosomally encoded resistance mechanisms. Clin Microbiol Rev 22(4):582–610
McCloskey B, Dar O, Zumla A, Heymann DL (2014) Emerging infectious diseases and pandemic potential: status quo and reducing risk of global spread. Lancet Infect Dis 14(10):1001–1010
Meftahi GH, Jangravi Z, Sahraei H, Bahari Z (2020) The possible pathophysiology mechanism of cytokine storm in elderly adults with COVID-19 infection: the contribution of "inflame-aging". Inflamm Res 69(9):825–839
Meganck RM, Baric RS (2021) Developing therapeutic approaches for twenty-first-century emerging infectious viral diseases. Nat Med 27(3):401–410
Mercorelli B, Palù G, Loregian A (2018) Drug repurposing for viral infectious diseases: how far are we? Trends Microbiol 26(10):865–876
Miller E. Global burden of disease: Part C. Potential and existing impact of vaccines on disease epidemiology. In: The vaccine book. Elsevier; 2003. p. 37–50
Morens DM (1994) Antibody-dependent enhancement of infection and the pathogenesis of viral disease. Clin Infect Dis 19(3):500–512
Morgan OW, Aguilera X, Ammon A, Amuasi J, Fall IS, Frieden T et al (2021) Disease surveillance for the COVID-19 era: time for bold changes. Lancet 397(10292):2317–2319
Moss R, Hickson RI, McVernon J, McCaw JM, Hort K, Black J et al (2016) Model-informed risk assessment and decision making for an emerging infectious disease in the Asia-Pacific region. PLoS Negl Trop Dis 10(9):e0005018
Moya A, Holmes EC, González-Candelas F (2004) The population genetics and evolutionary epidemiology of RNA viruses. Nat Rev Microbiol 2(4):279–288
Murray J, Cohen AL (2017) Infectious disease surveillance. Int Encycl Public Health 222
National Institute of Allergy and Infectious Diseases (NIAID). NIAID Emerging Infectious Diseases/ Pathogens [Internet]. 2018. Available from: https://www.niaid.nih.gov/research/emerging-infectious-diseases-pathogens
Navidinia M (2016) The clinical importance of emerging ESKAPE pathogens in nosocomial infections. Arch Adv Biosci 7(3):43–57
Observatories like Infectious Diseases Data Observatory (IDDO) (n.d.) [Internet]. Available from: https://www.iddo.org/
Palmer SR, Soulsby L, Simpson DIH (1998) Zoonoses: biology, clinical practice and public health control. Oxford University Press

Petersen E, Petrosillo N, Koopmans M, Beeching N, Di Caro A, Gkrania-Klotsas E et al (2018) Emerging infections—an increasingly important topic: review by the emerging infections task force. Clin Microbiol Infect 24(4):369–375

ProMED (Program for Monitoring Emerging Disease) (n.d.) [Internet]. Available from https://promedmail.org/

Ramirez D, Giron M. Enterobacter infections. In: StatPearls [Internet] StatPearls Publishing; 2021

Rodríguez-Hernández E, Quintas-Granados LI, Flores-Villalva S, Cantó-Alarcón JG, Milián-Suazo F (2020) Application of antigenic biomarkers for mycobacterium tuberculosis. J Zhejiang Univ-Sci B 21(11):856–870

Rosales C (2018) Neutrophil: a cell with many roles in inflammation or several cell types? Front Physiol 113

Rosenthal VD, Maki DG, Mehta Y, Leblebicioglu H, Memish ZA, Al-Mousa HH et al (2014) International nosocomial infection control Consortiu (INICC) report, data summary of 43 countries for 2007–2012. Device-associated module. Am J Infect Control 42(9):942–956

Saul S, Einav S (2020) Old drugs for a new virus: repurposed approaches for combating COVID-19. ACS Infect Dis 6(9):2304–2318

Schreiber A, Viemann D, Schöning J, Schloer S, Mecate Zambrano A, Brunotte L et al (2022) The MEK1/2-inhibitor ATR-002 efficiently blocks SARS-CoV-2 propagation and alleviates pro-inflammatory cytokine/chemokine responses. Cell Mol Life Sci 79(1):1–18

Sen V, Bozkurt IH, Aydogdu O, Yonguc T, Yarimoglu S, Sen P et al (2016) Significance of preoperative neutrophil–lymphocyte count ratio on predicting postoperative sepsis after percutaneous nephrolithotomy. Kaohsiung J Med Sci 32(10):507–513

Shope RE, Oaks SC (1992) Emerging infections: microbial threats to health in the United States. National Academy Press, Washington, DC, p 294

Shukla P, Nguyen HT, Torian U, Engle RE, Faulk K, Dalton HR et al (2011) Cross-species infections of cultured cells by hepatitis E virus and discovery of an infectious virus–host recombinant. Proc Natl Acad Sci 108(6):2438–2443

Silva V, Correia S, Pereira JE, Igrejas G, Poeta P. Surveillance and environmental risk assessment of antibiotics and amr/args related with mrsa: one health perspective. In: Antibiotics and antimicrobial resistance genes. Springer; 2020. p. 271–95.

Strittmatter SM (2014) Overcoming drug development bottlenecks with repurposing: old drugs learn new tricks. Nat Med 20(6):590–591

Sultana J, Crisafulli S, Gabbay F, Lynn E, Shakir S, Trifirò G (2020) Challenges for drug repurposing in the COVID-19 pandemic era. Front Pharmacol 1657

Swathi K, Nikitha B, Chandrakala B, Lakshmanadevi K, Malleswari M (2021) Repurposing antiviral drugs on recently emerged viral infections: a review article. Mater Today Proc

Swetha RG, Anbarasu A (2016) A review on infectious diseases and their importance in developing biological databases. Res J Pharm Technol 9(5):621–624

Taylor LH, Latham SM, Woolhouse ME (2001) Risk factors for human disease emergence. Philos Trans R Soc Lond Ser B Biol Sci 356(1411):983–989

Thacker SB, Berkelman RL (1988) Public health surveillance in the United States. Epidemiol Rev 10(1):164–190

Thacker SB, Stroup DF (1994) Future directions for comprehensive public health surveillance and health information systems in the United States. Am J Epidemiol 140(5):383–397

Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG Jr (2015) Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev 28(3):603–661

Turak O, Özcan F, İşleyen A, Başar FN, Gül M, Yilmaz S et al (2013) Usefulness of neutrophil-to-lymphocyte ratio to predict in-hospital outcomes in infective endocarditis. Can J Cardiol 29(12):1672–1678

Viale P, Giannella M, Lewis R, Trecarichi EM, Petrosillo N, Tumbarello M (2013) Predictors of mortality in multidrug-resistant Klebsiella pneumoniae bloodstream infections. Expert Rev Anti-Infect Ther 11(10):1053–1063

Vynnycky E, White R (2010) An introduction to infectious disease modelling. OUP Oxford
White LF, Wallinga J, Finelli L, Reed C, Riley S, Lipsitch M et al (2009) Estimation of the reproductive number and the serial interval in early phase of the 2009 influenza a/H1N1 pandemic in the USA. Influenza Other Respir Viruses 3(6):267–276
WHO (2021). Surveillance in Emergencies [Internet]. Available from: https://www.who.int/emergencies/surveillance
WHO (n.d.). The Global Health Observatory [Internet]. Available from: https://www.who.int/data/gho
Wolfe ND, Dunavan CP, Diamond J (2007) Origins of major human infectious diseases. Nature 447(7142):279–283
Wong JP, Viswanathan S, Wang M, Sun LQ, Clark GC, D'elia RV (2017) Current and future developments in the treatment of virus-induced hypercytokinemia. Future Med Chem 9(2): 169–178
Woolhouse M (2011) How to make predictions about future infectious disease risks. Philos Trans R Soc B Biol Sci 366(1573):2045–2054
Woolhouse ME (2002) Population biology of emerging and re-emerging pathogens. Trends Microbiol 10(10):s3–s7
Woolhouse ME, Gowtage-Sequeria S (2005) Host range and emerging and reemerging pathogens. Emerg Infect Dis 11(12):1842
Woolhouse ME, Rambaut A, Kellam P. Lessons from Ebola: improving infectious disease surveillance to inform outbreak management. Sci Transl Med 2015;7(307):307rv5-307rv5
World Health Organization. (2017) WHO publishes list of bacteria for which new antibiotics are urgently needed. [Internet]. Available from: https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed
World Health Organization, Regional Office for South-East Asia New Delhi (2005) Combating emerging infectious diseases in the South-East Asia region. World Health Organization
Yang KD, Yang B (2021) Immunopathogenesis of different emerging viral infections: evasion, fatal mechanism, and prevention. Front Immunol 12:2570
Yang KD, Yeh W, Yang M, Chen R, Shaio M (2001) Antibody-dependent enhancement of heterotypic dengue infections involved in suppression of IFNγ production. J Med Virol 63(2): 150–157
Yapıcı O, Berk H, Öztoprak N, Seyman D, Tahmaz A, Merdin A (2017) Can ratio of neutrophil-to-lymphocyte count and erythrocyte sedimentation rate in diabetic foot infecti on predict osteomyelitis and/or amputation? Hematol Rep 9(1):19
Yeh WT, Chen RF, Wang L, Liu JW, Shaio MF, Yang KD (2006) Implications of previous subclinical dengue infection but not virus load in dengue hemorrhagic fever. FEMS Immunol Med Microbiol 48(1):84–90
Zhou X, Willems RJ, Friedrich AW, Rossen JW, Bathoorn E (2020) Enterococcus faecium: from microbiological insights to practical recommendations for infection control and diagnostics. Antimicrob Resist Infect Control 9(1):1–13
Zou D, Ma L, Yu J, Zhang Z (2015) Biological databases for human research. Genomics Proteomics Bioinformatics 13(1):55–63
Zurfluh S, Baumgartner T, Meier MA, Ottiger M, Voegeli A, Bernasconi L et al (2018) The role of metabolomic markers for patients with infectious diseases: implications for risk stratification and therapeutic modulation. Expert Rev Anti-Infect Ther 16(2):133–142

Chapter 27
Repurposing of Minocycline, a Tetracycline Antibiotic, for Neurodegenerative Disorders

Ranjana Bhandari Sanshita, Paavan Sood, Deeksha Thakur, and Anurag Kuhad

Abstract Minocycline, a semisynthetic, broad spectrum tetracycline antibiotic has been widely used as a bacteriostatic drug. It exerts its anti-bacterial action on its attachment to the smaller subunit of prokaryotic ribosome (30S) and inhibits the translation process. Minocycline ($C_{23}H_{27}N_3O_7$) has a small size and shows high lipophilicity, due to which it can cross the blood–brain barrier and enter the CNS; therefore, its use can be repurposed in neurodegenerative disorders like Parkinson's disease, Huntington's disease, Alzheimer's disease, amyotrophic lateral sclerosis multiple sclerosis, etc. Drug repurposing involves identifying uses for drugs (approved and investigational) that are other than their original medication indications. Minocycline plays a role in neurodegenerative disorders by variable mechanisms such as exerting its anti-apoptotic action by inhibition of the intrinsic and extrinsic pathways of apoptosis, anti-inflammatory effects by modulation of microglia, cytokines, lipid mediators, metalloproteases, etc. It also acts as an anti-aggregatory drug and exerts its action on protein misfolding which is a major cause of neurodegenerative disorders. The repurposing of minocycline is being observed by performing clinical and pre-clinical trials which have also been discussed in this chapter.

Keywords Tetracycline · Minocycline · Neurodegenerative diseases · Alzheimer's disease · Parkinson's disease · Huntington's disease · Amyotrophic lateral sclerosis (ALS) · Multiple sclerosis

Abbreviations

Ach	Acetylcholine
AD	Alzheimer's disease

R. B. Sanshita · P. Sood · D. Thakur · A. Kuhad (✉)
Pharmacology Research Laboratory, University Institute of Pharmaceutical Sciences, UGC-Centre of Advanced Study, Panjab University, Chandigarh, Punjab, India
e-mail: anurag.kuhad@pu.ac.in

R. C. Sobti et al. (eds.), *Drug Repurposing for Emerging Infectious Diseases and Cancer*, https://doi.org/10.1007/978-981-19-5399-6_27

ALS	amyotrophic lateral sclerosis
ALS FRS-R	amyotrophic lateral sclerosis functioning rating scale
APOE	Apo-lipo-protein E
APP	Amyloid Precursor Protein
ATP	Adenosine Triphosphate
BBB	blood–brain barrier
BD	Twice a day
CGN	Cerebellar granule neurons
CN	cranial nerve
CNS	central nervous system
DA	Dopamine
DAT	Dopamine Transporter
DATATOP	Deprenyl and Tocopherol Antioxidative Therapy of Parkinsonism
DNA	Deoxyribose Nucleic Acid
EAE	encephalomyelitis
ETC	Electron Transport Chain
FDA	Food and Drug Administration
FVC	forced vital capacity
HD	Huntington's disease
IL	interleukin
IL-1 β	Interleukin-1beta
iNOS	Inducible Nitric Oxide Synthase
LMN	lower motor neuron
LRRK2	Leucine-rich repeat kinase 2
MAO-B	Monoamine oxidase-B
MAPK	Mitogen-activated protein kinase
MC	Matched control
MCI	Mild cognitive impairment
MMT	manual muscle testing
MPP^+	1-methyl-4-phenyl pyridinium
MPTP	1- Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MRI	Magnetic resonance Imaging
MRS	Magnetic resonance spectroscopy
MS	multiple sclerosis
NADPH oxidase	Nicotinamide adenine dinucleotide phosphate
NMDA Receptor	N-methyl-D-aspartate Receptor
PARK7	Parkinsonism associated Deglycase
PARP1	Poly Adenosine Diphosphate-Ribose polymerase
PD	Parkinson's Disease
PINK1	PTEN-induced kinase 1
PPMS	Primary progressive multiple sclerosis
PRKN	Parkin RBR E3 Ubiquitin Protein
PRMS	Progressive relapsing multiple sclerosis

RBANS	Repeatable battery for the assessment of Neuropsychological Status
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
RRMS	relapsing remitting multiple sclerosis
SNCA	Synuclein Alpha
SNpc	Substantia Nigra pars Compacta
SPMS	secondary progressive multiple sclerosis
TNF-α	Tumour Necrosis Factor-alpha
UMN	upper motor neuron
UPDRS	Unified Parkinson's Disease Rating Scale
WHO	World Health Organisation

27.1 Introduction

Minocycline is a semisynthetic, broad-spectrum, second-generation tetracycline antibiotic (Li et al. 2013). Tetracyclines are divided into two classes: first-generation antibiotics which include oxytetracycline, chlortetracycline and tetracycline, and second-generation antibiotics like minocycline, doxycycline and lymecycline (Garner et al. 2012). Minocycline is bacteriostatic in nature, i.e. stops growth of bacteria, and keeps them in the stationary phase of growth and bacterial cells fail to replicate.

Minocycline is effective against *Rickettsiae*, *Mycoplasma pneumoniae*, *Chlamydia trachomatis*, *Chlamydophila psittaci*, Chlamydia *trachomatis*, Ureaplasma *urealyticum*, *Borrelia recurrentis*, Campylobacter *fetus*, *Escherichia coli*, *Enterobacter aerogenes*, Shigella species, Acinetobacter species, *Haemophilus influenzae*, *Kelbsiella* species, etc. Chemically minocycline is 7-dimethyl-amino-6-demethyl-6-deoxytetracycline hydrochloride (Macdonald et al. 1973). For the molecular formula, molecular weight and structure of minocycline, refer to Fig. 27.1 below.

Minocycline shows its anti-bacterial action by inhibiting protein synthesis. In addition to anti-microbial activity, minocycline demonstrates neuroprotective actions in amyotrophic lateral sclerosis (ALS), ischaemia, multiple sclerosis,

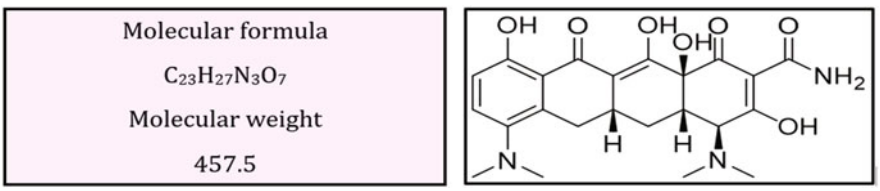


Fig. 27.1 Minocycline: molecular formula, molecular weight and structure (structure of minocycline created using ChemDraw)

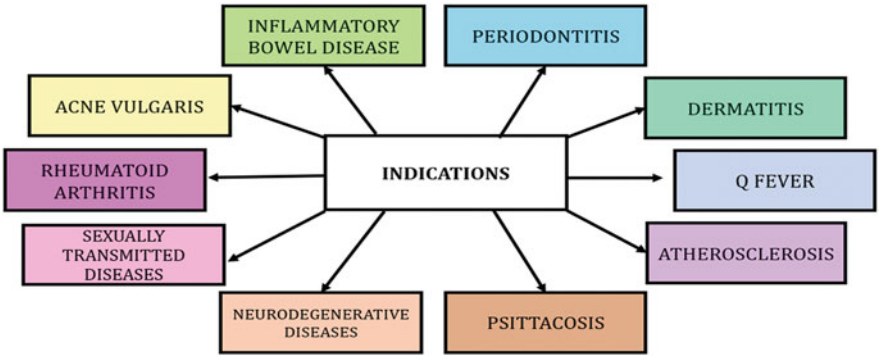


Fig. 27.2 Indications of minocycline

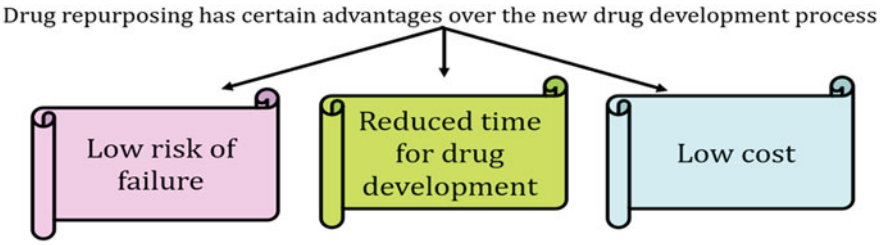


Fig. 27.3 Advantages of drug repurposing

Huntington's, Alzheimer's and Parkinson's disease (Bantubungi et al. 2005; Lampl et al. 2007; Cudkowicz 2010) (Fig. 27.2).

We will be focusing on the repurposing of minocycline, a tetracycline antibiotic in neurodegenerative disorders.

Drug repurposing is additionally called drug repositioning, re-profiling, re-tasking, drug rescuing, drug recycling, drug redirection and therapeutic switching, and is a concept which involves identifying uses for drugs (approved and investigational) that are other than their original medication indications (Pushpakom et al. 2018) (Fig. 27.3).

Neurodegenerative diseases, which are chronic and progressive, show selective and symmetric neuronal loss in motor, sensory or cognitive systems in the brain and spinal cord (Spivey et al. 1958; Li et al. 2013). Demyelination, loss of dendrites and neuronal death are the physiological signs of neurodegenerative conditions (Rekatsina et al. 2020).

Minocycline has small size (495 KDa), high lipophilicity and lipid solubility due to which it can cross the blood–brain barrier and enter the central nervous system where it targets various molecular pathways common to various neurodegenerative disorders (Noble et al. 2009b) and exerts anti-inflammatory, anti-apoptotic, proteolysis inhibitory actions, glutamate-antagonist properties, inhibiting microglial activation, proliferation and neuronal protection (Kim and Patel 2014; Shamsi et al. 2020).

27.2 Minocycline, a Tetracycline Antibiotic

27.2.1 Structure

Minocycline a tetracycline derivative depicts anti-bacterial property (Allen 1976). Tetracycline is a four-ring structure, chemically named as tetracyclic naphthacene carboxamide ring system (Fig. 27.4). The four rings are responsible for its antibiotic activity (Minocycline n.d.).

Tetracyclines have 2 zones—peripheral and upper zone. Modifications in the lower zone led to a decrease in the antibiotic and non-antibiotic properties of the drug. On the other hand, modifications in peripheral zone enhance the activity of biological target. In the tetracycline ring, OH, =O and OH at position 10, 11 and 12 form chelate with divalent and trivalent metal ions like Ca^{2+}, Mg^{2+}, Zn^{2+}, Fe^{2+} and Fe^{3+} and inhibit the calcium influx through NMDA responsive glutamate receptors. This chelation illustrates the neuroprotective action of minocycline against excitotoxity (Shultz and Zhong 2017).

Dimethylamino group attached at position 7 of tetracycline increases the antibacterial activity of the drug (Minocycline Hydrochloride Dihydrate n.d.). Phenol group is responsible for antioxidative property by removing hydrogen atom from phenolic group and leading to free radical production. But the free radicals produced during this process are less reactive due to stearic hindrance and resonance stabilization.

Carboxyamide functional group attached with aromatic ring inhibits PARP-1 [POLY(ADPribose) polymerase-1] (Alano et al. 2006) which is involved in DNA repairing. Inhibition of PARP-1 is also one of the important therapeutic targets responsible for neuroprotective action. Epimerization decreases the antibacterial

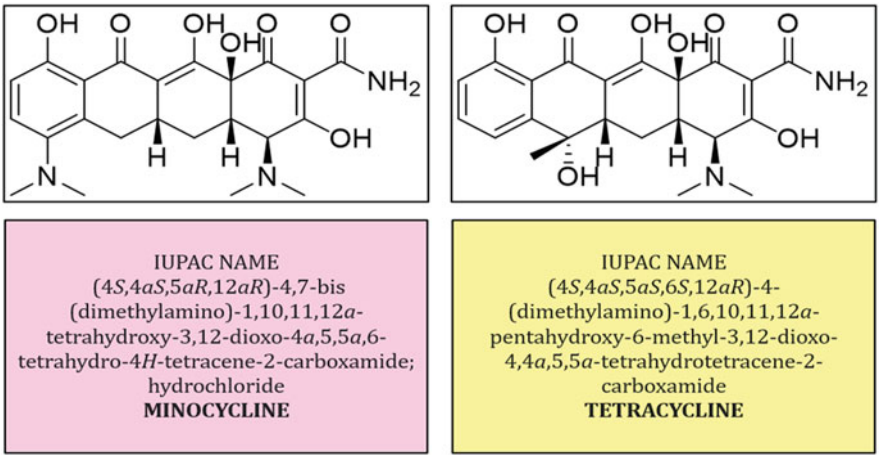


Fig. 27.4 Structure and IUPAC nomenclature of minocycline and tetracycline respectively (structures of minocycline and tetracycline created using ChemDraw)

activity against Gram-negative bacteria. Any modification at R1, R2 and R3 position lead to antifungal activity not antibacterial activity (Fuoco 2012).

27.2.2 Physicochemical Properties

Every drug molecule exhibits physicochemical properties depending on the nature of the drug, pKa value, solubility, molecular weight, molecular bonding, etc. The physicochemical properties help to determine the formulation system and the delivery route of the drug. The physicochemical properties of minocycline are given in Table 27.1.

27.2.3 ADME profile

27.2.3.1 Absorption

There exists a linear relation between minocycline absorption and the dose administered which can be seen from Table 27.1, and the absorption is considered to be complete post-oral administration (Saivin and Houin 1988; Gonzalez and Henwood 1989). Minocycline can be administered through oral and intravenous route (Saivin and Houin 1988). The absorption profile of minocycline can be seen in Table 27.2.

When understanding the absorption characteristics of minocycline, we consider C_{max}, T_{max} and AUC (Fig. 27.5).

Minocycline is well absorbed orally; however, the absorption is impaired in presence of an antacid or cations (divalent and trivalent). Tetracyclines form chelates (Chukwudi 2016) with divalent and trivalent cations which can stick to the teeth (leads to permanent staining) and in bones of growing children. Therefore, tetracyclines are not to be taken with milk and iron supplements due to chelate formation and are not given in pregnancy because they can stop foetal growth.

27.2.3.2 Distribution

Minocycline has a small size and high lipophilicity due to which it shows excellent distribution and has high bioavailability and can permeate across the blood–brain barrier (Li et al. 2013). High concentrations of minocycline are seen in liver, kidneys and the GIT, and minocycline shows approximately 70–80% plasma protein binding (Saivin and Houin 1988); it has been observed that minocycline has the maximum activity against bacterial infections among the other tetracyclines (Macdonald et al. 1973).

Table 27.1 Physicochemical properties of minocycline

Physicochemical properties	Property value	Description
1. Solubility (Li et al. 2021) (A) Methanol (B) Ethanol (C) Acetonitrile (D) Ethyl acetate (E) Water (Minocycline Hydrochloride n.d.)	$2.88X10^{-3}$ $1.90X10^{-3}$ $7.78X10^{-5}$ $2.63X10^{-5}$ 3.07 mg/ml Mole fraction	The solubility of alcohol is more as compared to ethyl acetate and other solvents because of alcohol can make hydrogen bond with minocycline (1)
2. $XlogP_3$ (Minocycline Hydrochloride Dihydrate n.d.) LogP LogS (Minocycline Hydrochloride n.d.)	−0.6 −0.03 −2.2	
3. Hydrogen bond donor count	5	
4. Hydrogen bond accepter count	9	
5. Molecular weight	457.18	
6. Colour	Bright yellow orange	
7. Crystalline nature (Hazardous Substances Data Bank (HSDB) n.d.)	Amorphous solid	
8. Optical rotation	Specific rotation = 166°	
9. Maximum absorption	352 and 263 nm 380 and 243 nm	0.1 N HCl 0.1 N NaOH
10. Odour	Odourless	
11. Nature	Hygroscopic	
12. p*K*a	2.8 7.8	Acidic Basic
13. Stability	Stable in air Sensitive to light	Cause darkened product
14. Boiling point	753.2	
15. Refractivity	122.54 A^0	

Table 27.2 Absorption profile of minocycline

Route of administration	Dose (mg)	C_{max} (mg/L)	T_{max} (h)	AUC (mg × h/L)
Oral	100	1.6	1.9	31.6
Oral	200	3.1	2.5	48.3

27.2.3.3 Metabolism

Minocycline biotransformation occurs in the liver and three inactive metabolites are produced. The principle metabolite is 9-hydroxy minocycline, and the other two metabolites are produced due to removal of methyl groups at position 9 and 4 of minocycline.

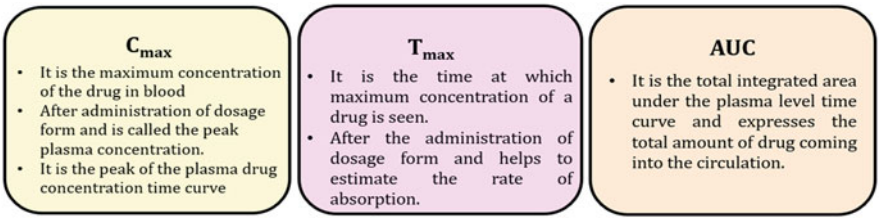


Fig. 27.5 C_{max}, T_{max} and AUC

27.2.3.4 Excretion

Only about 10% is excreted in unchanged form in urine (Welling et al. 1975). About 20–35% of minocycline is removed through the faecal route and the rest of minocycline is excreted in the form of metabolites through urine or faeces (Saivin and Houin 1988).

27.2.3.5 Half-Life and Clearance

Biological half-life of minocycline varies between 11 and 26 h with normal renal and hepatic functioning (Martins et al. 2021). Total clearance of minocycline is about 33.6–5.7 L/h (Saivin and Houin 1988).

27.2.3.6 Adverse Effects and Toxicity

Minocycline can lead to vestibular toxicity and produces ataxia, vertigo and nystagmus which revert to normal conditions on discontinuation of drug.

27.2.4 Mechanism of Action

27.2.4.1 Anti-microbial Action

Minocycline is a tetracycline antibiotic and after uptake into the cell (Fig. 27.6) acts by inhibiting protein synthesis reversibly by binding to the 30S bacterial ribosomal subunit. Tetracyclines are broad spectrum anti biotics (Li et al. 2013) and to penetrate the bacterial cell, they follow different paths in Gram-positive and Gram-negative bacteria.

To understand the mechanism by which minocycline exerts its anti-bacterial action, we must first understand the translation process (Fig. 27.7). Protein synthesis also known as translation is a ubiquitous process seen in almost all biological systems (Zhao and Krishnan 2014) and involves the 'decoding' of a messenger

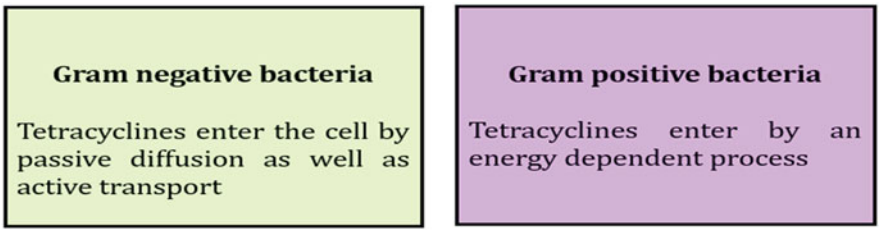


Fig. 27.6 Mode of uptake of tetracyclines

RNA (mRNA) (Ramakrishnan 2002) to build a **polypeptide** from polymerization of amino acids. The process of translation occurs in three steps initiation, elongation and termination, and involves the mRNA (genetic template), the ribosome (assembly machinery) and the aminoacyl transfer RNAs (aa-tRNAs) (Zhao and Krishnan 2014).

Ribosomes are cell organelles which are consisting of two subunits, one large and one small, which are present separately in the cytosol and combine only when protein synthesis has to take place (Fig. 27.8). The eukaryotes have 60s and 40S (together form 80S) subunits, whereas in prokaryotes (bacteria), they are 30S and 50S (together forms 70S) (Ramakrishnan 2002; Opron and Burton 2019) which forms basis of selective toxicity.

Ribosomes have three sites for tRNA binding. The A site (acceptor site) (Opron and Burton 2019) accepts the incoming aminoacyl-tRNA, which holds the new amino acid which will be added to the polypeptide chain, the P site (peptidyl site), which holds the tRNA with the nascent peptide chain (Zhao and Krishnan 2014), i.e. binds to the tRNA holding the growing polypeptide chain of amino acids and the E site (exit site), which holds the deacylated tRNA before it leaves the ribosome and serves final transitory step where a tRNA goes after it has transferred its polypeptide to another tRNA (which now occupies the P site) (Ramakrishnan 2002). The process of translation consists of three steps (Fig. 27.9).

Before the initiation step starts, charging of tRNA molecule takes place, i.e. before an amino acid is added to the growing polypeptide chain, it should get attached to a molecule called tRNA, in a process known as tRNA charging or aminoacylation. Amino acid is activated by its attachment to its corresponding tRNA. This is catalysed by enzymes called aminoacyl-tRNA synthetases. The charged tRNA participates in the chemistry of peptide bond formation in protein synthesis (Raina et al. 2014).

27.2.4.1.1 Translation

Step 1: Initiation

Ribosome, mRNA and initiator tRNA (Czworkowski and Moore 1996) together form the initiation complex. The 30S subunit of the bacterial ribosome attaches

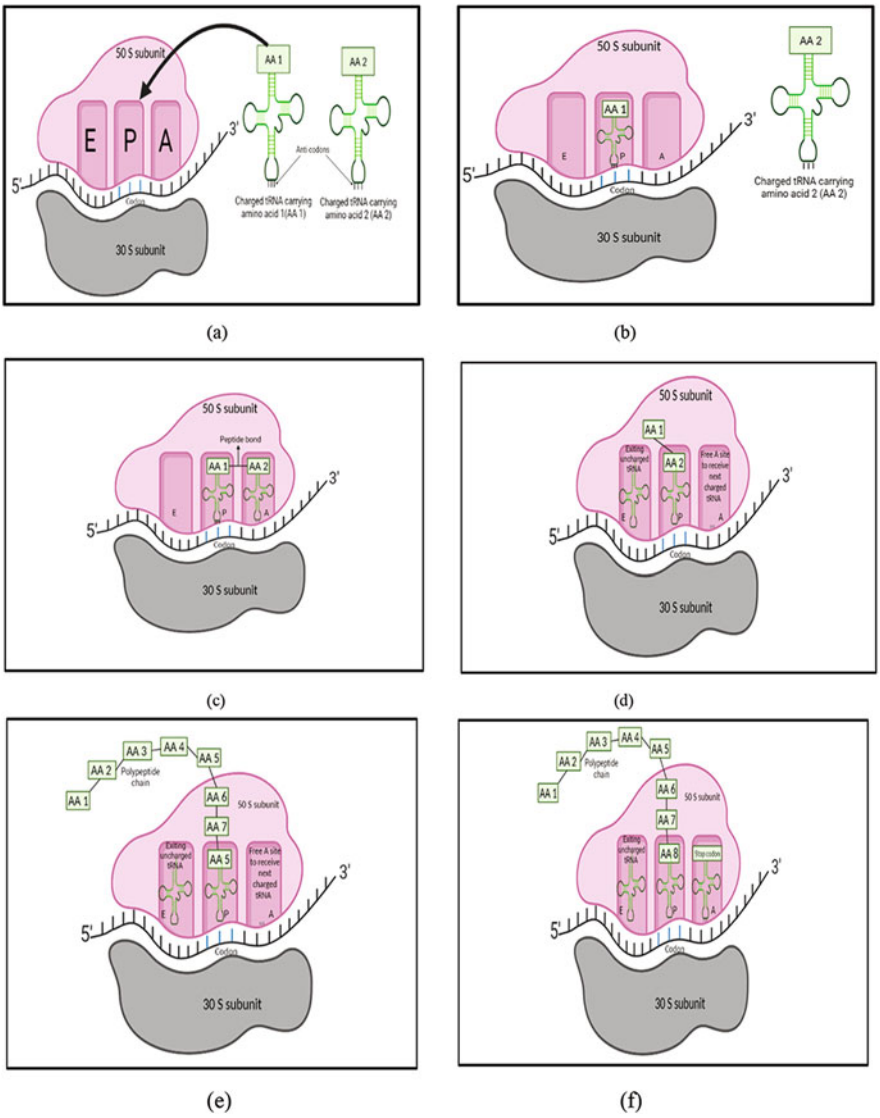


Fig. 27.7 Diagrammatic representation of translation. (**a**) Initiation of protein synthesis; (**b**) tRNA carrying AA 1 enters P site; (**c**) tRNA with AA 2 enters A site and peptide bond formation occurs between AA 1 and AA 2; (**d**) translocation: tRNA at A site along with dipeptide pulled towards P site; (**e**) elongation of polypeptide chain; (**f**) termination of translation. (Created using BioRender)

towards the 5' end of mRNA. Ribosome recognizes the initiator codon on mRNA and the charged initiator tRNA (Laursen et al. 2005) having anticodon brings the first amino acid to the site of initiator codon. Codon-anticodon recognition (Dever

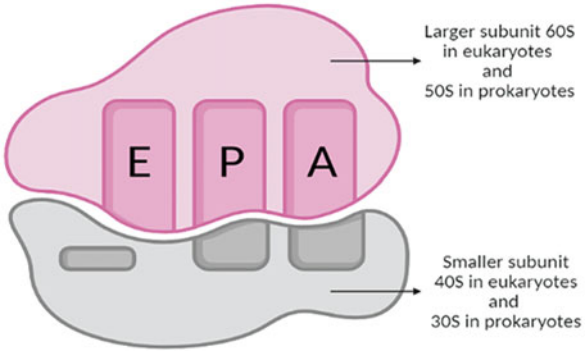


Fig. 27.8 Structure of ribosome (created using BioRender)

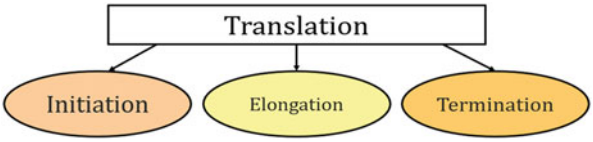


Fig. 27.9 Three steps of translation

et al. 2018) occurs to start polypeptide chain formation. Later bigger ribosomal subunit also attaches to the mRNA forming initiation complex.

Step 2: Elongation

Elongation of polypeptide chain occurs (Czworkowski and Moore 1996). Charged tRNA with first amino acid enters the ribosome at P site. Second amino acid enters A site. Peptide bond formation between the first amino acid located at P site and second amino acid at A site takes place. Peptidyl transferase (Czworkowski and Moore 1996) enzyme enables formation of peptide bonds between adjacent amino acids. After this, the first tRNA becomes free or uncharged and leaves the ribosome (Zhao and Krishnan 2014) and the A site is occupied by second tRNA and two amino acids linked by peptide bond.

Ribosome and mRNA now move until the second tRNA shifts from A site to P site and the A site is now ready to receive third charged tRNA with third amino acid. Now P site has second tRNA with 2 amino acids linked through a peptide bond and A site has third tRNA with third amino acid. Next step is formation of peptide bond between second and third amino acids. Again, ribosome and mRNA move till third tRNA with three amino acid occupies P site and second tRNA gets uncharged (Czworkowski and Moore 1996) and is free to take its task again. Now A site is ready to receive fourth charged tRNA. This process continues to further elongate the polypeptide chain.

Step 3: Termination

Polypeptide chain (Fig. 27.10) formation comes to an end by process of termination which occurs when stop codons in the mRNA enters the A site. Stop codons are recognized by proteins called release factors, which fit into the P site. The stop codons are UAA, UAG and UGA (Brown et al. 1990; Chavatte

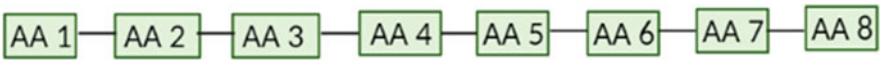


Fig. 27.10 Polypeptide chain (created using BioRender)

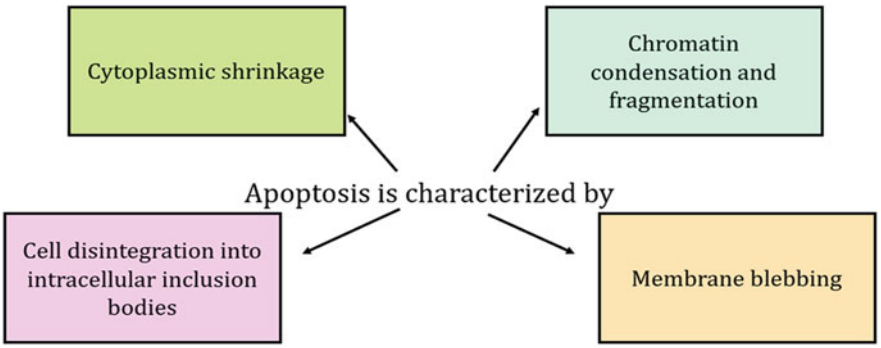


Fig. 27.11 Characteristics of apoptosis

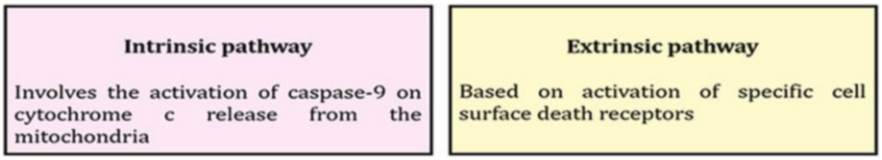


Fig. 27.12 Apoptotic pathways

et al. 2003). Tetracyclines bind to 30S ribosome and inhibit the aminoacyl tRNA attachment to A site; therefore, further steps of translation will not take place and bacterial growth ceases.

27.2.4.2 Anti-apoptotic Actions

Apoptosis (Fig. 27.11) refers to programmed cell death (Elewa et al. 2006) and the activation of apoptotic pathways plays a role in various neurodegenerative diseases like Alzheimer's disease, Parkinson's and Huntington's disease (Noble et al. 2009b).

Minocycline shows its anti-apoptotic action by inhibiting extrinsic (death receptor) and intrinsic pathway (mitochondrial) of apoptosis (Fig. 27.12) (Stirling et al. 2005; Inoue et al. 2009; Noble et al. 2009b).

Cytochrome c released from mitochondrial membrane (Elewa et al. 2006; Noble et al. 2009b) activates caspases, which are aspartate-specific cysteine proteases and promote neuronal cell death (Inoue et al. 2009). Minocycline stabilizes the mitochondrial membrane (Noble et al. 2009b) which inhibits the release of cytochrome c

and in turn caspases are not activated, thereby exerting its anti-apoptotic action. Neural apoptosis can be prevented by neuronal growth factors. These growth factors regulate the expression of the two gene products Bax and Bcl-2, Bax being proapoptotic and Bcl-2 being antiapoptotic. Minocycline also exerts its anti-apoptotic action by promoting the release of anti-apoptotic protein bcl-2 (Elewa et al. 2006).

27.2.4.3 Anti-inflammatory Action

Minocycline has been suggested to exert its anti-inflammatory effects (Fig. 27.13) by modulating microglia, immune cell activation and subsequent release of cytokines, chemokines, lipid mediators of inflammation, matrix metalloproteases (MMPs) and nitric oxide release (Stirling et al. 2005). Tetracyclines (Minocycline) show anti-inflammatory action in brain injury (acute and chronic) (Elewa et al. 2006) and various neurodegenerative disorders because they cross blood–brain barrier (Cheng et al. 2015).

Minocycline inhibits enzymes which play a role in inflammatory mechanisms like nitric oxide synthase which decreases the nitric oxide levels from monocytes (Elewa et al. 2006; Noble et al. 2009b).

It has also been seen that minocycline inhibits the release and formation of pro-inflammatory cytokines (tumour necrosis factor, interleukin 1, interleukin 6, etc.) (Dunston et al. 2011). Tetracyclines also inhibit lipopolysaccharide mediated activation of macrophages and microglial cells (Dunston et al. 2011).

Minocycline also suppresses free oxygen radical formation and neutrophilic migration and degranulation which helps to manage neutrophil mediated tissue injury (Elewa et al. 2006).

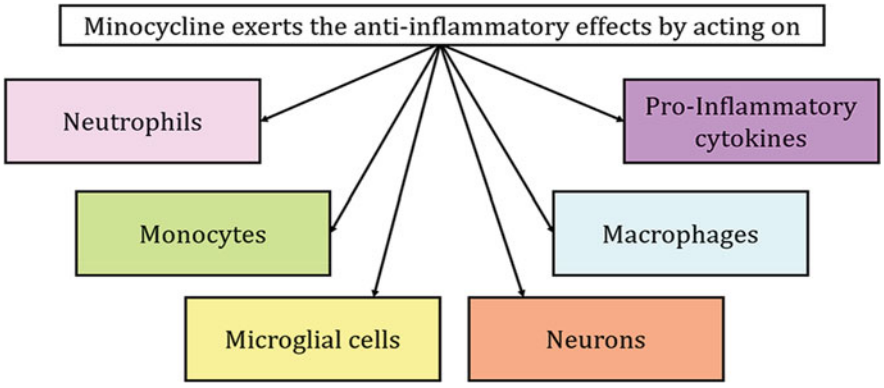


Fig. 27.13 Minocycline and its anti-inflammatory effect

27.2.4.4 Inhibition of Matrix Metalloproteinases

Matrix metalloproteinases are enzymes involved in degradation of the extracellular matrix (Matrisian 2000). These enzymes help in passage of leukocytes across the matrix barriers, and leukocytes are involved in diseases like multiple sclerosis (Brundula et al. 2002). Minocycline has been seen to inhibit MMP 9 and MMP 12 (Elewa et al. 2006).

27.2.4.5 Effect of Minocycline on Protein Misfolding

Protein misfolding is seen in many neurodegenerative diseases (Noble et al. 2009b). Misfolding means the adoption of abnormal conformations, by certain proteins, such that they tend to form large insoluble aggregates. Proper folding of proteins is needed to convert amino acids into functional proteins. If misfolding occurs, the proteins formed are non-functional, and aggregates will be formed which led to neuronal cell death. Alzheimer's disease, Huntington's disease, Parkinson's disease, Amyotrophic lateral sclerosis, etc. involve the misfolding of proteins and can be seen in Fig. 27.14 given below.

Minocycline acts on amyloid proteins by polar interactions and hydrogen bond formation. Minocycline acts as an anti-aggregatory agent and has also shown a decrease in huntingtin aggregation and also effective in reducing the development of abnormal tau species (Noble et al. 2009b). The different mechanisms of action of minocycline are summarized in Fig. 27.15.

27.2.5 Why Can Minocycline Be Repurposed in Neurodegenerative Diseases?

Minocycline has been in the market since last 30 years as a promising anti-microbial agent used in long-term treatment of chronic conditions like acne vulgaris and

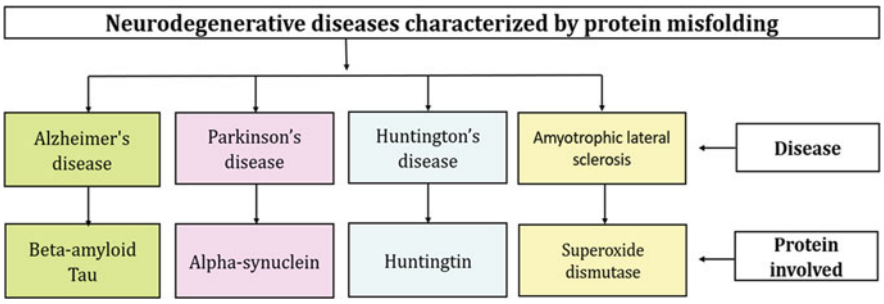


Fig. 27.14 Neurodegenerative diseases characterized by protein misfolding

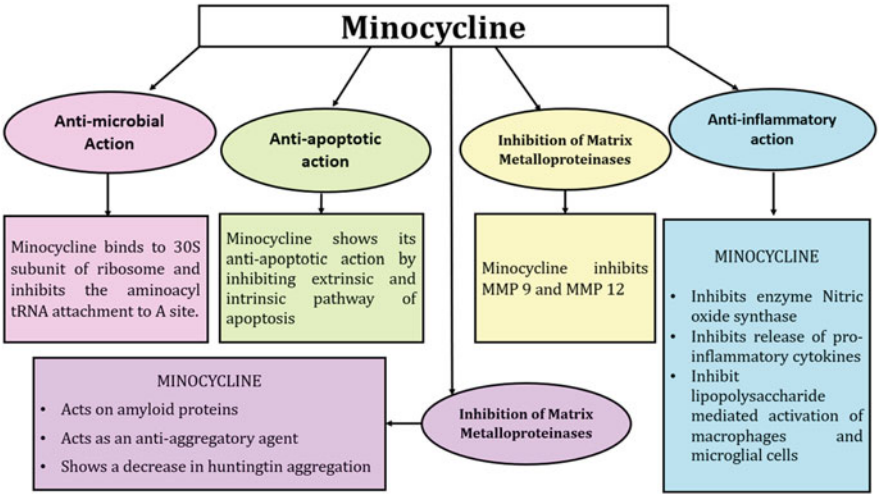


Fig. 27.15 Minocycline and its mechanisms of action

rosacea. The drug has rarely shown any autoimmune syndrome during the treatment. Minocycline has established the records of its safety in long-term use. It has edge over other tetracycline in terms of its small size (496 KDq) and high lipophilicity which is advantageous in conferring problems of bioavailability and blood–brain barrier permeability (Chisholm et al. 2011).

Other than antimicrobial activity, minocycline has also shown anti-inflammatory activity which marks the point of taking interest in repurposing it. The neuroprotective effect of minocycline in several models of acute and chronic neurodegenerative disease like multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), Huntington's disease (HD), Parkinson's disease (PD), epilepsy or haemorrhagic and ischaemic stroke, spinal cord injury and traumatic brain injury. The evidence from the pre-clinical studies is helping in favours of minocycline to use as potential neuroprotective agent based on its excellent combination with central nervous system (CNS) penetration and well-established clinical safety, making minocycline a viable candidate to use in therapy for neuroprotection (Chisholm et al. 2011).

Neurodegeneration comes with progressive loss of neurons from nervous system and neuroinflammation with abnormal protein assemblies. Due to high lipophilic nature of minocycline, it can readily cross the blood–brain barrier and exerts beneficial effects like anti-inflammatory and anti-apoptotic which finally leads to neuroprotection.

Minocycline has shown promising effects in proven by several studies like inhibition of neuroinflammation and neuron death in mouse models of AD with amyloidal plaques, ALS, HD, PD, Down's syndrome, and stroke. It also inhibits tau protein phosphorylation and prevents aggregation of insoluble tau, inhibit caspase

3 activation (Cheng et al. 2015). All these factors lead to great area of potential for minocycline.

27.3 Repurposing of Minocycline in Neurodegenerative Diseases (Pre-clinical & Clinical Evidence)

27.3.1 Alzheimer's & Other Related Dementias

Dementia is referred as an overall decline in memory characterized by progressive and deterioration of cognitive function. Patients with dementia have problems with cognition, behaviour and functional activities of everyday life (Dementia n.d.).

Alzheimer's disease (AD) is a neurodegenerative disorder that is mainly seen in geriatric population with dementia, behaviour change and cognitive impairment as major sign and symptoms. AD is also one of the common types of dementia (Schachter and Davis 2000). Beta amyloid protein is deposited along with neurofibrillary tangles in cerebral cortex and subcortical grey matter (Schachter and Davis 2000).

Five drugs are approved by FDA for improving cognitive functions:

- Cholinergic activators: Tacrine, rivastigmine, donepezil, galantamine
- Glutamate antagonist: Memantine

27.3.1.1 Pathology

(i) **Tau Protein Phosphorylation and Amyloid Beta Hypothesis** (Pressman and Rabinovici 2014)
In the cortical region of the brain, amyloid precursor protein (APP) is present in the cell membrane along with alpha, gamma and beta secretase enzymes. APP's main function is to grow and repair neurons. Alpha secretase enzyme divides the APP along with gamma secretase and form water-soluble peptide. But if beta secretase enzyme comes into play, it results in insoluble and sticky peptides called amyloid beta. This monomer of amyloid beta combines with more monomers in the synapse and form amyloid beta plaque (Fig. 27.16). These plaques come in between the neurons and disrupt the signalling as shown in Fig. 27.17.

Neurons are held together by cytoskeleton which is made up of microtubules along with tau proteins that prevents them from disintegrating and apoptosis. But due to the formation of amyloid beta plaque, it activates phosphokinase enzyme. This enzyme changes the shape of tau protein, and it stops supporting the microtubule and clumps up with other tau proteins or get neurofibrillary tangles. These tangles stop the functioning of microtubules and may lead to apoptosis of the neurons as shown in Fig. 27.18.

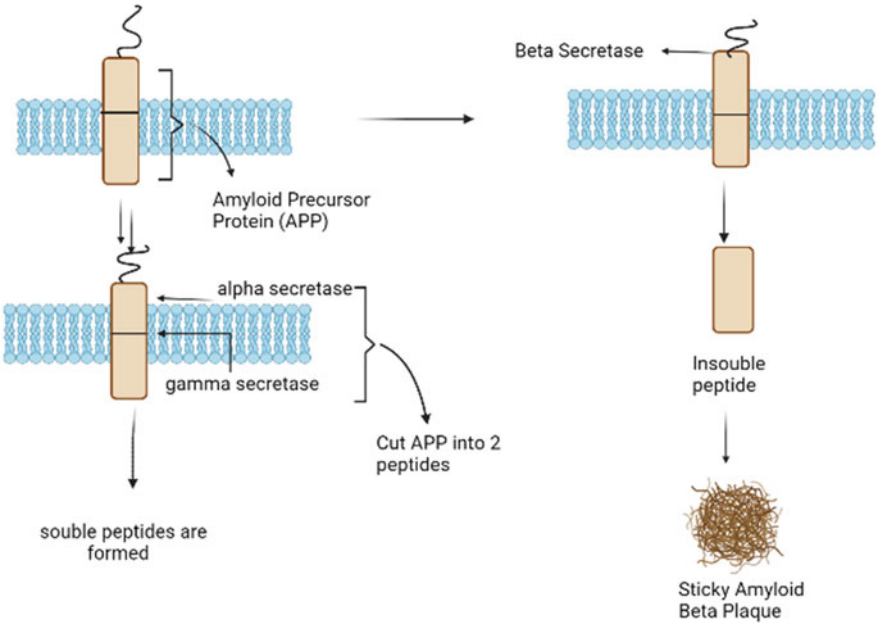
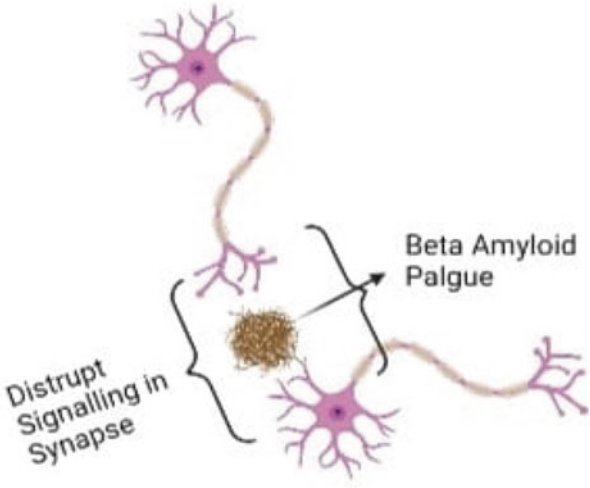


Fig. 27.16 Amyloid beta plaque formation (created using BioRender)

Fig. 27.17 Amyloid beta plaque (created using BioRender)



(ii) **Oxidative Stress Hypothesis** (Gella and Durany 2009)

In brain, many reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced that have two effects—(a) cellular signalling and (b) damage cellular structure that depend on the oxygen consumption of the

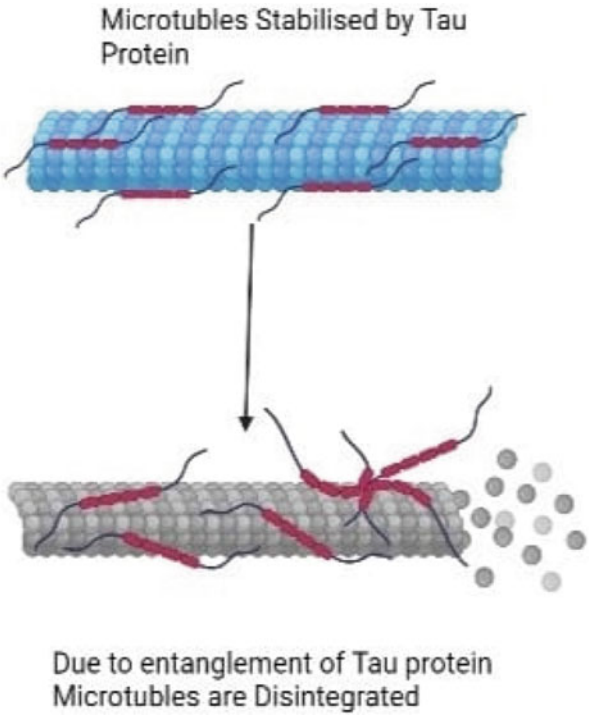


Fig. 27.18 Neurofibrillary tangles (created using BioRender)

brain. Neurons that are basic functional unit in brain will combine with ROS and lead to lipid peroxidation and apoptosis. If concentration of glutathione is also less, it can result in oxidative stress injury.

(iii) **Cholinergic Hypothesis**

Apo-lipo-protein E (APOE) (Kim et al. 2009) is important genotype that causes risk for Alzheimer disease specifically due to apoE4 gene. This gene decreases acetylcholine in the synaptic cleft, as less amount of Ach is available to bind in the receptor site.

Cholinergic receptor binding become less in the brain that results in impaired memory, learning and attention. So it is advised to give acetylcholinesterase inhibitors which will increase the availability of acetylcholine in the synapse. Four drugs are approved by FDA for this—*Tacrine, rivastigmine, donepezil, galantamine* (Hampel et al. 2018).

27.3.1.2 Pre-clinical Trails

Depending on the in-vivo and in-vitro studies, we determine that minocycline shows therapeutic efficacy against tau protein phosphorylation in mice. At primary stage, it was observed that minocycline prevents amyloid beta induced neuronal death, decreased capase-3 activation and lowered the production of caspase-3-cleaved tau

fragment. In case of in-vivo studies, we concluded that minocycline also reduces the caspase-3 activation and lowers the production of caspase-3-cleaved tau fragment. Through this experiment, it was finally concluded that minocycline is effective in doing confirmational changes in tau during the drug regimen, but the amount of tau protein is not directly related to the drug (Noble et al. 2009a).

In another experiment which was done on a mouse shows that minocycline also has anti-inflammatory activity. Htau mouse of AD was taken for the experiment and showed that minocycline reduces the abnormal tau species in htau mouse. Immunohistochemical analyses were done that showed that minocycline treatment leads to less activation of astrocytes in many cortical regions of htau mouse but shows no effect in Astro cytosis of hippocampus. Pro-inflammatory cytokines levels are also increased in htau mouse. These results showed that minocycline can target new cytokine to inhibit tau pathology in-vivo (Garwood et al. 2010).

To check the reduced anti-inflammatory parameters in brain structure and serum and reverse memory, impairment that is caused by administering amyloid beta in mice caused due to minocycline was noted. A male mouse was treated with oral minocycline drug for a period of 17 days after injecting amyloid beta oligomer via intracerebroventricular administration. It was observed in the mice that when minocycline was orally given, it reverses the increased levels of interleukin (IL-1β), tumour necrosis factor-alpha (TNF-α) and IL-10 caused by Aβ (1–42) in hippocampus, and in cortex, it increases the levels of IL-1β, TNF-α and IL-4. In serum, it also increases the levels of IL-1β and IL-4. Through these above-stated results, it is can be said that minocycline leads to improve spatial memory and cytokine levels in brain (Garcez et al. 2017).

27.3.1.3 Clinical Trials

Clinical trials on repurposing minocycline in AD patients were conducted at Huntington Medical Research Institutes Pasadena, California, United States. Thirteen participants with normal individuals, patients with mild cognitive impairment (MCI) or Alzheimer's disease (AD) were enrolled in phase 2 clinical trials. Clinical screening, neuropsychological tests, blood and urine analyses, quantitative magnetic resonance imaging (MRI) and proton (1H) and carbon 13 (13C) magnetic resonance spectroscopy (MRS) were performed to know the effect of minocycline in AD patients (Minocycline in Patients with Alzheimer's Disease n.d.).

Outcomes that were measured during the trails:

Repeatable battery for the assessment of neuropsychological status (RBANS). Hippocampal volumes measured in three groups: Alzheimer's disease (AD), mild cognitive impairment (MCI) and normal, age-matched controls (NC). Biomarker NAA/ml measured in three groups: Alzheimer's disease (AD), mild cognitive impairment (MCI) and normal, age-matched controls (NC).

27.3.2 Parkinson's Disease

Parkinson's disease (PD) is a neurodegenerative disorder in which dopaminergic neurons in the nigrostriatal pathway are degenerated and result in hypokinetic movements (Kouli et al. 2018). This type of disorder is more common in populations that are more than 50 years old. Major causes of PD are associated with other diseases such as co-poisoning, Mn in toxification, calcification of basal ganglia, brain tremors, strokes and viral diseases. Genes that cause mutation and result in PD are LRRK2, PARK7, PINK1, PRKN and SNCA gene (Dias et al. 2013).

Signs and symptoms include tremors, bradykinesia, i.e. slowness of movement, muscle rigidity, change in tone of speech, micrographia, uns posture and dementia.

One of the most frequent neurodegenerative diseases is Parkinson's disease. According to the Parkinson's Disease Foundation, the disease affects around 1 million people in the United States. In the United States, there are about 20 instances of Parkinson's disease per 100,000 persons per year (60,000 per year), with a typical onset age of around 60 years. In persons 60 years and older, the prevalence of Parkinson's disease is estimated to be around 1%, increasing to 1–3% in those aged 80 and up (DeMaagd and Philip 2015).

Pathogenesis

(i) In nigrostriatal pathway, a compound homology to dopamine called N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is preferentially transported by dopamine transporter (DAT) to presynaptic neuron.

MPTP in the presence of MOA-B in the synaptic cleft get converted into MPP^+ which replaces dopamine from synaptic cleft. MPP^+ is reuptaken by DAT in presynaptic neuron and is stored in the vesicles. MPP^+ inhibit ETC in mitochondria (mainly affect complex II) that result in decreased ATP production and degeneration of neurons as seen in Fig. 27.19. This all cascade is responsible for PD in elderly (Langston 2017).

(ii) **Reactive Oxygen Species (ROS)** (Dias et al. 2013)

Oxidative stress plays an important role in degeneration of dopaminergic neurons as our brain consumes 20% of oxygen supply that converts oxygen into ROS. ROS produced during this process leads to metabolism of dopamine in outer mitochondrial membrane with the help of MOA-A and MAO-B. Dopamine quinones that are generated after metabolism of dopamine are neurodegenerative in nature. ROS also leads to low concentration of glutathione and high levels of iron and calcium in substantia nigra pars compacta. A significant increase in cysteinyl adducts of dopamine in PD substantia nigra suggests accelerated oxidation according to post-mortem brain analysis.

(iii) **Molecular Theory**

Alpha-synuclein is a presynaptic neuronal protein that has shown genetical and neuropathological roles in PD (Stefanis 2012). This protein is transcribed by a gene named SNCA that is predominantly present in the brain. This gene is responsible for the modulation of synaptic vesicle transport and release of

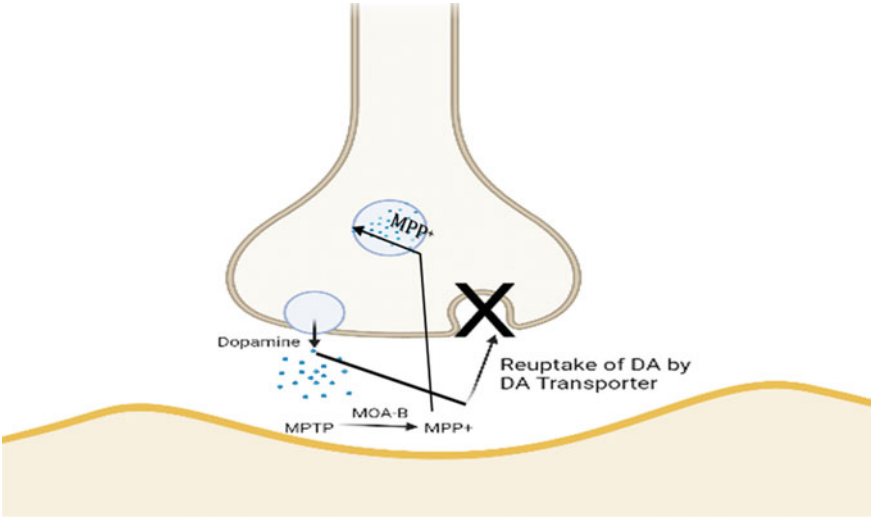


Fig. 27.19 Reuptake inhibition of dopamine via $MPTP^+$ (created by BioRender)

neurotransmitters. If any mutation is seen in the SNCA gene, then alpha synuclein will be converted into an amyloid form (misfolding of SNCA) gene, which will disrupt the vesicle storage of DA (Srinivasan et al. 2021). These beta sheets get polymerized into Lewis bodies that form aggregates in the neuron and disrupt the DA vesicles. Lewis bodies are marked as a hallmark in pathological studies of PD and are basically intraneuronal inclusions that include immunoreactive alpha synuclein protein aggregates (Wakabayashi et al. 2007). The disruption of vesicles leads to oxidative stress in the brain and results in the degeneration of dopaminergic neurons. Oxidative stress is produced due to the breakdown of peroxidase and catalase enzymes that results in the production of ROS. ROS produced will take electrons from the biomolecule and cause oxidative stress (Klein and Westenberger 2012).

27.3.2.1 Pre-clinical Studies

Many animal models have been used to see the neuroprotective action of minocycline in parkinsonism.

One of the primary causes of Parkinson's disease is the degeneration of dopamine in the nigrostriatal dopaminergic pathway. In an experiment, 8-week-old mate mice were taken to have iNOS deficiency. In this study, four intraperitoneal injections of MPTP-HCl saline in a 2-hour interval were given for MPTP intoxication in mice. It was observed that minocycline not only increased the number of surviving SNpc TH-positive neurons but also led to the formation of interleukin-1β. The activation of NADPH-oxidase and iNOS was also seen. From these above observations, we

concluded that iNOS is not the only culprit that is responsible for MPTP toxicity. Through this study, we came to know that microglial-related anti-inflammatory events play an important role in the MPTP neurotoxin effect, and minocycline shows neuroprotective activity (Wu et al. 2002).

In another experiment, in-vivo studies of mesencephalic and cerebellar granule neurons (CGN) were done that showed minocycline inhibits MPP^{+-}iNOS expression and NO-induced neurotoxicity, but MPP^{+-}induced toxicity will only be inhibited in the case of glia. Minocycline also inhibits NO-induced phosphorylation of p38 mitogen-activated protein kinase (MAPK) in CGN, and the p38 MAPK inhibitor, SB203580, blocks NO toxicity in CGN (Du et al. 2001).

27.3.2.2 Clinical Trails

A randomized, double-blind clinical trial was held by prioritizing creatine and minocycline in phase 2 clinical trials for Parkinson's disease in March 2003. About 195 ([CSL STYLE ERROR: reference with no printed form.]) people were taken for the trails and randomized to 3 groups. The participants that were enrolled in clinical trials were diagnosed PD within 5 years, but don't require medicine for the management of system. The primary outcome was the change in total Unified Parkinson's Disease Rating Scale (UPDRS) score from baseline to the time when there was enough disability to warrant symptomatic PD therapy or 12 months, whichever came first. The subjects were randomly assigned to one of three treatments: creatine 10 g/day, minocycline 200 mg/day or a matching placebo. Based on the placebo/tocopherol arm of the Deprenyl and Tocopherol Antioxidative Therapy Of Parkinsonism (DATATOP) trial, the futility threshold was set at a 30% reduction in UPDRS progression. p values less than or equal to 0.1 indicate futility (Ravina et al. 2006).

The result was that creatine and minocycline both can be considered to go for phase 3 trails where safety, tolerability, activity and cost are examined.

27.3.3 Huntington's Disease

Huntington's disease (HD) (Fig. 27.20) is an autosomal (Kim and Suh 2009) genetic neurodegenerative disease (Bantubungi et al. 2005) which occurs due to mutations in the gene coding for huntingtin protein (Cleren et al. 2010). Apoptosis, excitotoxicity, metabolic impairment and oxidative stress also play a role in the aetiology of HD (Kim and Suh 2009; Romero-Miguel et al. 2021). Neuronal degeneration is seen in spiny neurons of striatum (Romero-Miguel et al. 2021), cerebral cortex (Bantubungi et al. 2005) and in GABAergic neurons in caudate nucleus and putamen (Kim and Suh 2009).

The causative gene is located on chromosome 4 and codes an abnormal CAG triplet repeat expansion resulting in mutant huntingtin (Kim and Suh 2009) which

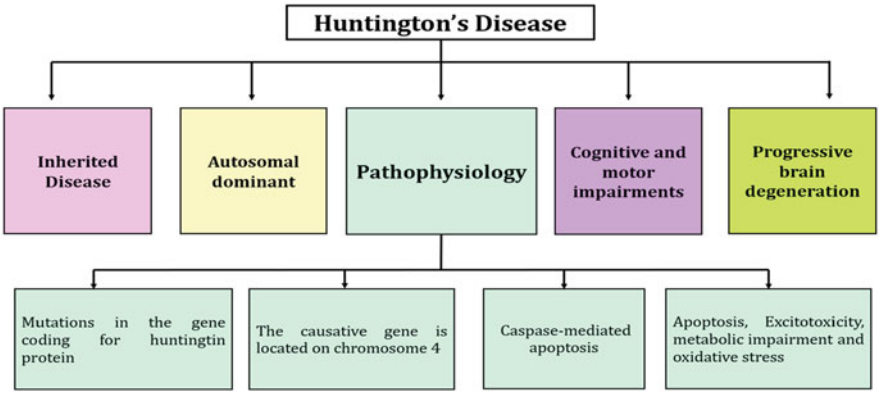


Fig. 27.20 Huntington's disease

aggregates, impairs proteasomal degradation and leads to caspase-mediated apoptosis (Thomas et al. 2004). This disease is characterized by involuntary motor changes. The movements start in the distal extremities such as fingers and toes, but also in small facial muscles. Other manifestations of this disease include unstable walking, extension of back muscles, difficulty in talking or swallowing, dysarthria and dysphagia. Hypokinesia, akinesia and rigidity are also observed (Roos 2010).

27.3.3.1 Pre-clinical Evidence

It was observed that minocycline inhibited the upregulation of caspase 1 and 3 in transgenic HD R6/2 mouse models in the year 2000 (Kim and Suh 2009), and it was observed that caspase 1 and caspase 3 inhibition were essential for neuroprotective action (Thomas et al. 2004). Minocycline acts as a caspase inhibitor and delayed HD progression and extended survival by 14% in the HD mouse models (Bonelli et al. 2003). It was also seen that the expression of iNOS was elevated in HD models of R6/2 mouse brains, and treatment with minocycline reduced iNOS activity by 14% (Kim and Suh 2009). To observe the anti-apoptotic effect of minocycline, two groups of HD phenotypic rat models were taken: the caspase-dependent and the calpain-dependent HD models. Minocycline exhibited neuroprotective action only in caspase-dependent models (Romero-Miguel et al. 2021). It was also observed that minocycline inhibits huntingtin aggregation at hippocampal slice culture model of R6/2 mouse HD model (Kim and Suh 2009).

The above observations were contraindicated by some studies where no improvements were seen after minocycline treatment in genetic HD mice models (Romero-Miguel et al. 2021).

27.3.3.2 Clinical Evidence

Depending on the results of in-vivo and in-vitro studies, minocycline was exposed to an open-label study where it was found that 10 out of 14 HD patients received minocycline showed an improvement in motor UHDRS (Unified Huntington's disease Rating Scale) (Kim and Suh 2009). This study was contradicted by Thomas et al. who reported that no significant change was observed in UHDRS score when patients were administered minocycline over a time span of 6 months (Bonelli et al. 2003; Kim and Suh 2009). The first clinical view was seen by an isolated study in 2002, which showed that after 1 year of treatment, remission of symptoms was observed (Romero-Miguel et al. 2021).

Very less number of clinical trials have been performed for the use of minocycline in HD, which contraindicated the beneficial and future effects of minocycline treatment; therefore, it is difficult to draw solid conclusions (Romero-Miguel et al. 2021).

27.3.4 Amyotrophic Lateral Sclerosis (ALS)

ALS disease is related to the death of upper motor neuron in the motor cortex of the brain and death of corresponding lower motor neurons in the brain stem and spinal cord.

ALS is involved with the damage of upper motor neurons as well as lower motor neurons which mainly consists of efferent system with two type of neuron system—proximal upper motor neuron (UMN) AND distal motor neuron (LMN). Motor neurons damage in a distal proximal pattern, i.e. the distal musculature became weaker and paralyzed before proximal musculature. The loss is asymmetrical in nature (Grad et al. 2017).

27.3.4.1 Pathophysiology

(i) The biochemical mechanism of ALS involved the formation of structures called inclusion bodies (abnormal protein aggregates of TDP-43, SOD1 or FUS) in the cytoplasm of motor neurons due to genetic mutations shown in Fig. 27.21. These abnormal proteins unable to fold correctly leads to altering the 3-D shape. These misfolded protein sticks to one another and forms larger aggregates called as inclusion bodies. These inclusion bodies are called as prions when they move to infect other cell (van den Bos et al. 2019).
These inclusion bodies damage motor cells following different mechanisms:

(ii) **Glutamate-mediated excitotoxicity**—this mechanism involves release of neurotransmitter glutamate from pre-synaptic neuron and binds to receptors on post-synaptic neurons. This glutamate is taken up by the astrocyte with the help

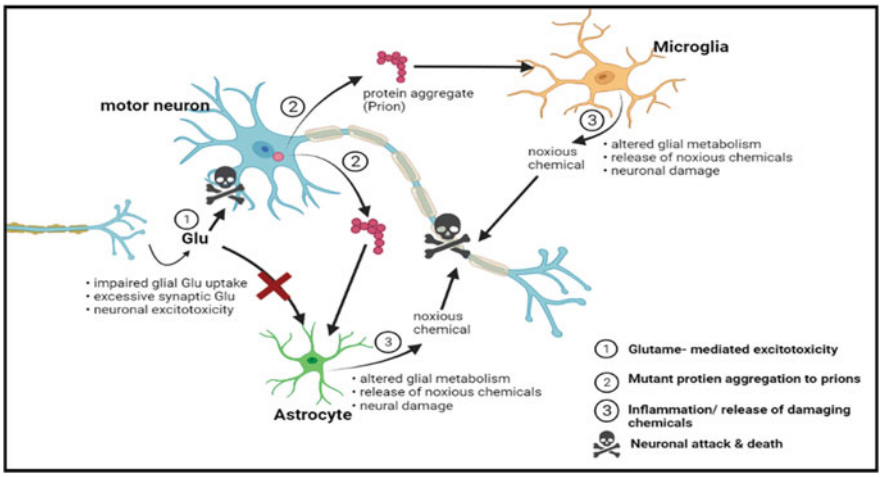


Fig. 27.21 Pathophysiology of amyotrophic lateral sclerosis (ALS) (created using BioRender)

of some protein. The mutations introduced in the protein transporter will hinder the uptake of glutamate into the astrocyte, leading to abnormally high levels of glutamate in the synapse. Due to increase in glutamate in synapse, more glutamate will bind to post-synaptic receptor which opens the calcium channels. The calcium influx inside the cytoplasm of motor neuron increases leading to excitotoxicity and death (Shaw et al. 1997).

(iii) **Mutant protein aggregation to prions**—the mutant gene encodes for mutant protein like SOD1 or FUS. These mutant proteins misfold and tend to stick with other protein ligand to become large protein aggregate called prion. When prion is inside the motor neuron, it causes the cell to enter into oxidative stress leading to death. The contents of dead motor neuron will leak out including prion and infect other motor neuron (Blokhuis et al. 2013).

(iv) **Prions** also cause activation of microglia and astrocyte which eventually start releasing noxious chemicals which will initiate an inflammatory response. It will further attack the axon of motor neuron leading to neural death (Rostalski et al. 2019).

(v) Laboratory studies have indicated the presence of inducible nitric oxide synthase iNOS and activation of caspase enzyme leading to motor neuron death in ALS (Chen et al. 2010).

Signs and symptoms: Corticospinal signs and symptoms induced in ALS can be seen in Fig. 27.22.

The corticospinal tract consists of upper motor neuron originating in the motor cortex and lower motor neuron that goes to skeletal muscle. In ALS, the damage of these neurons will result in problems of skeletal muscle contractions which lead to different signs as shown in Table 27.3.

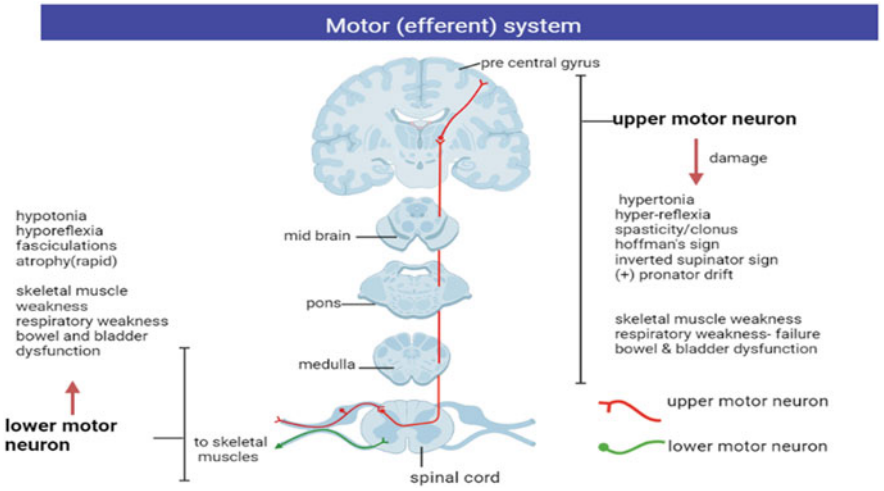


Fig. 27.22 Corticospinal signs and symptoms induces in ALS (created using BioRender)

Table 27.3 Corticospinal signs of upper and lower motor neurons induced in ALS

Upper motor neuron damage—signs	Lower motor neuron damage—signs
Hypertonia	Hypotonia
Hyper-reflexia	Hypo-reflexia
Spasticity/clonus	Fasciculation
Hoffman's sign	atrophy
Inverted supinator sign	
(+) pronator drift	

All these signs shown in Fig. 27.22 can manifest as skeletal muscle weakness eventually leading to paralysis, respiratory weakness leading to respiratory failure and also have bowel and bladder dysfunction (Garg et al. 2017).

ALS is also associated with bulbar dysfunction—it involves damage to corticobulbar tracts similar to corticospinal tracts. it also includes upper motor neuron and lower motor neuron but the difference is that the lower motor neurons in corticobulbar tracts go to muscles in head, face and neck (cranial area) (Kühnlein et al. 2008). So bulbar dysfunction in a nutshell is a dysfunction of cranial nerves. The damage to these nerves leads to signs shown in Table 27.4.

27.3.4.1.1 Causes

The average age of onset of ALS is about 60 years; it affects about two people per one hundred per year but only 5–10% cases were genetically associated and the other 90% of the cases are due to environmental component that triggers the onset of disease. The exact environmental component and its roles in genetic are known.

Table 27.4 Corticobulbar signs of upper and lower motor neurons induced in ALS

Bulbar dysfunction—upper motor neuron	Bulbar dysfunction—lower motor neurons
Dysphagia (CN IX, X)	Dysphagia (CN IX, X)
Dysarthria (CN X) & laryngospasm	Dysarthria (CN X) & hoarseness
Pseudo bulbar affect	Incomplete eye closure (CN VII)
Jaw stiffness (CN VII)	Poor lip closure (CN VII)
Brady kinetic tongue (CN XII) & sialorrhea	Hypotonic tongue and deviation (CN XII)
Facial droop/weakness (CN VII)	Facial droop/weakness (CN VII)
Soft palate droop (CN X)	Soft palate droop (CN X)

There is no cure for ALS at present, and the average survival from the onset to death is about 2–4 years. But some patients do live longer than the average survival, because 5–10% of ALS is variant cases in which the disease progresses until it gets very worse and then suddenly stops progressing due to fatigue (Haverkamp et al. 1995).

27.3.4.1.2 Treatment

Riluzole—it produces modest lengthening of survival. It acts by diminishing glutamate release and thereby decreasing excitotoxic neural cell death.

Antisense oligonucleotides—it acts by diminishing expression of mutant SOD1 protein.

Rehabilitation aids—like foot-drop splints facilitate ambulation, finger extension splints potentiate grip.

27.3.4.1.3 Minocycline in ALS

In ALS, the motor neuron follows the apoptotic pathway to final cell death under the influence of oxidative stress. The molecules which cause apoptosis increase the levels of caspase 1 and 3 activity in spinal cord, resulting in altering the balance of pro-apoptotic and anti-apoptotic regulatory protein which favours apoptosis (Chisholm et al. 2011).

Minocycline acts as an anti-apoptotic agent by inhibiting caspase through intraventricular administration. Through the study on mouse model of ALS, chronic inhibition of caspase will significantly inhibit neuroprotective effects of genetic manipulation leading to overexpression of anti-apoptotic agent in transgenic mice.

Minocycline also acts by inhibiting the activity of iNOS and caspase enzyme. It has shown promised affects in protection of nerve cells by reducing cell death and prolonging survival.

The clinical trials of minocycline have been conducted on two preliminary humans who have proven its safe use in ALS. Its tolerability is well when conjugated with riluzule—only FDA-approved drug for ALS.

A randomized, placebo-controlled phase 3 trial was also conducted; after 4 months running the trial, the outcome was measured in two parts:

(a) The difference of ALS functioning rating scale (ALSFRS-R).
(b) Forced vital capacity (FVC), manual muscle testing (MMT) and the quality of life, safety and survival.

In minocycline group, the ALSFRS-R was recorded faster, whereas the FVC declined in comparison to placebo group which lead to greater mortality rate in patients having minocycline. The minocycline group had also faced adverse events like non-serious gastrointestinal and neurological problems and decrease in quality-of-life score (Gordon et al. 2007).

Another randomized placebo-controlled tolerability trial of minocycline was conducted in which 78 in first trial and 23 in second trials were taken in.

In first trial, among 78, 19 people received riluzule drug 200 mg daily or placebo. After 6 months, no adverse events were recorded over placebo.

In second trial, 23 patients were taken for cross-over trial for 8 months in which they were given 400 mg drug for 4 months (mean tolerable dose—387 mg daily) which gave result about adverse gastrointestinal effects at higher, increased blood urea nitrogen and liver enzyme (Gordon et al. 2004, 2007).

27.3.4.1.4 Minocycline and Riluzule (Rilitek)

The combination of riluzule—500 mg /BD (drug used in ALS) and minocycline—100 mg has shown no significant increase in adverse effects. A pilot study was conducted to evaluate the safety of this combination on 20 ALS patients for 6 months (Pontieri et al. 2005).

27.3.5 Multiple Sclerosis

Multiple sclerosis is a chronic complex neurodegenerative disease. Multiple sclerosis is a demyelinating disease of central nervous system which includes brain and spinal cord. It affects myelin, a protective sheath that surrounds the axons of neurons allowing them to quickly send electrical impulses. The myelin is produced by oligodendrocytes (group of cells that supports neurons) (Huang et al. 2017).

Pathophysiology: The immune system inappropriately attacks and destroys the myelin, which makes the communication between neurons to break down, ultimately leading to all sorts of sensory, motor and cognitive problems as shown in Fig. 27.23.

The auto reactive immune cell like t-lymphocytes and b-lymphocytes crosses the blood–brain barrier (BBB) by attaching to the right ligand or surfaced molecule. Once they cross the BBB, t-cells get activated by myelin which in result do some alteration in BBB membrane which expresses more receptors for immune cells to

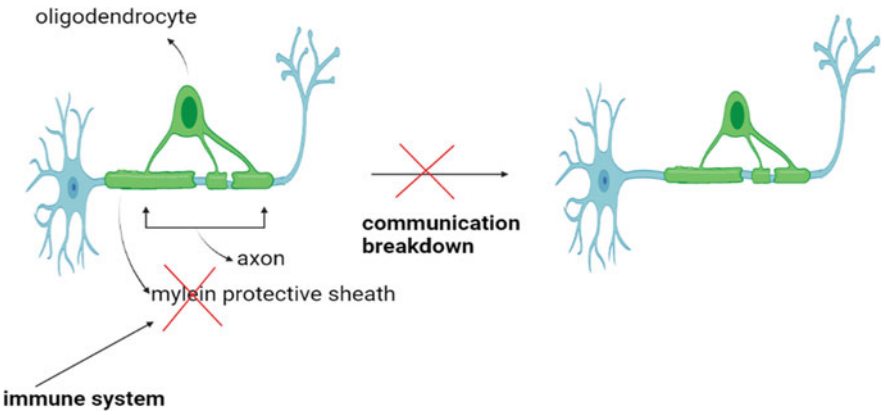


Fig. 27.23 Immune system attacking myelin leading to breakdown of communication between two neurons. (Created using BioRender)

bind and cross BBB to cause local inflammation resulting in demyelination, gliotic scarring and axonal loss (Lucchinetti et al. 2000; Glass et al. 2010).

Multiple sclerosis is a type 4 hypersensitivity reactions, i.e. cell-mediated hypersensitivity. The myelin-specific t-cells release inflammatory cytokines like IL-1, IL-6, TNF-alpha and interferon-gamma, which dilates blood vessels and helps more immune cells to get in (Ferguson et al. 1997; Mars et al. 2007). These inflammatory mediators cause damage to oligodendrocytes. The cytokines further attracts b-cells and macrophages which includes establishment of ectopic lymphoid follicles within the CNS , antigen presentation, cytokine and antibody production (Lassmann et al. 2012). The b-cells begin to make antibodies that mark the myelin sheath proteins, and macrophages use those antibodies markers to engulf and destroy the oligodendrocytes. Without oligodendrocytes, there is no myelin to cover the neurons that leaves the area of scar tissue called plaques or sclera. These immune attacks happen in bouts. The attacks on oligodendrocytes might happen and then regulatory t-cells will come in to inhibit or calm down the other immune cells leading to reduction in inflammation. Early on in multiple sclerosis, the oligodendrocytes will heal and extend out new myelin to cover the neurons. This process is called remyelination (Chari 2007). But overtime, oligodendrocytes die off, the remyelination stops and the damage becomes irreversible with the loss of axons as shown in Fig. 27.24.

Cause: The exact cause of multiple sclerosis is unknown but linked to both genetic and environmental factors.

(a) **Genetic factors**—being female and having genes that encodes specific immune molecule called HLDA-DR2 (used to identify and bind to foreign molecules).
(b) **Environmental factors**—infections, vitamin D deficiency (Ghasemi et al. 2017; Sintzel et al. 2018)

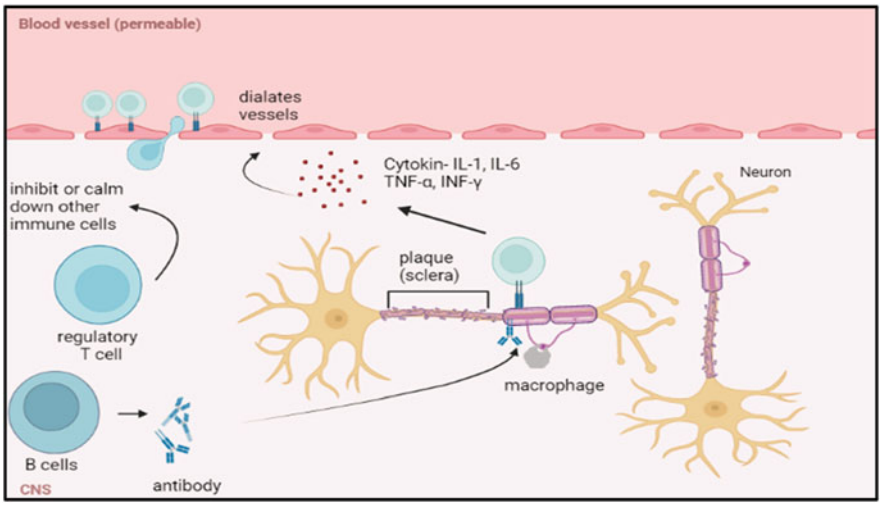


Fig. 27.24 Pathophysiology of multiple sclerosis (created using BioRender)

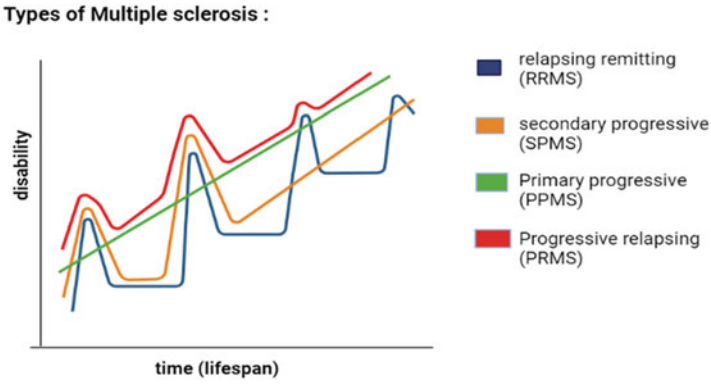


Fig. 27.25 Types of multiple sclerosis (MS) (created using BioRender)

Types of Multiple Sclerosis (Based on Pattern of Symptoms Over Time) Shown in Fig. 27.25.

(i) **Relapsing remitting multiple sclerosis, RRMS**—This is the most common type of MS. In this the bouts of autoimmune attacks happens for months or even years, apart from causing an increase in the level of disability. For e.g., during a bout, a person may lose some vision followed by improvement in case if there is remyelination shown in Fig. 27.26. More often, the remyelination process is not completed which leads to some residual disability. As more attacks take place, the central system gets more irreversibly damaged. There is no increase in disability between bouts indicated by flat lines during that time (Cunill et al. 2018).

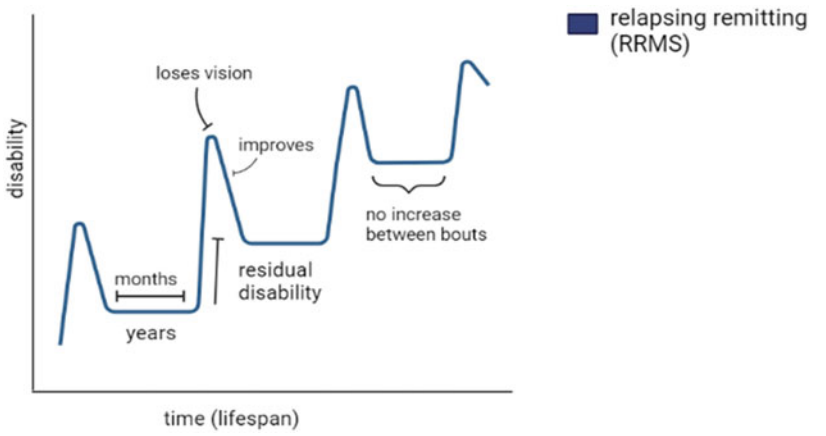


Fig. 27.26 Bouts in relapsing remitting type (RRMS) (created using BioRender)

(ii) **Secondary progressive multiple sclerosis, SPMS**—similar to RRMS, but over time the immune attack becomes constant causes steady progression of disability (Inojosa et al. 2021).
(iii) **Primary progressive multiple sclerosis, PPMS—**in this, there is one constant attack on myelin which causes steady progression of disability over patient's lifetime (Ontaneda and Fox 2015).
(iv) **Progressive relapsing multiple sclerosis, PRMS**—it is also one constant attack with bouts superimposed in between causes increased disability in faster way (Andersson et al. 1999).

Symptoms vary from person to person and location of plaques affects 2.5 million individuals worldwide mainly 20–40-year-old, affecting female twice as more often than men (Tafti et al. 2021).

Symptoms: can worsen over weeks, linger for months.

(i) **Charcot's neurological triad includes dysarthria, nystagmus and intention tremor.**

(a) **Dysarthria**—it is due to plaques in the brainstem, affects nerve fibre that control muscles of mouth and throat, interfere with conscious movements like eating, talking and leads to stutter and unconscious movements like swallowing (Hartelius et al. 2000).
(b) **Nystagmus**—it is due to plaques around the nerves controlling eye movements. Plaques on optic nerve causes loss of vision due to damage of optic nerve called optic neuritis. Some symptoms also include blurring or greying of the vision and dark point in the centre of vision. There is also damage to nerves that controls eye movement. The eye movement can be painful and even causes double vision. Eyes can no longer move in coordinated way (Serra et al. 2018).

(c) **Intention tremors**—caused by plaques along the motor pathways in the spinal cord. It affects out band signals like skeletal muscle control. Motor symptoms include muscle weakness, muscle spasms, tremors, ataxia (loss of balance and coordination). In serious cases, it can lead to paralysis (McCreary et al. 2018).

(ii) **Plaques** in sensory pathways can affect inbound signals sensations from the skin causes numbness, pins and needles, paresthesias (tingling feeling which can be painful, itching or burning sensation) (Frohman et al. 2006). Plaques also involve autonomic nervous symptoms—bowl and bladder symptoms like constipation and urinary incontinence and sexual symptoms like sexual dysfunction.

(iii) **Lhermitte's sign**—it is like an electric shock which runs down the back and radiates to the limbs when a person bends their neck forward (Khare and Seth 2015).

(iv) High order activities of brain can also be affected causing poor concentration and critical thinking. It also causes depression and anxiety.

27.3.5.1 Diagnosis

Diagnosis is primarily clinical and depends on neurological signs and symptoms. To differentiate multiple sclerosis from other conditions, various criteria are used including McDonald criteria which is based on demonstration of lesions (Milo and Miller 2014).

(i) **MRI**—uses gadolinium; contrast agent, it shows multiple central system lesions called white matter plaques. The RRMS can be diagnosed at early stages after one relapse.

(ii) **Cerebrospinal fluid**—contains high level of antibodies.

(iii) **Visual evoked potential**—it measures nervous system's response to visual stimuli (Milo and Miller 2014).

Treatment—several drugs are being used which act by different mechanism.

There is no cure for multiple sclerosis, but there are medications typically effective in RRMS because they lessen the severity of relapses and make them happen less frequently as shown in Table 27.5.

Table 27.5 Disease modifying treatment of multiple sclerosis (MS)

Treatment	Mode of action
Interferon—beta	Immune modulation
Glatiramer acetate	Immune modulation
Fingolimod	Immune modulation
Monoclonal antibody to alpha4-integrin (nataizumab)	Immune modulation (blocks lymphocytes entry into CNS)
Mitoxantrone	Immune suppression(cytotoxic)

(i) RRMS—corticosteroids, cyclophosphamide (cell cycle inhibitor), intravenous immunoglobulin can all be used to help blunt the autoimmune process. First-line drugs for RRMS—interferon—beta-1 a (avenex), glatiramer acetate.
(ii) Plasmapheresis can be effective (plasma filtered to remove disease causing auto antibodies).
(iii) Chronic treatment includes—immunosuppressant like recombinant beta-IFN which decreases the level of inflammatory cytokines in the brain and increases the function of t-regulatory cells.
(iv) Other immunosuppressant block t-cells from getting into the brain by interfering with cell surface molecules that gain passage through the BBB. Brain inflammation is also reduced by agents like daclizumab; humanized antibody acting against IL2R alpha in addition to mild functional blockade of CD4 T cells.
(v) PPMS—fewer options available and treatment targeted at managing the symptoms like depression, bladder dysfunction (Goldenberg 2012). Physical therapy and cognitive rehabilitation therapy can be helpful with sensory, motor and cognitive symptoms.
(vi) Vitamin D has also proven beneficial to bring out therapeutic effects.

27.3.5.2 Minocycline in Multiple Sclerosis

Although there are approved drugs available in the market for multiple sclerosis but some patients don't respond to this drug which opens up more area to explore for better treatment. As minocycline has shown anti-inflammatory action, in addition, it had also shown immunomodulatory neuroprotective effects in several pre-clinical and clinical studies by the various mechanisms like suppression of migratory inflammatory cells, modulating peripheral immune response and inhibiting microglia activation within the CNS.

27.3.5.3 Mechanism of Action

Minocycline crosses the BBB and inhibits reactive microgliosis, production of IL-1β, upregulate nitric oxide synthase and activate CD4+ T cells.

It also acts as anti-apoptic agent.

On the basis of clinical studies, minocycline had decreased gadolinium-enhancing activity for over 6 months in patients having relapsing-remitting MS (RRMS) (Zabad et al. 2007).

It attenuates the conversion of demyelination from the very event to clinically definite MS. Minocycline had also reduced the EAE (encephalomyelitis) severity because of immune-modulating activity. On oral consumption of minocycline, no relapses were observed between 6 months and 2 years despite the moderately high relapse rate of 1.3/year. It also antagonizes the pro-inflammatory IL-12 receptors

which were reportedly found to be high MS and decreases the activity of matrix metalloproteinase-9 (Hahn et al. 2016).

27.3.5.4 Pilot Study of Minocycline in RRMS

To test minocycline on the areas of safety and tolerability, pilot scale study was conducted which ran on 10 potential relapsing remitting type MS patients. The results came out to be safe and tolerable with annual relapse rate of 0.25 percent during treatment despite of 1.2 percent rate before treatment. The active lesions were also lowered during the first 6 months of treatment. The trial was run on small scale and hence, was not enough to support its potential and safety in RRMS (Zhang et al. 2008).

27.3.5.5 Minocycline and Interferon-β

A randomized, double-blind and placebo controlled study was also conducted in which IFN β1-a (44μg, sc) was administered three times weekly for first 3 months along with RRMS patient also randomized to give minocycline 100 mg, BD or placebo. The whole treatment lasted for 96 weeks which resulted in changing brain volume due to enlargement of T2-weighted lesions and qualifying relapses which holds no significant difference for minocycline versus placebo. Although no unexpected adverse effects were reported, but many has discontinued therapy due to adverse effects resulted from minocycline versus placebo (Sørensen et al. 2016).

27.4 Future Perspectives

As more areas are being explored with different clinical or pre-clinical research for the use of minocycline in different areas, its road to future is going to go in long run. From the antimicrobial agent, it is being explored in neuroprotective region which opens up huge market of minocycline like:

(i) Minocycline has also been identified as novel treatment of depression. The on-going trials of minocycline for bipolar and unipolar depression have been conducted which showed statically significant antidepressant effect (Rosenblat 2018).
(ii) In fragile x-knock out mouse, minocycline has rescued the dendrite spine and synaptic structural abnormalities. This also improved language and behavioural patterns. This created a huge area of research in both behavioural and language measure (Utari et al. 2010).
(iii) Minocycline has also shown non-infectious cytoprotective action in myocardial ischaemia by the mechanism of reperfusing injury (Thind et al. 2015).

(iv) The current on-going study of minocycline in animal model of schizophrenia has also claimed its action by reversing cognitive effects of MK801 (Levkovitz et al. 2007).
(v) Combined treatment of minocycline and atorvastatin is also being explored to suppress the severity of EAE (encephalomyelitis) induced in mice by exhibiting anti-inflammatory and neuroprotective action (Luccarini et al. 2008).

27.5 Conclusion

Minocycline can be repurposed in neurodegenerative diseases, as it delays the alteration of motor activities, inflammation and apoptosis of neurons in Huntington's disease, Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease and multiple sclerosis after performing clinical and pre-clinical trials. In AD, minocycline has shown good results in pre-clinical trials in mice having tau protein phosphorylation and amyloid beta aggregation, but in phase 2 clinical trials when minocycline was given to patients with mild AD for 2 years, good results were not obtained. So minocycline was not used further. In Parkinson's disease, pre-clinical trials were successful in decreasing MPTP neurotoxicity that is induced by iNOS and results in neuroprotective activity. Clinical trials for Parkinson's disease with minocycline and creatine yielded positive results, prompting the decision to move forward with phase 3 trials to assess safety, tolerability, activity and cost. Clinical and pre-clinical trials of Huntington's disease showed contraindicating studies that make it difficult to have a solid conclusion on this. When minocycline was given with riluzule in mice, then good results were seen, but in clinical trials, the combinations of these two drugs have shown no such good results. In clinical and pre-clinical trials, minocycline alone has manifested beneficial effects. In multiple sclerosis, minocycline is really effective in clinical and pre-clinical trials.

References

Alano CC, Kauppinen TM, Valls AV, Swanson RA (2006) Minocycline inhibits poly(ADP-ribose) polymerase-1 at nanomolar concentrations. Proc Natl Acad Sci U S A 103:9685–9690. https://doi.org/10.1073/PNAS.0600554103

Allen JC (1976) Minocycline. Ann Intern Med 85:482–487. https://doi.org/10.7326/0003-4819-85-4-482

Andersson PB, Waubant E, Gee L, Goodkin DE (1999) Multiple sclerosis that is progressive from the time of onset: clinical characteristics and progression of disability. Arch Neurol 56:1138–1142. https://doi.org/10.1001/ARCHNEUR.56.9.1138

Bantubungi K, Jacquard C, Greco A et al (2005) Minocycline in phenotypic models of Huntington's disease. Neurobiol Dis 18:206–217. https://doi.org/10.1016/j.nbd.2004.09.017

Blokhuis AM, Groen EJN, Koppers M et al (2013) Protein aggregation in amyotrophic lateral sclerosis. Acta Neuropathol 125:777–794. https://doi.org/10.1007/S00401-013-1125-6

Bonelli RM, Heuberger C, Reisecker F (2003) Minocycline for Huntington's disease: an open label study. Neurology 60:883–884. https://doi.org/10.1212/01.WNL.0000049936.85487.7A

Brown CM, Stockwell PA, Trotman CNA, Tate WP (1990) The signal for the termination of protein synthesis in procaryotes. Nucleic Acids Res 18:2079–2086. https://doi.org/10.1093/NAR/18.8.2079

Brundula V, Rewcastle NB, Metz LM et al (2002) Targeting leukocyte MMPs and transmigration minocycline as a potential therapy for multiple sclerosis. Brain 125:1297–1308. https://doi.org/10.1093/brain/awf133

Chari DM (2007) Remyelination in multiple sclerosis. Int Rev Neurobiol 79:589–620. https://doi.org/10.1016/S0074-7742(07)79026-8

Chavatte L, Kervestin S, Favre A, Jean-Jean O (2003) Stop codon selection in eukaryotic translation termination: Comparison of the discriminating potential between human and ciliate eRF1s. EMBO J 22:1644–1653. https://doi.org/10.1093/EMBOJ/CDG146

Chen K, Northington FJ, Martin LJ (2010) Inducible nitric oxide synthase is present in motor neuron mitochondria and Schwann cells and contributes to disease mechanisms in ALS mice. Brain Struct Funct 214:219. https://doi.org/10.1007/S00429-009-0226-4

Cheng S, Hou J, Zhang C et al (2015) Minocycline reduces neuroinflammation but does not ameliorate neuron loss in a mouse model of neurodegeneration. Sci Rep 5. https://doi.org/10.1038/SREP10535

Chisholm V, Butala N, Smith TM (2011) The clinical potential of minocycline in amyotrophic lateral sclerosis. Neurology 17:3. https://doi.org/10.17925/USN.2021.17.1.3

Chukwudi CU (2016) rRNA binding sites and the molecular mechanism of action of the tetracyclines. Antimicrob Agents Chemother 60:4433–4441. https://doi.org/10.1128/AAC.00594-16

Cleren C, Calingasan NY, Starkov A et al (2010) Promethazine protects against 3-nitropropionic acid-induced neurotoxicity. Neurochem Int 56:208–212. https://doi.org/10.1016/j.neuint.2009.10.006

Cudkowicz M (2010) A futility study of minocycline in huntington's disease. Mov Disord 25:2219–2224. https://doi.org/10.1002/mds.23236

Cunill V, Massot M, Clemente A et al (2018) Relapsing–remitting multiple sclerosis is characterized by a T follicular cell pro-inflammatory shift, reverted by dimethyl fumarate treatment. Front Immunol 9. https://doi.org/10.3389/FIMMU.2018.01097

Czworkowski J, Moore PB (1996) The elongation phase of protein synthesis. Prog Nucleic Acid Res Mol Biol 54:293–332. https://doi.org/10.1016/s0079-6603(08)60366-9

DeMaagd G, Philip A (2015) Parkinson's disease and its management: Part 1: Disease entity, risk factors, pathophysiology, clinical presentation, and diagnosis. Pharm Ther 40:504

Dementia (n.d.)

Dever TE, Dinman JD, Green R (2018) Translation elongation and recoding in eukaryotes. Cold Spring Harb Perspect Biol 10. https://doi.org/10.1101/CSHPERSPECT.A032649

Dias V, Junn E, Mouradian MM (2013) The role of oxidative stress in Parkinson's disease. J Parkinsons Dis 3:461. https://doi.org/10.3233/JPD-130230

Du Y, Ma Z, Lin S et al (2001) Minocycline prevents nigrostriatal dopaminergic neurodegeneration in the MPTP model of Parkinson's disease. Proc Natl Acad Sci U S A 98:14669–14674. https://doi.org/10.1073/PNAS.251341998

Dunston CR, Griffiths HR, Lambert PA et al (2011) Proteomic analysis of the anti-inflammatory action of minocycline. Proteomics 11:42–51. https://doi.org/10.1002/PMIC.201000273

Elewa HF, Hilali H, Hess DC, Machado LS, Fagan SC et al (2006) Minocycline for short-term neuroprotection. Pharmacotherapy 26:515–521

Ferguson B, Matyszak MK, Esiri MM, Perry VH (1997) Axonal damage in acute multiple sclerosis lesions. Brain 120:393–399. https://doi.org/10.1093/BRAIN/120.3.393

Frohman E, Racke MK, Raine CS (2006) Multiple sclerosis—the plaque and its pathogenesis. Mass Med Soc 354:942–955. https://doi.org/10.1056/NEJMra052130

Fuoco D (2012) Classification framework and chemical biology of tetracycline-structure-based drugs. Antibiotics 1:1–13. https://doi.org/10.3390/ANTIBIOTICS1010001

Garcez ML, Mina F, Bellettini-Santos T et al (2017) Minocycline reduces inflammatory parameters in the brain structures and serum and reverses memory impairment caused by the administration of amyloid β (1-42) in mice. Prog Neuro-Psychopharmacology Biol Psychiatry 77:23–31. https://doi.org/10.1016/j.pnpbp.2017.03.010

Garg N, Park SB, Vucic S et al (2017) Differentiating lower motor neuron syndromes. J Neurol Neurosurg Psychiatry 88:474–483. https://doi.org/10.1136/JNNP-2016-313526

Garner SE, Eady A, Bennett C et al (2012) Minocycline for acne vulgaris: Efficacy and safety. Cochrane Database Syst Rev 2012. https://doi.org/10.1002/14651858.CD002086.pub2

Garwood CJ, Cooper JD, Hanger DP, Noble W (2010) Anti-inflammatory impact of minocycline in a mouse model of tauopathy. Front psychiatry 1. https://doi.org/10.3389/FPSYT.2010.00136

Gella A, Durany N (2009) Oxidative stress in Alzheimer disease. Cell Adh Migr 3:88. https://doi.org/10.4161/CAM.3.1.7402

Ghasemi N, Razavi S, Nikzad E (2017) Multiple sclerosis: pathogenesis, symptoms, diagnoses and cell-based therapy. Cell J 19:1. https://doi.org/10.22074/CELLJ.2016.4867

Glass CK, Saijo K, Winner B et al (2010) Mechanisms underlying inflammation in neurodegeneration. Cell 140:918–934. https://doi.org/10.1016/J.CELL.2010.02.016

Goldenberg MM (2012) Multiple sclerosis review. Pharm Ther 37:175

Gonzalez JP, Henwood JM (1989) Pefloxacin: a review of its antibacterial activity, pharmacokinetic properties and therapeutic use. Drugs 37:628–668. https://doi.org/10.2165/00003495-198937050-00003

Gordon P, Moore D, Gelinas D et al (2004) Placebo-controlled phase I/II studies of minocycline in amyotrophic lateral sclerosis. Neurology 62(10):1845–1847

Gordon PH, Moore DH, Miller RG et al (2007) Efficacy of minocycline in patients with amyotrophic lateral sclerosis: a phase III randomised trial. Lancet Neurol 6:1045–1053. https://doi.org/10.1016/S1474-4422(07)70270-3

Grad LI, Rouleau GA, Ravits J, Cashman NR (2017) Clinical spectrum of Amyotrophic Lateral Sclerosis (ALS). Cold Spring Harb Perspect Med 7. https://doi.org/10.1101/CSHPERSPECT.A024117

Hahn JN, Kaushik DK, Mishra MK et al (2016) Impact of minocycline on extracellular matrix metalloproteinase inducer, a factor implicated in multiple sclerosis immunopathogenesis. J Immunol 197:3850–3860. https://doi.org/10.4049/JIMMUNOL.1600436/-/DCSUPPLEMENTAL

Hampel H, Mesulam MM, Cuello AC et al (2018) The cholinergic system in the pathophysiology and treatment of Alzheimer's disease. Brain 141:1917–1933. https://doi.org/10.1093/BRAIN/AWY132

Hartelius L, Runmarker B, Andersen O (2000) Prevalence and characteristics of dysarthria in a multiple-sclerosis incidence cohort: relation to neurological data. Folia Phoniatr Logop 52:160–177. https://doi.org/10.1159/000021531

Haverkamp LJ, Appel V, Appel SH (1995) Natural history of amyotrophic lateral sclerosis in a database population validation of a scoring system and a model for survival prediction. Brain 118:707–719. https://doi.org/10.1093/BRAIN/118.3.707

Hazardous Substances Data Bank (HSDB) (n.d.): 3130—PubChem

Huang W-J, Chen W-W, Zhang X (2017) Multiple sclerosis: Pathology, diagnosis and treatments. Exp Ther Med 13:3163. https://doi.org/10.3892/ETM.2017.4410

Inojosa H, Proschmann U, Akgün K, Ziemssen T (2021) A focus on secondary progressive multiple sclerosis (SPMS): challenges in diagnosis and definition. J Neurol 268:1210–1221. https://doi.org/10.1007/S00415-019-09489-5

Inoue S, Browne G, Melino G, Cohen GM (2009) Ordering of caspases in cells undergoing apoptosis by the intrinsic pathway. Cell Death Differ 16:1053–1061. https://doi.org/10.1038/CDD.2009.29

Khare S, Seth D (2015) Lhermitte's sign: the current status. Ann Indian Acad Neurol 18:154. https://doi.org/10.4103/0972-2327.150622

Kim DW, Patel SP (2014) Profile of selumetinib and its potential in the treatment of melanoma. Onco Targets Ther 7:1631–1639. https://doi.org/10.2147/OTT.S51596

Kim HS, Suh YH (2009) Minocycline and neurodegenerative diseases. Behav Brain Res 196:168–179. https://doi.org/10.1016/j.bbr.2008.09.040

Kim J, Basak JM, Holtzman DM (2009) The role of apolipoprotein E in Alzheimer's disease. Neuron 63:287. https://doi.org/10.1016/J.NEURON.2009.06.026

Klein C, Westenberger A (2012) Genetics of Parkinson's disease. Cold Spring Harb Perspect Med 2. https://doi.org/10.1101/CSHPERSPECT.A008888

Kouli A, Torsney KM, Kuan W-L (2018) Parkinson's disease: etiology, neuropathology, and pathogenesis. Park Dis Pathog Clin Asp:3–26. https://doi.org/10.15586/CODONPUBLICATIONS.PARKINSONSDISEASE.2018.CH1

Kühnlein P, Gdynia HJ, Sperfeld AD et al (2008) Diagnosis and treatment of bulbar symptoms in amyotrophic lateral sclerosis. Nat Clin Pract Neurol 4:366–374. https://doi.org/10.1038/NCPNEURO0853

Lampl Y, Boaz M, Gilad R et al (2007) Minocycline treatment in acute stroke: an open-label, evaluator-blinded study. Neurology 69:1404–1410. https://doi.org/10.1212/01.wnl.0000277487.04281.db

Langston JW (2017) The MPTP story. J Parkinsons Dis 7:S11. https://doi.org/10.3233/JPD-179006

Lassmann H, Van Horssen J, Mahad D (2012) Progressive multiple sclerosis: pathology and pathogenesis. Nat Rev Neurol 8(11):647–656

Laursen BS, Sørensen HP, Mortensen KK, Sperling-Petersen HU (2005) Initiation of protein synthesis in bacteria. Microbiol Mol Biol Rev 69:101–123. https://doi.org/10.1128/MMBR.69.1.101-123.2005

Levkovitz Y, Levi U, Braw Y et al (2007) Minocycline, a second-generation tetracycline, as a neuroprotective agent in an animal model of schizophrenia. Brain Res 1154:154–162

Li C, Yuan K, Schluesener H (2013) Impact of minocycline on neurodegenerative diseases in rodents: a meta-analysis. Rev Neurosci 24:553–562. https://doi.org/10.1515/revneuro-2013-0040

Li R, Yin X, Jin Y et al (2021) The solubility profile and dissolution thermodynamic properties of minocycline hydrochloride in some pure and mixed solvents at several temperatures. J Chem Thermodyn 157:106399. https://doi.org/10.1016/J.JCT.2021.106399

Luccarini I, Ballerini C, Biagioli T et al (2008) Combined treatment with atorvastatin and minocycline suppresses severity of EAE. Exp Neurol 211(1):214–226

Lucchinetti C, Brück W, Parisi J, Scheithauer B, Rodriguez M, Lassmann H (2000) Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. Wiley Online Libr. https://doi.org/10.1002/1531-8249(200006)47:6<707::AID-ANA3>3.0.CO;2-Q

Macdonald H, Kelly RG, Stewart Allen E et al (1973) Pharmacokinetic studies on minocycline, in man. Clin Pharmacol Ther 14:852–861. https://doi.org/10.1002/cpt1973145852

Mars LT, Bauer J, Gross DA et al (2007) CD8 T cell responses to myelin oligodendrocyte glycoprotein-derived peptides in humanized HLA-A*0201-transgenic mice. J Immunol 179:5090–5098. https://doi.org/10.4049/JIMMUNOL.179.8.5090

Martins AM, Marto JM, Johnson JL, Graber EM (2021) A review of systemic minocycline side effects and topical minocycline as a safer alternative for treating acne and rosacea. Antibiotics 10. https://doi.org/10.3390/antibiotics10070757

Matrisian LM (2000) Matrix metalloproteinases. Curr Biol 10:160–166. https://doi.org/10.1016/S0960-9822(00)00720-X

McCreary JK, Rogers JA, Forwell SJ (2018) Upper limb intention tremor in multiple sclerosis: an evidence-based review of assessment and treatment. Int J MS Care 20:211. https://doi.org/10.7224/1537-2073.2017-024

Milo R, Miller A (2014) Revised diagnostic criteria of multiple sclerosis. Autoimmun Rev 13:518–524. https://doi.org/10.1016/J.AUTREV.2014.01.012

Minocycline (n.d.): Uses, Interactions, Mechanism of Action | DrugBank Online. https://go.drugbank.com/drugs/DB01017. Accessed 30 Oct 2021a

Minocycline Hydrochloride (n.d.) | DrugBank Online
Minocycline Hydrochloride Dihydrate (n.d.) | C23H32ClN3O9—PubChem
Minocycline in Patients with Alzheimer's Disease (n.d.)—Full Text View—ClinicalTrials.gov
Noble W, Garwood C, Stephenson J et al (2009a) Minocycline reduces the development of abnormal tau species in models of Alzheimer's disease. FASEB J 23:739–750. https://doi.org/10.1096/fj.08-113795
Noble W, Garwood CJ, Hanger DP (2009b) Minocycline as a potential therapeutic agent in neurodegenerative disorders characterised by protein misfolding. Prion 3:78–83. https://doi.org/10.4161/pri.3.2.8820
Ontaneda D, Fox RJ (2015) Progressive multiple sclerosis. Curr Opin Neurol 28:237. https://doi.org/10.1097/WCO.0000000000000195
Opron K, Burton ZF (2019) Ribosome structure, function, and early evolution. Int J Mol Sci 20. https://doi.org/10.3390/IJMS20010040
Pontieri FE, Ricci A, Pellicano C et al (2005) Minocycline in amyotrophic lateral sclerosis: a pilot study. Neurol Sci 26:285–287. https://doi.org/10.1007/S10072-005-0474-X
Pressman P, Rabinovici GD (2014) Alzheimer's disease. Encycl Neurol Sci:122–127. https://doi.org/10.1016/B978-0-12-385157-4.00475-9
Pushpakom S, Iorio F, Eyers PA et al (2018) Drug repurposing: progress, challenges and recommendations. Nat Publ Gr. https://doi.org/10.1038/nrd.2018.168
Raina M, Ibba M, Yoshihisa T (2014) tRNAs as regulators of biological processes. Front Genet 5:171. https://doi.org/10.3389/fgene.2014.00171
Ramakrishnan V (2002) Ribosome structure and the mechanism of translation. Cell 108:557–572. https://doi.org/10.1016/S0092-8674(02)00619-0
Ravina B, Kieburtz K, Tilley B et al (2006) A randomized, double-blind, futility clinical trial of creatine and minocycline in early Parkinson disease. Neurology 66:664–671. https://doi.org/10.1212/01.WNL.0000201252.57661.E1
Rekatsina M, Paladini A, Piroli A et al (2020) Pathophysiology and therapeutic perspectives of oxidative stress and neurodegenerative diseases: a narrative review. Adv Ther 37:113–139. https://doi.org/10.1007/s12325-019-01148-5
Romero-Miguel D, Lamanna-Rama N, Casquero-Veiga M et al (2021) Minocycline in neurodegenerative and psychiatric diseases: an update. Eur J Neurol 28:1056–1081. https://doi.org/10.1111/ENE.14642
Roos RAC (2010) Huntington's disease: a clinical review. Orphanet J Rare Dis 5:2–9. https://doi.org/10.1186/1750-1172-5-40
Rosenblat J (2018) Efficacy and tolerability of minocycline for depression: a systematic review and meta-analysis of clinical trials. Elsevier
Rostalski H, Leskelä S, Huber N et al (2019) Astrocytes and microglia as potential contributors to the pathogenesis of C9orf72 repeat expansion-associated FTLD and ALS. Front Neurosci 13. https://doi.org/10.3389/FNINS.2019.00486/FULL
Saivin S, Houin G (1988) Clinical pharmacokinetics of doxycycline and minocycline. Clin Pharmacokinet 15:355–366. https://doi.org/10.2165/00003088-198815060-00001
Schachter AS, Davis KL (2000) Alzheimer's disease. Dialogues Clin Neurosci 2:91. https://doi.org/10.31887/DCNS.2000.2.2/ASSCHACHTER
Serra A, Chisari CG, Matta M (2018) Eye movement abnormalities in multiple sclerosis: pathogenesis, modeling, and treatment. Front Neurol 9:31. https://doi.org/10.3389/FNEUR.2018.00031
Shamsi F, Zeraatpisheh Z, Alipour H et al (2020) The effects of minocycline on proliferation, differentiation and migration of neural stem/progenitor cells. Int J Neurosci 130:601–609. https://doi.org/10.1080/00207454.2019.1699083
Shaw PJ, Shaw PJ, Ince PG (1997) Role of RNA binding proteins in neurodegenerative diseases View project Glutamate, excitotoxicity and amyotrophic lateral sclerosis. Artic J Neurol 244. https://doi.org/10.1007/BF03160574

Shultz RB, Zhong Y (2017) Minocycline targets multiple secondary injury mechanisms in traumatic spinal cord injury. 12. https://doi.org/10.4103/1673-5374.206633
Sintzel MB, Rametta M, Reder AT (2018) Vitamin D and multiple sclerosis: a comprehensive Review. Neurol Ther 7:59–85. https://doi.org/10.1007/S40120-017-0086-4
Sørensen PS, Sellebjerg F, Lycke J et al (2016) Minocycline added to subcutaneous interferon β-1a in multiple sclerosis: Randomized RECYCLINE study. Eur J Neurol 23:861–870. https://doi.org/10.1111/ENE.12953
Spivey HE, Katayama MC, Yoshida M, Harper AE (1958) Significance of the protein-sparing effect of dextrin. Am J Physiol 193:479–482. https://doi.org/10.1152/ajplegacy.1958.193.3.479
Srinivasan E, Chandrasekhar G, Chandrasekar P et al (2021) Alpha-Synuclein aggregation in Parkinson's disease. Front Med 8. https://doi.org/10.3389/FMED.2021.736978
Stefanis L (2012) α-Synuclein in Parkinson's disease. Cold Spring Harb Perspect Med 2. https://doi.org/10.1101/CSHPERSPECT.A009399
Stirling DP, Koochesfahani KM, Steeves JD, Tetzlaff W (2005) Minocycline as a neuroprotective agent. Neuroscientist 11:308–322. https://doi.org/10.1177/1073858405275175
Tafti D, Ehsan M, Xixis KL (2021) Multiple sclerosis 15.
Thind G, Agrawal P, Hirsh B et al (2015) Mechanisms of myocardial ischemia–reperfusion injury and the cytoprotective role of minocycline: scope and limitations. Futur Med 11:61–76. https://doi.org/10.2217/FCA.14.76
Thomas M, Ashizawa T, Jankovic J (2004) Minocycline in Huntington's disease: a pilot study. Mov Disord 19:692–695. https://doi.org/10.1002/mds.20018
Utari A, Chonchaiya W, Rivera SM, Schneider A, Hagerman RJ, Faradz SM, Ethell IM, Nguyen DV (2010) Side effects of minocycline treatment in patients with fragile X syndrome and exploration of outcome measures. Am J Intellect Dev Disabil 115(5):433–443
van den Bos MAJ, Geevasinga N, Higashihara M et al (2019) Pathophysiology and diagnosis of ALS: insights from advances in neurophysiological techniques. Int J Mol Sci 20. https://doi.org/10.3390/IJMS20112818
Wakabayashi K, Tanji K, Mori F, Takahashi H (2007) The Lewy body in Parkinson's disease: molecules implicated in the formation and degradation of alpha-synuclein aggregates. Neuropathology 27:494–506. https://doi.org/10.1111/J.1440-1789.2007.00803.X
Welling PG, Shaw WR, Uman SJ et al (1975) Pharmacokinetics of minocycline in renal failure. AntimicrobAgents Chemother 8:532–537. https://doi.org/10.1128/AAC.8.5.532
Wu DC, Jackson-Lewis V, Vila M et al (2002) Blockade of microglial activation is neuroprotective in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson disease. J Neurosci 22:1763–1771. https://doi.org/10.1523/JNEUROSCI.22-05-01763.2002
Zabad R, Metz L et al (2007) The clinical response to minocycline in multiple sclerosis is accompanied by beneficial immune changes: a pilot study. Multiple Scler J 13(4):517–526. https://doi.org/10.1177/1352458506070319
Zhang Y, Metz LM, Yong VW et al (2008) Pilot study of minocycline in relapsing-remitting multiple sclerosis. Can J Neurol Sci 35:185–191. https://doi.org/10.1017/S0317167100008611
Zhao YB, Krishnan J (2014) mRNA translation and protein synthesis: an analysis of different modelling methodologies and a new PBN based approach. BMC Syst Biol 8:25. https://doi.org/10.1186/1752-0509-8-25