# Hormone Related Cancer Mechanistic and Nanomedicines

## Challenges and Prospects

Mahfoozur Rahman
Waleed H Almalki
Majed Alrobaian
Sarwar Beg
Khalid S Alharbi
*Editors*

Springer

# Hormone Related Cancer Mechanistic and Nanomedicines

Mahfoozur Rahman • Waleed H Almalki •
Majed Alrobaian • Sarwar Beg •
Khalid S Alharbi
Editors

# Hormone Related Cancer Mechanistic and Nanomedicines

## Challenges and Prospects

Springer

*Editors*
Mahfoozur Rahman
Department of Pharmaceutical Sciences
Faculty of Health Sciences, SHUATS
Allahabad, Uttar Pradesh, India

Waleed H Almalki
Department of Pharmacology and Toxicology,
College of Pharmacy
Umm al-Qura University
Mecca, Saudi Arabia

Majed Alrobaian
Department of Pharmaceutics & and
Pharmaceutical Technology, College of
Pharmacy
Taif University
Taif, Saudi Arabia

Sarwar Beg
Department of Pharmaceutics
School of Pharmaceutical Education and
Research, Jamia Hamdard
New Delhi, India

Khalid S Alharbi
Department of Pharmacology, College of
Pharmacy
Jouf University
Sakakah, Saudi Arabia

ISBN 978-981-19-5557-0 ISBN 978-981-19-5558-7 (eBook)
https://doi.org/10.1007/978-981-19-5558-7



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

*Most Rev. Prof. (Dr.) Rajendra B. Lal*
*Vice Chancellor, SHUATS, Prayagraj*
A great thinker, reformer, visionary, God fearing, and great theologist

# Preface

This book is on the progress made in hormone-related cancers and their management by nanomedicine-based therapy for targeting the hormone-regulated cancer. Chapters by experts on different specific aspects of hormonal anticancer agents and their effective delivery by nanomedicines for better healthcare applications have been presented to our widespread readers and healthcare professionals. The nanotheranostics sector provides a very promising strategy for monitoring drug biodistribution and pathology longitudinal processes by integrating the imaging and drug delivery functions in nanoformulation, providing vital insights into the identification of tumor and predicting the efficacy of nanomedicine. For their unique properties, which include their small size and biocompatibility and the ability to permeate the cellular membrane with carrying drugs, nanomaterials have been used for various biomedical applications. Metallic nanoparticles are now commonly used as therapeutics due to their interesting surface reactivity and low cytotoxicity effects. There are several reports that AuNPs are antiproliferative in numerous model diseases such as prostate cancer with anti-angiogenic and anti-inflammatory. There is a trend toward optimizing the design such that more nanomedicines reach the tumor while minimizing systemic loss with significant regulation of hormones. Cancer nanomedicines are currently facing challenges because of some recent clinical failures.

A succinct account of key highlights of each of the chapter included in the book has been discussed in the text mentioned below.

Chapter 1 entitled "Challenges and opportunities in the delivery of oral anticancer therapeutics" extensively covers drug delivery challenges related to oral routes of administration, marketing, or in development that answer those challenges.

Chapter 2 of Dr. Jain on "Nanotechnology in the Management of Hormonal Cancer" covers owing to the limitations of the traditional drug delivery system, advanced targeting drug delivery systems are employed in the management of hormonal cancer.

Chapter 3 of Dr. Jindal entitled "Progress of Cancer Nanomedicine, Clinical Hurdles, and Opportunities" covers the challenges encountered during clinical testing and development of cancer nanomedicines, as well as future opportunities.

In Chap. 4, Dr. Akbar describes the role of nanohybrids in hormonal cancer therapy that overcomes the drug resistance as one of the major contributing factors in the conventional anticancer product.

Dr. Sandeep in Chap. 5 summarizes that the combination of administration and targeting of the functionalized nanoparticles to the tumor may cause decreased systemic toxicities and result in enhanced drug payload.

Dr. Verma in Chap. 6, "Pancreatic Cancer: Nanoparticle-Targeted Therapy via Epidermal Growth Factor Receptor," summarizes that pancreatic cancer is the malignant neoplasm of the pancreas and c specifically targets and blocks the EGFR signaling cascade that represents various strategies exploited for targeting EGFR overexpressed on cancer cells as a therapeutic intervention approach for cancer treatment. Dr. Rahman and Dr. Sandeep in Chap. 7 entitled "Nanocarriers-Based Targeted Therapies for Pancreatic Cancer and Challenges Ahead" and Chap. 8 "Pancreatic Cancer Treatment By Using Theragnostic Nanoparticles" describe the new possibilities of targeting pancreatic cancer via nanocarriers and the challenges ahead.

Dr. Iqbal in Chap. 9 discusses "Nanomedicine-Based Combinational Therapy for Breast Cancer" with an insight into the general pathophysiology of breast cancer and available combinational drug therapy. Further authors have elaborated on the applications of nano-based combinational drug therapy for breast cancer treatment. This chapter provides an insight into the clinical trial status and limitations of nano-based combinational drug therapy for breast cancer.

In Chap. 10, "Nano-Liposomal System for Breast Cancer Therapy," Dr. Ahmad highlights the recent advances in the nanoliposomes-based therapeutic system for breast cancer, including gene and theranostic delivery perspectives. The challenges associated with the development of liposome-based products for future clinical settings are also discussed.

Dr. Sandeep in Chap. 11 discusses conventional to nanotherapeutic strategies against triple-negative breast cancer further with special emphasis on biological processes and nanotechnology advancements and future nanomedicine treatments for TNBC.

Dr. Verma in Chap. 12 discusses the effect of thymoquinone and its delivery by using nanomedicine in benign prostatic hyperplasia, which may be successfully established using a nanoscale transport stage layout for TQ, which could manipulate its biopharmaceutical limitations boundaries.

Dr. Shukla in Chap. 13 describes the concept of nanomedicine in endocrine hormone cancer treatment. Although the nanomedicines could be an ideal strategy to treat the endocrine cancer using different forms of nanoproducts.

In Chap. 14, Dr. Saini Patel discusses the "Neurocognitive Underpinning of Neurological Disorders: The Role of Default Mode Network" and how it altered DMN may be plausibly extended as an indicator of neurological disorders.

Dr. Akbar in Chap.15 summarizes neuroendocrine carcinoma of the endometrium and their therapeutics from conventional treatment approach to nanomedicine.

In Chap. 16, Amita Verma describes the "Effective Luteinizing Hormone Drug Delivery by Nanocarriers in Hormonal Cancer Treatment" even though several nanomedicines have been developed to improve the anticancer effect. Hence, synthesized nanoparticles could serve as potential carriers with advantages of improved biodegradability, biocompatibility, improved loading capacity, targeting ability, scalability, and stability.

Dr. Shukla in Chap. 17 discusses regulatory landscapes in approval of cancer vaccines. Hence, bilateral talks with the regulatory body are a mandatory requirement to discuss and deliberate the clinical development plan on a case-by-case basis. Therefore, consider the specific issues related to the quality of product under development. All such regulatory aspects about the development and approval of cancer vaccines are discussed hereby in this book chapter.

This book focuses on the advanced progress made in hormone-related cancers and their management by nanomedicine therapy for targeting the hormone-related cancer. The book, therefore, carries a lot of potential as a repertoire of knowledge and package of information for the pharmaceutical scientists, nano-scientists and nanobiotechnologists, endocrine scientists to provide holistic information on interest.

Finally, volume editors would like to extend their appreciation to Springer and their staff for providing a professional platform for communication with the experts in the field.

Allahabad, India — Mahfoozur Rahman
Mecca, Saudi Arabia — Waleed H Almalki
Taif, Saudi Arabia — Majed Alrobaian
New Delhi, India — Sarwar Beg
Sakakah, Saudi Arabia — Khalid S Alharbi

# Contents

# About the Editors

**Mahfoozur Rahman Ph.D.** is serving as Assistant Professor at the Department of Pharmaceutical Science, Faculty of Health Sciences, Sam Higginbottom University of Agriculture, Technology and Sciences (SHUATS), Allahabad, India. His major areas of research interest include development and characterization of nanosized drug delivery systems for cancer and inflammatory disorders including psoriasis, arthritis, and neurodegenerative disorders, etc. To date, he has published over 150 publications in peer-reviewed journals, 50 book chapters, 07 International books, and 04 articles in international magazine. He has Google Scholar *H-index of 35* and over 3500 citations to his credit. Dr. Rahman also served as Guest Editor of several journals including *Seminar in cancer Biology*. Besides, Dr. Rahman got travel grant from various international congress such as IAPRD, MDS, Nano Today Conference, KSN, WCN, on the basis of his research work and contribution in the field.

**Waleed H Almalki Ph.D.** is a Professor of Pharmacology at the College of Pharmacy, Umm Al-Qura University, Makkah, Saudi Arabia. He earned his doctoral degree at the University of Glasgow, United Kingdom, having a dissertation on ocular pharmacology. His current research is focused on the broad areas of host-virus networks in hepacivirus C disease progression as well as host-virus genes expression during oxidative stress, hepatic steatosis, fibrosis, and hepatocellular carcinoma. He is also enthusiastically involved in the studies of tyrosine kinase inhibitors for the treatment of breast and colon cancer, in the Saudi population. Dr. Almalki has published many research and review articles in peer-reviewed international journals on HCV pathogenesis, treatment, and drug designing, as well as edited various book chapters about angiogenesis, pharmacological assays, and infectious disease epidemiology.

**Majed Alrobaian Ph.D.** is the Dean of the Faculty of Pharmacy at Taif University in Saudi Arabia. He obtained his B.Sc. from King Saud University in Riyadh, Saudi Arabia, and his M.Sc. in Pharmaceutical Sciences from the University of Strathclyde in the United Kingdom in 2010, and his Ph.D. from the same university in 2014. His research interests focus on drug delivery and, in particular, the delivery of therapeutic genes for the treatment of cancer. Before obtaining his Ph.D., he worked as

Director of Pharmacy at King Abdul-Aziz Hospital. Currently, he is appointed as the Science Faculty Ambassador for the University of Strathclyde. He is a member of a number of committees in the Saudi Commission for Health Specialties.

**Sarwar Beg Ph.D.** is currently serving as Assistant Professor at the Department of Pharmaceutics, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi. He has over a decade of teaching and research experience in the field of pharmaceutics and biopharmaceutics, especially in the development of novel and nanostructured drug delivery systems using Quality-by-Design paradigms. Before joining Jamia Hamdard, Dr. Beg worked as a Research Scientist at Jubilant Generics Limited, Noida, India. He has authored over 160 publications, 48 book chapters, 12 books, 03 Indian patent applications and served as Guest Editors of the various international journals. He has a Google Scholar *H-Index* of 29 and over 3200 citations to his credit.

**Khalid S Alharbi Ph.D.** is an Assistant Professor at the Department of Pharmacology and Toxicology, College of Pharmacy, Jouf University, Sakaka, Saudi Arabia. He obtained both his B.Sc. (Pharmaceutical Sciences, 2002) and M.Sc. (Toxicology, 2009) at the College of Pharmacy at King Saud University (KSU), Riyadh, Saudi Arabia. He was awarded his Ph.D. (Pharmacy and Biomedical Sciences, 2016) from the University of Strathclyde, United Kingdom. Dr. Alharbi's major area of research is cancer, including drug discovery, nanomedicine, and preclinical pharmacological and toxicological studies of anticancer agents. He has published several articles in peer-reviewed journals and presented papers at numerous international conferences.

# 1 Challenges and Opportunities in the Delivery of Oral Anticancer Therapeutics

Mahfoozur Rahman, Kainat Alam, Shipra Daniel, Afroze Alam, and Sarwar Beg

**Abstract**

Global cancer prevalence has continuously increased in the last decades despite substantial progress achieved for patient care. Cancer is no longer recognized as a singular disease, but as a plurality of different ones, leading to the important choice of the drug administration route and promoting the development of novel drug-delivery systems (DDS). Due to their structural diversity, therapeutic cancer drugs present specific challenges in physicochemical properties that can adversely affect their efficacy and toxicity profile. These challenges are addressed by innovative DDS to improve bioavailability, pharmacokinetics, and biodistribution profiles. Here, we define the drug delivery challenges related to oral routes of administration and review innovative DDS, marketed or in development, that answer those challenges.

M. Rahman (✉)
Department of Pharmaceutical Sciences, Faculty of Health Sciences, SHUATS, Allahabad, Uttar Pradesh, India

K. Alam
KKECS College of Nursing, Bengaluru, India

S. Daniel
Christian College of Nursing, Faculty of Health Sciences, Sam Higginbottom University of Agriculture, Technology & Sciences, Allahabad, Uttar Pradesh, India

A. Alam
School of Pharmacy, Al-karim University, Katihar, India

S. Beg
Department of Pharmaceutics, School of Pharmaceutical Education and Research, Nanomedicine Research Lab, Jamia Hamdard, New Delhi, India



M. Rahman et al. (eds.), *Hormone Related Cancer Mechanistic and Nanomedicines*,
https://doi.org/10.1007/978-981-19-5558-7_1

**Keywords**

Tumours · Novel drug delivery · Toxicity · Bioavailability and pharmacokinetics

## 1.1 Introduction

With 9.6 million victims globally in 2018, cancer is the second biggest cause of death after cardiovascular illnesses, and cancer fatalities are expected to rise by 60% in the next two decades (Thanki et al. 2013). Since 2012, the pharmaceutical industry has been aggressively focusing its resources on the research, development, and marketing of novel oncology treatment products to meet unmet medical needs, with the US FDA having approved 90 new cancer medicines (Thanki et al. 2013). More than 30% of these novel medications are for the treatment of blood and lymph node malignancies (leukaemia, myeloma, and lymphoma), whereas 12–13% are for the treatment of lung and skin cancers. Other indications account for fewer than 10% of all newly authorized medicines (Thanki et al. 2013). Small molecules, monoclonal antibodies (mAbs), and peptides are examples of active pharmaceutical ingredients (API) utilized in cancer therapy. Small molecules are still the most common kind of cancer treatment, accounting for roughly 63% of newly authorized medications, while antibodies (Ab) are also a rapidly increasing industry, with 23 new Ab-based therapies approved since 2012. Tyrosine kinase inhibitors, such as CabometyxR, and programmed death receptor-1 (PD-1)-blocking antibodies are the most often produced therapy options within those two therapeutic groups, accounting for 33 of 46 small molecules and 9 of 22 antibody-based medicines, respectively. With the approval of Zaltrap®R, Xofigo®R, and Kymriah®R, respectively (Subklewe et al. 2019), new treatment classes such as proteins, systemic radiotherapies, or cell-based therapies are becoming increasingly common.The majority of today's cancer medicines are utilized in combination regimens (Subklewe et al. 2019). Indeed, around 30% of newly authorized medications are given in combination with one or more other therapeutic agents in order to improve therapy efficacy (Subklewe et al. 2019). Nevertheless, and in order to be effective, these therapeutic entities always require: the selection of the ideal administration routes for monotherapy and/or combination and the adjustment of the formulation and the drug-delivery systems (DDS) to provide optimal access to the targeted cancer cells (Subklewe et al. 2019). In cancer therapy, oral and intravenous (i.v) administrations are routinely employed, but subcutaneous (sc.) administration has only been used in a few cases (Subklewe et al. 2019). There are several factors to consider when deciding how to administer a therapy. There are a number of reasons why oral delivery of small molecules is the gold standard, whereas intravenous administration (IV) remains the preferred method for more problematic molecules such as biologics or nano-DDS (Subklewe et al. 2019). As a result, new formulations and DDS would be needed to enable a wider range of administration choices and to ensure that the drugs reach the cancer cells or tissues in the most effective way possible. Liposomal nano-DDS, such as Vyxeos®, Onivyde®, and Marqibo®, or

albumin-based nanoparticles, have recently been used to boost drug delivery to cancer cells, increasing therapeutic effectiveness (Porter et al. 2007). There are a number of benefits and potential to increase cancer therapy efficacy independent of the molecular class of the API that may be gained by using DDS. It is not only cancer therapies that face difficulties (Porter et al. 2007). Oral use of these drugs is not recommended due to their low stability in the gastrointestinal system and poor permeability through the intestinal epithelium (Porter et al. 2007). For example, mAbs, such as ocrelizumab (Ocrevus®) or atezolizumab (Tecentriq®), which are utilized as immunotherapeutic drugs and administered intravenously to boost the cancer immune system, are an example of this (Porter et al. 2007). It is possible that APIs have undesirable physicochemical or pharmacokinetic characteristics. Paclitaxel (Taxol®) or doxorubicin (Adriamycin®) at their therapeutic dosage, these small compounds require chemical modification or formulation development (Date et al. 2016). Due to a low therapeutic index, these active molecules may potentially have detrimental effects on normal tissues, limiting their effectiveness (Date et al. 2016). Many tumours, such as pancreatic cancer, have a thick stromal compartment or a weak or non-functional vascularization, making it difficult to administer many forms of therapy to the condition (Vaz et al. 2015). DDS is under growing pressure because of these challenges to guarantee that the proper medicine is delivered at the right time and place while reducing adverse effects on healthy cells by developing effective and hopefully non-invasive techniques (Giordano et al. 2017). There are several obstacles and possibilities involved with delivering cancer drugs via various administration routes, which we explore in this chapter in conjunction with an appropriate drug delivery system (DDS) (Giordano et al. 2017). Special attention will be given on new delivery options that might significantly contribute to transforming the cancer treatment environment.

## 1.2 Oral Anticancer Drug Delivery Route: Challenges & Opportunities

The oral route is commonly believed to be the ideal mode of administration for patients, since it is the least intrusive delivery route, and it adheres to cost-containment rules in place in most industrialized nations, supporting the transfer of cancer treatment away from hospital-based settings. Orally given medications may be designed to have a systemic impact or a local effect when their targets are in the GI tract (Thanki et al. 2013; Banerjee et al. 2017). After oral administration, drugs meant to have a systemic impact are expected to reach the circulation via passing the stomach-lining epithelium or more commonly the intestinal epithelium, and then be exposed to first-pass metabolism by the liver, depending on enzymatic stability (Thanki et al. 2013). To present, the oral route has been predominantly employed to transport tiny molecules with a molecular weight of less than 500 μg/mol, while novel drug-delivery techniques are currently contemplated to broaden this delivery route to bigger molecules, such as peptides and proteins (Thanki et al. 2013). The solubility and stability of small drugs in intestinal medium, as well as their

**Table 1.1** Challenges faced and overcome in oral anticancer drug delivery [Ref (Thanki et al. 2013), (Junyaprasert and Morakul 2015), (Maher et al. 2016), 8]

| Challenges faced | Formulation | Marketed formulation | Ref |
|---|---|---|---|
| • Poor drug stability, enzymatic degradation | • Drug-loaded nanocarriers | Polyarginine nano capsules | Thanki et al. (2013) |
| • Poor permeability across epithelium | • Permeation enhancers,<br>• Mucus-penetrating formulations, intraepithelial microneedles | Peptelligence®, Mycapssa®, Eligen®, Rybelsus®<br>Covalent coupling to CPP<br>RaniPill™, SOMA | [8], Junyaprasert and Morakul (2015) |
| • Poor solubility/poor dissolution rate | • Nanonization and lipid-based formulation | Nano crystal®<br>SMEDDS | Maher et al. (2016) |
| • GI-specific targeting drug delivery | • Gastroretentive formulation<br>• Enteric formulations<br>• Dual-responsive hydrogels (pH and microbiota responsive) for colon drug delivery.<br>• Electronic smart pills. | Star-shaped ultralong-lasting capsule,alginate/ polyacrylamide hydrogel<br>Eudragit®<br>Guar gum/PVA hydrogel<br>Intellicaps® | Thakral et al. (2012a) |

permeability across the intestinal membrane, drive their oral bioavailability, (Junyaprasert and Morakul 2015). In terms of solubility and permeability, the biopharmaceutical classifies pharmaceuticals into four categories. Oral transport of 90% of molecules was predicted to be restricted by intestinal fluid solubility (Maher et al. 2016; Thakral et al. 2012a). Table 1.1 summarizes four significant issues linked to oral medication bioavailability: insufficiency of drug solubility, GI tract drug stability, absorption, and enzyme degradation; necessity for tailored therapy administration to specific GI tract areas where cancer is present. The primary strategies proposed to overcome such issues are the creation of formulations able to boost drug solubility and permeability, the use of novel nanocarriers to improve/crossing of the intestinal epithelium, and the development of site-specific delivery systems. In order to improve low drug solubility, the drug nanonization procedure is frequently employed. Drug particle size reduction has been effectively applied to diverse molecules for many years, allowing the expansion of drug substance surface area, thereby boosting dissolving rate or perceived solubility. As a result of the increased apparent solubility, bioavailability increases 5–10 times (Junyaprasert and Morakul 2015). Medication nanocrystals have an average size of less than 1 μm and are made entirely of the drug. Solid dosage forms (capsules, tablets) or liquid suspensions of these nanocrystals can be stabilized due to their possible physical instability (polymorphism, Ostwald ripening, aggregation). Because they don't require organic solvents or extremely high pH ranges for solubilization, drug nanosuspension formulations can provide higher doses of nanonized medication in less amounts.

Mucoadhesion is thought to be aided by increased surface area due to nanocrystal particle size reduction and/or the presence of surfactants in addition to an apparent increase in solubility (Maher et al. 2016). Since the first nanocrystal medication (Emend®R) was released in 2000 (Thakral et al. 2012a), many more have followed. Recent approvals include NanoCrystals®R from Alkermes, DissoCubeTM from SkyePharma, and SolumatrixTM from iCeutica. It was also proven that making poorly soluble medications into homogenous nanosuspensions reduces interpatient variability (Rabinow 2004), which is important for cancer patients on long-term therapy. Lipid-based formulations are another way to alleviate low medication aqueous solubility (Junghanns and Muller 2008). For example, simple oily solutions of oils and cosolvents, surfactants, and cosurfactants can be prepared (Jannin et al. 2008). Microemulsions and self-micro emulsifying DDS have been extensively studied for GI medication solubility. The formulations can be packed into hard or soft capsules for oral administration due to their isotropic combination stability.

Drug solubilization, penetration, and absorption are improved by these systems, which generate lipid droplets in interaction with intestinal fluids. Self-micro emulsifying DDS can be made with drug peptides and proteins. Because lipidic formulations are mostly hydrophilic, drug loading is frequently restricted unless hydrophobic ion pairing methods and other formulation optimization approaches are applied (Griesser et al. 2017). Some examples of commercial success include the immunosuppressive peptide cyclosporine A (NeoralR) and the antiviral medicines ritonavir (NorvirR), saquinavir (FortovaseR), and tipranavir (AptivusR). These lipidic systems with permeation enhancers have the benefit of addressing both solubility and permeability (Aungst 2000). In order to boost bioavailability without causing toxicity, this technique requires simultaneous administration of the medicinal ingredient and the permeation enhancer to the absorption site. Peptides, peptide analogues, and other polar high-molecular-weight compounds are common permeation enhancers. The most promising technologies are Peptelligence R from Enteris BioPharma and TPER from Chiasma. TPER and PeptelligenceR (Maher et al. 2016) use enteric-coated solid dose forms with a permeation enhancer, acyl carnitine for PeptelligenceR (Maher et al. 2016). Solubilization and absorption of the peptide drug are improved by simultaneous release of the permeability enhancer and the peptide drug (TJ). To treat neuroendocrine tumours (NET) and acromegaly (Clinical trials. Octreotide capsules n.d.), a rare, severe illness caused by a benign pituitary gland tumour, the FDA has accepted a resubmission by Chisma. The oral GLP-1 analogue is delivered by forming a noncovalent compound with salcaprozate sodium, shielding it from digestive enzymes and enhancing its permeability through the intestinal epithelium. An oncology peptide, protein, or other big molecule might be used (Twarog et al. 2019; Davies et al. 2017). Oral delivery of fragile molecules, such as peptides or proteins, appears to be a future problem due to their limited stability in the GI tract and low bioavailability (Richard 2016). Several techniques to improve molecule stability and permeability are now being investigated (Date et al. 2016). To create prodrugs, nonpharmacologically active compounds are covalently linked to the drug, increasing permeability and decreasing metabolism (Duncan 2006). PEGylation (Benny et al. 2010) and cell-penetrating peptides (CPPs), such

as the HIV transactivator of transcription (Khafagy and Morishita 2012), were two early development tactics investigated. Identifying functional reactive groups for covalent modification of the medication without affecting its pharmacological action is crucial, as is selecting a linker strategy. This method maintains conjugate stability in the GI system while allowing for timely drug release in a therapeutically active state.

These benefits are crucial in cancer therapeutic peptide and protein delivery. Other CPPs include polyarginine and penetratin, which were employed to transport proteins or peptides like insulin (Khafagy et al. 2010; Nielsen et al. 2014; Kamei et al. 2013). Small hydrophilic medications are linked to lipidic squalene to generate an amphiphilic prodrug able to self-assemble and form nanoparticles (Couvreur et al. 2006). This method has been used for cisplatin oral administration (Kotelevets et al. 2017). Due to the carrier's nanoscale size and improved epithelial permeability associated to a specific interaction mechanism or cell-penetrating capabilities, nanocarriers have also been widely studied to boost drug bioavailability after oral delivery. One of these, polyarginine (PArg) nano capsule, was recently employed in early research for oral administration of an antitumoural peptide, elisidepsin. Their guanidine functional groups (CPPs) stimulate cellular internalization and their oily core of Mygliol®R and lecithin promotes cellular internalization. After oral administration of PArg nano capsules, in vivo studies in mice showed longer GI retention. It is probable that they developed TJ protein connections that promote paracellular transport of hydrophilic medicines due to their contact with the GI mucosa. Despite improved bioavailability over free drugs, the intestinal membrane penetration to reach the systemic circulation remains a problem for such systems. Due to their nanometre size range, nanocarriers often struggle to permeate intestinal mucus, especially in the thickest regions of the GI tract walls (Ensign et al. 2012; Lai et al. 2009). Thus, optimizing nanocarrier size and surface characteristics is required to efficiently permeate the mucus layer before turnover (Lollo et al. 2017). Oral nanocarriers like eRAPA, encapsulated rapamycin, are also in Phase II clinical trials for non-muscle invasive bladder cancer (Rapamycin Holdings n.d.). Methyl metacrylate is a pH-sensitive polymer used in eRAPA particles. When taken orally as a free medication, rapamycin is very poorly bioavailable due to its short half-life and high first-pass hepatic clearance. With improved bioavailability and safety in Phase Ib clinical trials, eRAPA (encapsulated rapamycin) has been developed (Bohan et al. 2020). Some anticancer medicines struggle to penetrate mucus in the intestinal lumen. Several new delivery techniques are now being studied in preclinical and clinical settings. These methods employ dissolvable drug-loaded microneedles embedded in gastro-resistant capsules. The microneedles are implanted into the intestinal epithelium when they reach the GI tract. The medicine inside the microneedles dissolves and enters the systemic circulation. For oral insulin delivery, MIT and Novo Nordisk have developed a self-orienting millimetre-scale applicator (SOMA). Similar to regular SC delivery, this potential technology revealed similar insulin pharmacokinetic characteristics (Abramson et al. 2019). Aside from systemic distribution, oral administration can deliver drugs to particular GI tumours including gastric, intestinal, or colorectal cancer. Several medication

delivery techniques are currently being tested. The stomach is the simplest GI area to access following oral medication delivery. However, medication residence duration in the stomach is generally brief, limiting therapeutic effectiveness. A gastroretentive dose form is included in most sophisticated systems (Abramson et al. 2019). One of such systems is an ultralong-acting capsule that delivers a continuous therapeutic dose of ivermectin against malaria for up to 14 days (Lopes et al. 2016) (not designed for cancer therapy). Another approach uses two types of alginate and polyacrylamide networks interconnected and crosslinked to generate a hydrogel capable of withstanding in vivo stomach stresses (Bellinger et al. 2016). This system can be easily dismantled by ingesting a biocompatible chelator and a reducing agent. This characteristic is essential in cancer therapy, but not limited to, halt the treatment if an adverse response occurs. The main issue in treating intestinal cancer is to avoid the harsh stomach environment and deliver the medicine solely in the more neutral gut. Polyanionic pH-sensitive polymers, which dissolve exclusively at neutral pH, are commonly used to coat enteric-coated products (Liu et al. 2017; Liu et al. 2017). These polymers (e.g. Evonik's Eudragit R) effectively shield pharmaceuticals from the acidic environment of the stomach while enabling drug release in the intestinal fluid (Thakral et al. 2012b). Oral administration may also reach the intestinal lymphatic system, which can help treat lymphoma and certain metastatic malignancies (Trevaskis et al. 2015). This delivery method might be appealing for medications sensitive to hepatic processing; however, the bioavailability of the molecules is minimal. Using lipid-based formulations and chemical changes of medications to generate lipid prodrugs is the most common strategy to target the intestinal lymphatic system (Chaudhary et al. 2014). Finally, targeting the colon may increase medication delivery to colorectal tissues, notably in colorectal cancer (Esseku and Adeyeye 2012). Reduced proteolytic activity, decreased expression of efflux transporters, and increased residence time can enhance systemic macromolecule absorption. The medicine must traverse the thick mucus layer in this portion of the GI tract to reach the colon without being discharged in the stomach or intestine (Sinha et al. 2004). To administer the anticancer medication 5-fluorouracil (5-FU) (Soppimath et al. 2000), guar gum, a natural polysaccharide, was employed. With polymers like poly(vinyl) alcohol, it can generate a pH-responsive hydrogel (Palo et al. 2017). These tactics must be modified to the illness condition, which might alter the pH and bacteria of the colon. The next generation of oral drugs may use cutting-edge technology like 3D printing (US FDA n.d.). Beyond quick prototyping, 3D printing allows for unparalleled flexibility in drug design and production, which is ideal for individualized treatments. Thus, 3D printing is gaining traction in the pharmaceutical industry, especially after the FDA-approved 3D-printed pills for epileptic seizures in 2015 (Becker et al. 2014). The Spritam tablet is created by layering powder liquid until the correct dose is reached. It provides for a large dosage of medication in a little tablet. Porous, the tablet dissolves fast in fluids, making it easier for patients who have problems swallowing to follow their treatment regimen. In the near future, individualized medicine delivery digital devices are being investigated. This includes the electronic smart pill Intellicap®R from Medi metrics Personalized Drug Delivery, which can measure individual GI residence durations,

temperature, and local pH (Mazzaferro et al. 2013). This should allow locating the capsule and regulating medication distribution to a specific area. With this smart medication delivery pill, the traditional oral administration pill will become a vital building element of a future tailored and integrated healthcare system. Table 1.1 summarizes challenges and solutions for oral anticancer medication delivery. The oral route is favoured for most drugs due to its simplicity, non-invasiveness, and improved quality of life for cancer patients (Mazzaferro et al. 2013). This mode of administration is ideal for treating gastrointestinal and colorectal malignancies, as it allows direct access to the tumour, despite the fact that medication absorption in the colon is variable. It must also be suitable for localized distribution, necessitating complicated and costly technology such as smart pills. Using lipid-based formulations, this pathway can also enable selective entrance into the systemic lymphatic system via the intestinal lymphatic network. It is also used to treat non-small-cell lung cancer, renal cell carcinoma, and hepatocellular carcinoma. Oral administration of big molecules like peptides and biologics has several obstacles in order to compete with IV administration in terms of bioavailability. The large dosage of macromolecules or small molecules to be supplied must also be considered while creating a novel oral formulation (Brayden et al. 2020). Oral administration of cytotoxic drugs with a short therapeutic window may be constrained by intra- and interpatient absorption variability, as well as treatment regimen and tolerability. Conversely, newer cancer treatments, such as noncytotoxic targeted therapies or hormone therapy, may benefit greatly from innovative oral formulations, such as nanotechnologies (O'neill and Twelves 2002; Huda et al. 2020).

### 1.2.1 Current Challenges in Oral Anticancer Drug Delivery

- The variety of available medications and their physicochemical features, the safety of administration, the possible difficulty of reaching a specific tumour site, and the complexity of treatment schemes are all challenges.
- These issues should be taken into account while developing a medicine delivery strategy and selecting an administration route.
- *The oral route is chosen by patients due to its ease and non-invasiveness.* This method confronts two significant obstacles: poor drug bioavailability (solubility and/or permeability difficulties depending on the molecule) and targeted delivery to a specific GI tract segment.
- Drug nanonization, lipid-based formulations, permeability enhancers, and gastroretentive dose forms are the key new techniques.
- Oral cancer treatment is widely utilized and suitable for gastrointestinal malignancies. It also allows specialized lymphatic system access for lymphoma and several metastatic malignancies.
- Newer treatment techniques include noncytotoxic-targeted medicines, immunotherapy, and hormone therapy which may benefit from innovative oral formulations.

## 1.3 Conclusion

Increasing knowledge of cancer pathology and development of innovative therapeutic techniques has led to the assumption that cancer is a multi-disease entity that requires well-defined and reasoned cancer therapies. In order to provide anticancer medications orally, intravenously or subcutaneously, DDS must be used efficiently. As discussed in this study, developing novel formulations has shown to increase effectiveness and toxicity profiles of various treatment approaches. It's probable that improving oral cancer drug bioavailability will remain a critical problem. To perfect delivery methods for specific drugs and indications, we believe a greater knowledge of GI barrier crossing processes is needed. Also, limited bioavailability of drugs necessitates frequent administration of large dosages of biologics or small molecules. In order to dramatically enhance patient quality of life, drug distribution difficulties must be solved. Only one anticancer medicine has been authorized by the FDA since 2013, Amgen's XgevaR.

## References

Abramson A, Caffarel-salvador E, Khang M et al (2019) An ingestible self-orienting system for oral delivery of macromolecules. Science 363:611–615

Aungst BJ (2000) Intestinal permeation enhancers. J Pharm Sci 89(4):429–442

Banerjee A, Pathak S, Subramanium VD, Dharanivasan G, Murugesan R, Verma RS (2017) Strategies for targeted drug delivery in treatment of colon cancer: current trends and future perspectives. Drug Discov Today 22(8):1224–1232

Becker D, Zhang J, Heimbach T et al (2014) Novel orally swallowable IntelliCapR device to quantify regional drug absorption in human GI tract using diltiazem as model drug. AAPS Pharm Sci Tech 15(6):1490–1497

Bellinger AM, Jafari M, Grant TM et al (2016) Oral, ultra–long-lasting drug delivery: application toward malaria elimination goals. Sci Transl Med 8(365):365ra157–365ra157

Benny O, Fainaru O, Adini A et al (2010) An orally delivered small-molecule formulation with antiangiogenic and anticancer activity. Nat Biotechnol 46(2):220–231

Bohan PMK, Cindass JL, Chick RC et al (2020) Results of a phase Ib trial of encapsulated rapamycin in prostate cancer patients under active surveillance to prevent progression. J Clin Oncol 38(Suppl. 5):34–34

Brayden DJ, Hill TA, Fairlie DP, Maher S, Mrsny RJ (2020) Systemic delivery of peptides by the oral route: formulation and medicinal chemistry approaches. Adv Drug Deliv Rev. https://doi.org/10.1016/j.addr.2020.05.007

Chaudhary S, Garg T, Murthy RSR, Rath G, Goyal AK (2014) Recent approaches of lipid-based delivery system for lymphatic targeting via oral route. J Drug Target 22(10):871–882

Clinical trials. Octreotide capsules (n.d.). https://clinicaltrials.gov/ct2/show/NCT03252353

Couvreur P, Stella B, Harivardhan Reddy L et al (2006) Squalenoyl nanomedicines as potential therapeutics. Nano Lett 6(11):2544–2548

Date AA, Hanes J, Ensign LM (2016) Nanoparticles for oral delivery: design, evaluation and state-of-the-art. J Control Release 240:504–526

Davies M, Pieber TR, Hartoft-Nielsen ML, Hansen OKH, Jabbour S, Rosenstock J (2017) Effect of oral semaglutide compared with placebo and subcutaneous semaglutide on glycemic control in patients with type 2 diabetes a randomized clinical trial. J Am Med Assoc 318(15):1460–1470

Duncan R (2006) Polymer conjugates as anticancer nanomedicines. Nat Rev Cancer 6(9):688–701

Ensign LM, Cone R, Hanes J (2012) Oral drug delivery with polymeric nanoparticles: the gastrointestinal mucus barriers. Adv Drug Deliv Rev 64(6):557–570
Esseku F, Adeyeye MC (2012) Bacteria and pH-sensitive polysaccharide-polymer films for colon targeted delivery. Crit Rev Ther Drug Carr Syst 28(5):395–445
Giordano G, Pancione M, Olivieri N et al (2017) Nano albumin bound-paclitaxel in pancreatic cancer: current evidences and future directions. World J Gastroenterol 23(32):5875–5886
Griesser J, Hetenyi G, Moser M, Demarne F, Jannin V, Bernkop-Schnurch A (2017) Hydrophobic ion pairing: key to highly pay loaded self-emulsifying peptide drug delivery systems. Int J Pharm 520(1–2):267–274
Huda S, Alam A, Sharma PK (2020) Smart nanocarriers-based drug delivery for cancer therapy: an innovative and developing strategy. J Drug Deliv Sci Technol 60:102018
Jannin V, Musakhanian J, Marchaud D (2008) Approaches for the development of solid and semi-solid lipid-based formulations. Adv Drug Deliv Rev 60(6):734–746
Junghanns JUAH, Muller RH (2008) Nanocrystal technology, drug delivery and clinical applications. Int J Nanomedicine 3:295–309
Junyaprasert VB, Morakul B (2015) Nanocrystals for enhancement of oral bioavailability of poorly water-soluble drugs. Asian J Pharm Sci 10(1):13–23
Kamei N, Kikuchi S, Takeda-Morishita M et al (2013) Determination of the optimal cell-penetrating peptide sequence for intestinal insulin delivery based on molecular orbital analysis with self-organizing maps. J Pharm Sci 102(2):469–479
Khafagy ES, Morishita M (2012) Oral bio drug delivery using cell-penetrating peptide. Adv Drug Deliv Rev 64(6):531–539
Khafagy ES, Morishita M, Ida N, Nishio R, Isowa K, Takayama K (2010) Structural requirements of penetratin absorption enhancement efficiency for insulin delivery. J Control Release 143(3): 302–310
Kotelevets L, Chastre E, Caron J et al (2017) A squalene-based nanomedicine for oral treatment of colon cancer. Cancer Res 77(11):2964–2975
Lai SK, Wang YY, Hanes J (2009) Mucus-penetrating nanoparticles for drug and gene delivery to mucosal tissues. Adv Drug Deliv Rev 61(2):158–171
Liu J, Pang Y, Zhang S et al (2017) Triggerable tough hydrogels for gastric resident dosage forms. Nat Commun 8(1):1–9
Liu L, Yao WD, Rao YF, Lu XY, Gao JQ (2017) pH-responsive carriers for oral drug delivery: challenges and opportunities of current platforms. Drug Deliv 24(1):569–581
Lollo G, Gonzalez-Paredes A, Garcia-Fuentes M, Calvo P, Torres D, Alonso MJ (2017) Polyarginine nanocapsules as a potential oral peptide delivery carrier. J Pharm Sci 106(2): 611–618
Lopes CM, Bettencourt C, Rossi A, Buttini F, Barata P (2016) Overview on gastroretentive drug delivery systems for improving drug bioavailability. Int J Pharm 510(1):144–158
Maher S, Mrsny RJ, Brayden DJ (2016) Intestinal permeation enhancers for oral peptide delivery. Adv Drug Deliv Rev 106:277–319
Mazzaferro S, Bouchemal K, Ponchel G (2013) Oral delivery of anticancer drugs I: general considerations. Drug Discov Today 18(1–2):25–34
Nielsen EJB, Yoshida S, Kamei N et al (2014) *In vivo* proof of concept of oral insulin delivery based on a co-administration strategy with the cell-penetrating peptide penetratin. J Control Release 189:19–24
Oneill VJ, Twelves CJ (2002) Oral cancer treatment: developments in chemotherapy and beyond. Br J Cancer 87(9):933–937
Palo M, Hollander J, Suominen J, Yliruusi J, Sandler N (2017) 3D printed drug delivery devices: perspectives and technical challenges. ¨. Expert Rev Med Devices 14(9):685–696
Porter CJH, Trevaskis NL, Charman WN (2007) Lipids and lipid-based formulations: optimizing the oral delivery of lipophilic drugs. Nat Rev Drug Discov 6(3):231–248
Rabinow BE (2004) Nanosuspensions in drug delivery. Nat Rev Drug Discov 3(9):785–796

Rapamycin Holdings I DBA EB (n.d.) NCT04375813: trial of encapsulated rapamycin (eRAPA) for bladder cancer prevention. Clinical Trialsgouv. https://clinicaltrials.gov/ct2/show/NCT04375813

Richard J (2016) Challenges in oral peptide delivery: lessons learnt from the clinic and fututre prospects. Ther Deliv 7(2):117–138

Sinha VR, Mittal BR, Bhutani KK, Kumria R (2004) Colonic drug delivery of 5-fluorouracil: an *in vitro* evaluation. Int J Pharm 269(1):101–108

Soppimath KS, Kulkarni AR, Aminabhavi TM (2000) Controlled release of antihypertensive drug from the interpenetrating network poly(vinyl alcohol)–guar gum hydrogel microspheres. J Biomater Sci Polym Ed 11(1):27–43

Subklewe M, Von Bergwelt-Baildon M, Humpe A (2019) Chimeric antigen receptor T cells: a race to revolutionize cancer therapy. Transfus Med Hemotherapy 46(1):15–24

Thakral S, Thakral NK, Majumdar DK (2012a) Eudragit®: a technology evaluation. Expert Opin Drug Deliv 10(1):131–149

Thakral S, Thakral NK, Majumdar DK (2012b) EudragitR: a technology evaluation. Expert Opin Drug Deliv 10(1):131–149

Thanki K, Gangwal RP, Sangamwar AT, Jain S (2013) Oral delivery of anticancer drugs: challenges and opportunities. J Control Release 170(1):15–40

Trevaskis NL, Kaminskas LM, Porter CJH (2015) From sewer to saviour-targeting the lymphatic system to promote drug exposure and activity. Nat Rev Drug Discov 14(11):781–803

Twarog C, Fattah S, Heade J, Maher S, Fattal E, Brayden DJ (2019) Intestinal permeation enhancers for oral delivery of macromolecules: a comparison between salcaprozate sodium (SNAC) and sodium caprate (c10). Pharmaceutics 11(2):1–21

US FDA. Spritam. (n.d.). www.accessdata.fda.gov/drugsatfdadocs/nda/2015/207958Orig1s000TOC.cfm

Vaz J, Ansari D, Sasor A, Andersson R (2015) SPARC: a potential prognostic and therapeutic target in pancreatic cancer. Pancreas 44(7):1024–1035

# Nanotechnology in the Management of Hormonal Cancer

2

Anas Ahmad, Rajan Swami, Teenu Sharma, and Atul Jain

**Abstract**

Hormone-related cancers, specifically breast, endometrium, ovary, and prostate, show a distinctive mechanism of carcinogenesis. The occurrence of cancer depends upon the endogenous and exogenous hormones-driven cell proliferation that also leads to genetic modification. Management of hormonal cancer includes the implementation of newer strategies for early detection and prevention of hormonal cancer metastasis. Owing to the limitation of the traditional drug delivery system, advanced targeting drug delivery systems is employed in the management of hormonal cancer. Nanotechnology-based drug delivery systems like lipid-based carrier systems (solid lipid nanoparticles), liposomes, polymeric nanoparticles, inorganic nanoparticles, caron nanotubes, and other carrier systems are routinely employed. Besides, peptide and nucleic acid-based (DNA and siRNA) technology is recently used to target the desired receptors which are over-expressed over tumour mass.

**Keywords**

Hormonal cancer · Nanotechnology · Nanocarriers · Receptor · Hormone

A. Ahmad
Department of Pharmacology, Chandigarh College of Pharmacy, Landran, Mohali, India

R. Swami
Chitkara College of Pharmacy, Chitkara University, Punjab, India

T. Sharma
Chitkara College of Pharmacy, Chitkara University, Punjab, India

A. Jain (✉)
Department of Pharmaceutics, Delhi Institute of Pharmaceutical Science and Research, New Delhi, India



M. Rahman et al. (eds.), *Hormone Related Cancer Mechanistic and Nanomedicines*,
https://doi.org/10.1007/978-981-19-5558-7_2

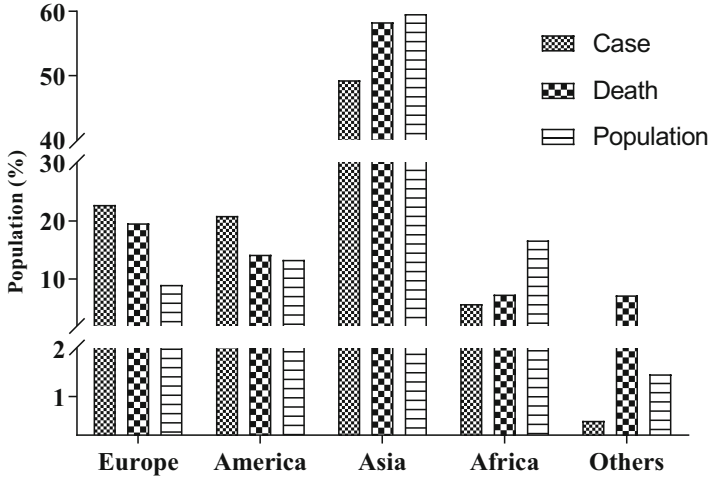



**Fig. 2.1** Population-case-death data of cancer patients across various continent

## 2.1 Introduction

Cancer, a proliferative syndrome, has a multifaceted character with unlimited growth and affects mankind for decades as a leading cause of death worldwide (Khurana et al. 2018). It comprises of a group of varied tissue responses due to mutation(s) in genetic material, resulting eventually in the uncontrolled growth, invasion and destruction of adjacent tissues, and at times, metastasis to other locations in the body via liquid tissues (Qiao et al. 2015). Globally, the cancer burden is estimated to have risen to 19.3 million new cancer cases and 10.0 million deaths in 2020 and is anticipated that there will be 28.4 million new cancer cases by 2040 and approximately 17 million cancer-caused mortalities every year (Sung et al. 2021). Figure 2.1 illustrates the overall percentage of the population affected by cancer, across the globe. Besides, in India, it is estimated that around 13.24 Lac cases are bearing the disease in the year 2020 with an annual progression of 1.2 million new cases and 8.5 Lac deaths (National Institute of Cancer Prevention and Research (NICPR). Cancer Statistics India 2020). Further, in India alone, it has been estimated that by 2020, the number of patients affected with cancer may reach beyond 15 million. The most prevalent cancer among males includes prostate, colon, rectum, and melanoma, while among the females, predominant ones are breast, uterine corpus, and colorectal carcinoma (Howlader et al. 2021).

### 2.1.1 Cancer Classification and Pathophysiology

Cancer can be classified into two categories, based on the type of tissue and location, viz. (a) Histological type (the type of tissue) and (b) primary site (location in the body). Cancer cells differ from normal cells in size, structure, function, and growth rate (Cooper 2000). Malignant cells grow uncontrollably and invade adjacent structures and destroy surrounding tissues and organs through the cardiovascular or lymphatic system metastasis (Seyfried and Huysentruyt 2013). Cancerous cells lose their ability to differentiate and prevent themselves from performing the normal essential functions required by the tissues, resulting in a variety of other tissue changes in the body, viz., pain, cachexia, lowered immunity, anaemia, leukopenia and thrombocytopenia, etc. However, a cluster of abnormalities is required before a normal cell transforms into an uncontrolled proliferating mass (Shigdar et al. 2014; Anand et al. 2008). Primarily, the development of cancer depends both on internal (genetic factors-inherited mutations, immune conditions, and hormones) and external (lifestyle factors like use of tobacco, diet, infectious organisms, environmental exposure to chemicals and radiation) factors and later on abnormalities in the genetic material (tumour initiator mutagenic and tumour promoter non-mutagenic agents) caused by the said factor together resulting in the tumour formation (Ali et al. 2011). Inherited cancers tend to occur earlier in life and typically cause multiple growths in the same organ. Although some cancers appear to follow racial and ethnic lines, it is difficult to segregate genetic influences from environmental and lifestyle factors as the major cause of cancer (Maskarinec et al. 2011). Alteration in the genetic makeup of normal cells can cause their transformation into cancerous cells (American Cancer Society. Breast Cancer 2019a). However, most DNA changes linked to breast cancer are acquired, which take place over time. There is a set of genes that regulate the growth, division, and apoptosis of cells. Changes in genetic makeup can cause the cells to depart from their physiology and are linked to cancer (Davis and Lin 2011). Malignant cells lose their ability to perform the regular functions required by the tissue resulting in pain, cachexia, lowered immunity, anaemia, thrombocytopenia, and leucopenia (Baba and Câtoi 2007). This uncontrolled cellular growth and spread of cancer cells are regulated by proteins produced in cells, which are altered or mutated by environmental factors, alteration in gene replication, or repair processes. Uncontrolled growth can eventually interfere with one or more physiological functions and possibly lead to death too. The primary sites of cancer metastasis are bone, lymph nodes, liver, lungs, and brain (Learner 2019).

### 2.1.2 Theory of Cancer Initiation

The development of cancer is a complex process and is interconnected with factors related to environmental exposure, lifestyle practices, medical interventions, genetic traits, viruses, familial susceptibility, and ageing. Interactions between repeated carcinogenic exposure and an individual's susceptibility status might be the most probable reason for the development of cancer (Garg et al. 2019). According to Rous

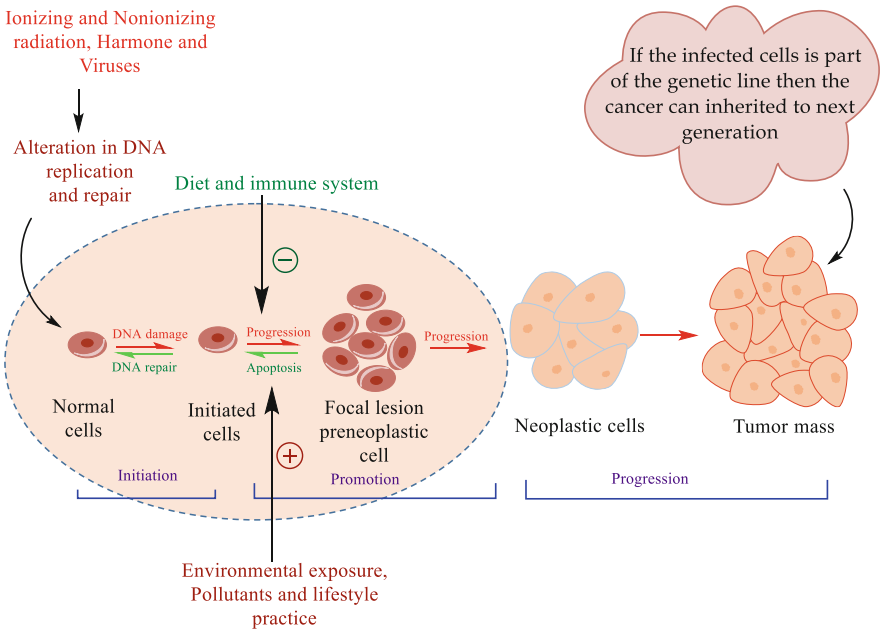



**Fig. 2.2** Theory of Initiation-Promotion-Progression

**Table 2.1** Role of the commonly associated genes in cancer development

| Name of Gene | Impression |
|---|---|
| Proto-oncogenes | Responsible for normal cell growth; on mutation, it converts into a "bad" gene and called an oncogene. When oncogene remains active, even its activity is not required, resulting in uncontrolled cell growth that may lead to cancer |
| Tumour suppression genes | BRCA genes (BRCA1 and BRCA2) are normal tumour suppressor genes that slow down cell growth, repair DNA alterations, and help in the management of apoptosis. Alteration in BRCA genes leads to abnormal cell growth resulting in cancer |
| Inherited gene | Transfer of inherited mutated altered gene takes place from a parent to a child that increases the chance of occurrence of cancer |
| Acquired gene | Mutation in a gene related to breast cancer takes place during her life-span and leads to changes which may arise due to environmental or lifestyle of the person |

and Kidd (Rous and Kidd 1941), the probable theory behind the cause of cancer is the *"Initiation-Promotion-Progression"* theory, where the multiple exposure and mechanisms factors are responsible for developing cancer, as shown in Fig. 2.2. Besides, Table 2.1 enlists a description of some common genes associated with cancer development (Sledge et al. 2014).

## 2.2 Hormonal Cancer

A hormone is a natural chemical substance, produced in the body, transported in tissue fluids to regulate each vital activity of our body system. However, they're also handy for some types of cancer to grow and spread, leading to hormone-sensitive or hormone-dependent cancer. Hormone-dependent cancers are identified as the presence of proteins on their cell surfaces known as receptors. Receptors play with hormones like a "lock and key" (Casey et al. 2015). Hormone-dependent cancer shows a distinctive mechanism of carcinogenesis. Cell proliferation via endogenous and exogenous hormones drives cell proliferation, and thus the opportunity for the accumulation of random genetic errors (Henderson and Feigelson 2000). Cancer affected by hormones show some common side effects such as nausea, weakness, unexplained weight loss or weight gain, loss of body hair, feeling cold, feeling tired or weak, menstrual changes or loss of menstrual periods in women, and erectile dysfunction (trouble with erections) in men. Most common cancers which are fueled by hormones include breast cancer (estrogen and progesterone), ovarian cancer (estrogen), uterine or endometrial cancer (estrogen and progesterone), and prostate cancer (testosterone and similar hormones) and can help in growing and spreading. Other hormone-based cancers include cancer of the thyroid, pancreas, testis, pituitary, osteosarcoma, and adrenal (Biggers 2020; Nwadike 2018).

### 2.2.1 Types of Hormonal Cancer

#### 2.2.1.1 Breast Cancer

Breast cancer is one of the leading causes of death in women (15%), followed by lung cancer (Sung et al. 2020). It holds the second and fifth position in occurrence and mortality, respectively, and it is the leading type at a global scale in terms of the occurrence of new cases (WHO 2018). Although it is not an epidemic, the reports in recent years are spine chilling with approximately 2.3 lac females suffering from breast cancer in the USA alone. In the Western world, approximately 1 in 11 develop the malignancy and 1 in 30 dying from the disease (Brown et al. 2017). The tumour is malignant if the cells can grow into (invade) surrounding tissues or spread (metastasize) to distant areas of the body where cells usually form a tumour that can often be seen on an X-ray or felt as a lump. A small number of cancers start in other tissues in the breast. These cancers are called sarcomas and lymphomas and are not really thought of as breast cancers. Non-cancerous breast tumours have abnormal growths, but they do not spread outside of the breast and they are not life-threatening (Society AC 2019). However, some benign breast lumps can increase a woman's risk of getting breast cancer. Most of the breast cancers begin in the ducts (ductal cancers) or glands (lobular cancers). Clinically, estrogen and progesterone are the most suitable cell surface markers in breast cancer, which are used to predict response to hormone therapy. Besides, genes also play a significant role in the pathophysiology of the cancer.

Propagation of breast cancer takes place when the cancer cells get metastasized via blood or lymph to different parts of the body, like lungs, liver, bone, brain, and skin (Leyrer et al. 2017). The lymphatic system has higher susceptibility to participate in the spreading of cancer to other parts of your body, as lymph nodes are small, bean-shaped collections of immune system cells (metastasized) (Sandhu et al. 2017). Most of the lymph vessels of the breast drain into axillary lymph nodes (present under the arm), supraclavicular and infraclavicular lymph nodes (present around the collar bone), and internal mammary lymph nodes (present in the chest near the breast bone) (American Cancer Society 2019a, b).

#### 2.2.1.2 Cervical Cancer

Cervical cancer is the fourth most common cancer in women worldwide. Cervical cancer is nearly always caused by infection with human papillomavirus (HPV). Despite being highly preventable, cervical cancer is the fourth most common cancer and cause of cancer death in women globally (Castle et al. 2021). The most carcinogenic form of HPV is the high-risk human papillomavirus 16 (hrHPV16). However, only a few hrHPV16 infections develop into cancer. Demographic variations within the HPV16 genotype have been proposed to give differentiation into carcinogenicity (Singh et al. 2017).

#### 2.2.1.3 Endometrial Cancer

The uterus is the pear-shaped pelvic hollow muscular organ, where the development of fetal occurs. Endometrial cancer begins in the uterus, where malignant cancer cells form in the tissues of the endometrium (lining of the uterus) (Ameer et al. 2020). Endometrial cancer begins in the layer of cells that form the lining of the uterus. Endometrial cancer is sometimes called uterine cancer and it is different from cancer of the muscle of the uterus known as sarcoma of the uterus. Signs and symptoms of endometrial cancer include unusual vaginal bleeding or pain in the pelvis. Besides, obesity, a person having metabolic syndrome, women taking tamoxifen or estrogen alone (without progesterone) for breast cancer may increase the risk of endometrial cancer (Kim et al. 2013). Other risk factors for endometrial cancer include hormone replacement therapy only after menopause, use of tamoxifen in the management of breast cancer, type 2 diabetes, exposure of endometrial tissue to estrogen produced in the body by never giving birth, menstruating at an early age and starting menopause at a later age, presence of polycystic ovarian syndrome, family history of endometrial cancer, genetic symptoms like Lynch syndrome, endometrial hyperplasia, etc.

#### 2.2.1.4 Prostate Cancer

Prostate cancer is dependent on testosterone and similar hormones that can help it grow and spread. Androgens-dependent prostate cancer is one of the most common cancers that occur in men. It is affected by testosterone and similar related hormones. As compared to other cancer, its progression rate is very slow and finding and treating it before symptoms occur may not improve men's health. Adenocarcinoma and neuroendocrine are the most common and less common histologic types

observed in prostate cancer (Rawla 2019). Cells of the prostate gland secrete Prostate-specific antigen (PSA), a protein in the bloodstream. The highest concentration is indicative of prostate cancer. Besides, ejaculation can temporarily enhance the PSA levels. Overall management of prostate cancer is purely based on the size, location, and the type of cancer (malignant and Benign). Hormone therapy is associated with persons with large advanced tumours, also known as androgen deprivation therapy (ADT), along with radiation therapy. Further, if it no longer responds to ADT, it is termed castration-resistant prostate cancer. The latter was treated with chemotherapy, immunotherapy, radiation therapy, or other newer treatment options, such as new drugs that target androgen. Incontinence, bone pain and weakness, and sexual problem are the major side effects that can be managed with the suggestion of health care professionals (Society AC 2021).

## 2.3 Limitations of Conventional Treatment and Opportunities for Cancer Treatment

Surgery, radiation therapy, chemotherapy, hormonal therapy, and immunotherapy are considered to be the most frequently employed therapies for the treatment of breast cancer. Surgical removal of the tumour and surrounding tissue is preferred for cancer treatment in the chronic stages (Gonzalez-Angulo et al. 2007). However, the foremost drawbacks of surgery are severe pain, the necessity of hospitalization, and chances of remaining cancerous tissue in the affected area. On the other hand, treatment with radiation therapy, involving the use of high-energy electromagnetic waves to eradicate cancer cells, is challengeable in the context of patient safety (Sledge et al. 2014). Among all the approaches, the most frequently employed is chemotherapeutic treatment using diverse anticancer drugs. Nevertheless, the major pitfalls associated with chemotherapy include extra-vascularization, higher drug resistance, lack of site-specific targeting, drug elicited and solvent-associated adverse effects, and nonselective cytotoxicity, leading eventually to poor patient compliance and submaximal therapeutic benefits (Selimah Fauzee et al. 2011; Mettler et al. 2014).

## 2.4 Targeted Drug Delivery

Targeted drug delivery plays an important role in the management of cancer treatment as drug molecule gathers at the specified site of the body selectively, with a low toxicity profile, independent of the site and mode of its administration (Bae and Park 2011). Delivery of anticancer molecule should be specific, precise in a sustained and controlled manner via suitable biological events (receptor targeting) which also overcome the non-specific toxic effect and accumulation of drug molecule as observed conventional drug delivery (Senapati et al. 2018). Table 2.2 Advanced nanocarriers systems facilitate the accumulation of drug molecules in the desired site and remain in the bloodstream for the longest time because of the

**Table 2.2** Various types of nanocarriers used in drug delivery

| Type of Carrier | Type of the carrier | Name of the carrier |
|---|---|---|
| Colloidal carrier | Vesicular | Liposome, Niosome, Pharmacosome, Virosomes, Immunoliposome |
| | Particulate carrier | Biodegradable polymeric /lipidic nanoparticle, Nanocapsule, nanocomposite, microsphere, microparticle, microsponge |
| Cellular carrier | Biological | Resealed erythrocyte, albumin, antibodies, platelets, leukocytes |
| Supra molecular delivery system | Bio-polymer | Polymeric micelles, lipid crystals, lipoprotein |
| Macromolecular carrier | Protein/ glycoprotein | Neo-glycoprotein, artificial viral envelops |
| Others | Glycosylated hydrophilic polymer | Poly-L-lysine, |
| | Monoclonal antibodies | Antibody-antigen complex, bispecific antibodies |
| | Lectin | Polysaccharides |

EPR effect (Golombek et al. 2018). Different levels of targeting can be achieved after an alteration of nanocarriers as targeting towards discrete (i) organ (ii) specific cell type within a tissue or organ, and (iii) specific intracellular compartment in the cells (Jain et al. 2018). Table 2.3 summarizes the current chemotherapy option available for major hormonal cancer.

## 2.5 Advanced Drug Delivery System for the Management of Hormonal Cancers

Despite the multiple challenges for effective delivery of the existing chemotherapeutic agents, it has been revealed from the vast literature reports that delivery of such drugs through nanostructured drug delivery systems (DDS) has demonstrated immense therapeutic benefits (Singh et al. 2009). Frequently explored nanocarrier systems include lipid-based nanoparticles (SLNs and NLCs), liposomes, polymeric nanoparticles, lipid-polymer hybrid systems, lyotropic-liquid crystals, carbon nanotubes, dendrimers, nanomaterials, nanocomposites, etc. (Jain et al. 2020), owing to their minuscule size, high aspect ratio, ability to get functioned and rescued from the macrophage defence mechanism, and enable these nanocarriers to move into the cancer cells transcellular (Kapse-Mistry et al. 2014). Besides, the nanostructured systems also provide the desired enhancement in the absorption potential of the "difficult" drugs, site-specific delivery of the drugs to the affected site, dose reduction, controlled release action, excellent encapsulation ability, enhanced distribution potential, and the overall improvement in the biopharmaceutical performance (Mussi et al. 2013). In the industrial milieu, these nanostructured systems are also considered to be relatively more amenable to product development,

**Table 2.3** List of different major hormonal cancer and associated chemotherapy

| Name of the cancer | Name of the therapy/ Drug | Examples | Therapy | Side/ Adverse effects |
|---|---|---|---|---|
| Breast cancer | Aromatase inhibitors | Anastrozole (Arimidex®), letrozole (Femara®) and Exemestane (Aromasin®) | Used in menopause condition when production of estrogen reduces significantly In premenopausal women, production of aromatase is high to inhibitors to work effectively These are used to shrink tumors before surgery, and after surgery to prevent reoccurrence of breast cancer | Hot flashes, night sweats and vaginal dryness or atrophy, similar to symptoms of menopause (amount of estrogen decrease in the body). Increase the risk for heart attack, chest pain (angina), heart failure, high cholesterol, bone loss, joint pain, depression, and mood swings. |
| | Selective estrogen receptor modulators (SERMs) | Tamoxifen (Nolvadex®), Taloxifene (Evista®), and toremifene (Fareston®) | To prevent reoccurrence of breast cancer, it is given after surgery for early ER-positive breast cancer in men or women. They're also approved to treat advanced breast cancer and may be used to prevent breast cancer in high-risk individuals. | Hot flashes, blood clots, stroke, bone loss, mood changes, depression and loss of sex drive, headaches, nausea, vomiting, rashes, impotence, and loss of sex drive (tamoxifen side effect). Stroke or developing potentially fatal blood clots in the lungs or legs (Raloxifene). |
| | Toremifene is only approved for advanced stage breast cancer that has spread | | | |
| | Fulvestrant | Faslodex | Fulvestrant is approved for female candidate who has advanced ER-positive breast cancer after treatment hormone therapy. Also prescribed for postmenopausal | Nausea, vomiting, constipation, fatigue, back pain, bone pain, joint pain, headaches, hot flashes, and breathing issues |

(continued)

**Table 2.3** (continued)

| Name of the cancer | Name of the therapy/ Drug | Examples | Therapy | Side/ Adverse effects |
|---|---|---|---|---|
| | | | women with ER-positive, HER2-negative cancers | |
| Cervical cancer | Chemo with targeted therapy, biosimilar | Bevacizumab (Avastin), Bevacizumab-awwb (Mvasi) and bevacizumab-bvzr (Zirabev), Tisotumab vedotin (HuMax-TF) | Treatment checks the growth and spreading of the cancer cells especially in recurrent cancer patients, and for the treatment of recurrent or metastatic cervical cancer that has progressed during or after chemotherapy | Skin reactions, flu-like symptoms, diarrhea, and weight changes |
| Endometrial cancer | Tamoxifen, sLHRH agonists, aromatase inhibitors | Anastrozole (Arimidex®), letrozole (Femara®) and Exemestane (Aromasin®) | Hormone therapy is typically reserved for advanced uterine or endometrial cancer, or for reoccurrence cases, generally it's often combined with chemotherapy | Constipation, fatigue, back pain, bone pain, joint pain, headaches, hot flashes, and breathing issues |
| Prostate cancer | Androgen deprivation therapy (ADT) (LHRH agonists, or GnRH agonists) | Leuprolide (Lupron®, Eligard®), Goserelin (Zoladex®), Triptorelin (Trelstar®) or Histrelin (Vantas®). | Used to lower the amount of testosterone, drugs are given as a small implant skin | Lack of sexual interest, shrinkage of testicles and penis, breast tenderness, bone thinning and fractures, low red blood cells (anemia), brain fog, loss of muscle mass, weight gain, fatigue, high cholesterol, depression |
| | Androgen blockers | Testosterone Enzalutamide (Xtandi®), Apalutamide (Erleada®) and | Androgen blockers if an orchiectomy or an LHRH agonist or | Diarrhea, fatigue, rash and worsening hot flashes, dizziness and seizures are |

(continued)

**Table 2.3** (continued)

| Name of the cancer | Name of the therapy/ Drug | Examples | Therapy | Side/ Adverse effects |
|---|---|---|---|---|
| | | Darolutamide (Nubeqa®). | antagonist is no longer working | more severe, but less common, side effects |

pilot plant scale-up, and subsequent commercialization. These systems have been metamorphosed as one of the most effective drug delivery technologies of the twenty-first century to combat multiple cancers (Nakamura et al. 2012).

## 2.6 Nanomedicine-Based Therapies for Hormonal Cancers

The multifold challenge is evident for targeting to investigate selective target, to find the drug specific to the disease/condition, and (Cavanagh et al. 1995) to have information about drug carrier (Tannock 1998). The effective tumour targeting of NPs leads to having better control over the release of the drugs from a carrier, effective in vivo fate (pharmacokinetics and pharmacodynamics), augmented uptake, and cytoplasmic delivery with alleviated toxicity levels. Cancer targeting involves "passive targeting" and its interrelated but refined and effective way of targeting, i.e. "Active targeting". Tumour nanotechnology-based therapeutics are swiftly moving forward with the capacity to answer numerous restrictions of conventional therapeutics such as unspecific biodistribution, low therapeutic indices, and lack of water solubility.

Passive targeting consists of the transport of nanocarriers through leaky tumour capillary fenestrations into the tumour interstitium and cells by convection or passive diffusion. Selective accumulation of nanocarriers and drugs then occurs by the enhanced permeability and retention effect (EPR) that has become the gold standard in tumour targeting, especially in all the rapidly growing solid tumours (Haley and Frenkel 2008; Maeda et al. 2000, 2009). Indeed, the EPR effect is pertinent in practically all human cancers except hypovascular tumours, i.e. pancreatic cancer or prostate cancer, etc.(Maeda et al. 2001). Passive targeting always consolidates the significantly lower toxicity with active targeting as the latter assists in increasing the drug titer in tumour viz *a* viz passive targeting. This can be achieved using second-order (cell-specific) targeting of the drug-taking help of tumour microenvironment characteristics like pH, temperature, and receptor overexpression, leading to the reduction of dose and side effects of the drug. Although the role of the receptor and ligands has already been explained, carriers do perform an imperative role in carrying a drug to the site of action, i.e. specific cells.

Currently, cancer therapy has become a multidisciplinary challenge requiring close collaboration among clinicians, biological and materials scientists, and biomedical engineers. Conventional chemotherapeutic agents are distributed

non-specifically in the body affecting both normal and tumour cells. Given the potency of modern pharmacological agents, tissue selectivity is a major issue. Hence, the dose achievable within the solid tumour is limited resulting in suboptimal treatment due to excessive toxicities. The ultimate goal of cancer therapeutics is to increase the survival time and improve the quality of life of the patient by reducing the systemic toxicity of chemotherapy (Byrne et al. 2008). The idea of exploiting vascular abnormalities of tumours, avoiding penetration into normal tissue interstitium while allowing access to tumours, becomes particularly attractive. In this context, the tumour targeting of nanomedicine-based therapeutics has emerged as one approach to overcome the lack of specificity of conventional chemotherapeutic agents. Carrier is one of the most important entities required for the successful transportation of loaded drugs.

The choice of carrier system depends upon the target cells that should be reached and the choice of drug that needs to be delivered. Drug delivery carriers can be classified into two major types, i.e. soluble and cellular carriers. Soluble carrier helps to deliver the drug to the systemic circulation or assist in drug delivery to the tumour micro-environment. However, cellular carriers help in the localization of a drug to the defined cells. Other strategies, like, polymeric or lipidic carriers carrying physically or chemically enclosed/conjugated drugs are apt choices against tumour targeting. There are a heap of carrier systems that can be taken into account to design an active drug targeting system. But every nanoparticulate carrier system has its advantages and disadvantages. However, their specific characteristics assist to choose the desired system for specific cancer.

Nanotherapeutics emerged as a refuge for the conventional drug delivery to target the conventional drugs to target them to tumour environ. Nanoparticles are solid architectures with at least one dimension having a size less than 1000 nm. They can either be "nanospheres" with the drug dispersed in a polymeric lipid matrix, or "nanocapsules" where the drug is enclosed in a cavity made by the layer of polymer or lipids (Meier 2000; Caban et al. 2014). Predominantly, they comprise of a framework (polymer or lipids), where contrast agents/drugs, etc. are attached or encapsulated in the matrix of polymeric/lipidic. This architecture assists in improving the pharmacokinetics and biodistribution of the drug. Furthermore, a ligand potentiates directing the nanoparticle specifically to the tumour cell itself. Depending upon the selection of scaffold, many nanocarriers have emerged in the arena of drug delivery. However, drug delivery systems having significance with current work are explained here onwards.

Targeting through ordinary or conventional nanotherapeutic is non-specific and can cause toxicity due to extra pyramidal side effects. Cell machinery got impaired in a diseased state and this resulted in diseased machinery that can be explored for designing new vehicles. A few years ago, Hanahan and Weinberg (Hanahan and Weinberg 2011) tallied six important hallmarks of cancer (which was further increased by the same group); these hallmarks are vital for a cell to acquire its way to master its destiny to become a tumour. These hallmarks include (1) ability of cancer cells to stimulate growth signals; (2) uncontrolled multiplication ability; (3) evading apoptosis; (4) downregulate anti-growth signals; (5) angiogenesis to

develop new blood vessels to supply nutrients to the tumour; and (6) stimulate metastasis and invasion. A keen understanding of the hallmarks helps researchers to develop better and efficient targeted cellular drug delivery carrier systems.

Through these hallmarks, cancer cells become controllers in developing the disease by deregulating tumour pathogenesis. Due to all such hallmarks, the cancer cells become rigid and gather the ability to mutate. Abundant understanding in the area of tumour pathogenesis has not helped us to translate the knowledge into clinical findings against cancer. The non-specific distribution of the chemotherapy in the body leads to unwanted toxicity to normal cells. Although as mentioned earlier, by developing the hallmarks the cancers cells become the controller of their fate, these traits can be used against the tumour itself by developing a carrier system that contains ligand specific to over-expressed receptors over tumour cells or by EPR effect. These ligands include small chemical entities, carbohydrates, polysaccharides, peptides, nucleic acid, monoclonal antibodies, or hormones that have an affinity for the over-expressed receptors in the cell surface. Thus, ligand-associated nanoparticles further potentiate the action of NPs by active targeting, which has a special role in targeting 'Hormonal Cancers'. Jain et al. discussed a brief account of ligands, receptors, and different types of targeting in drug delivery in the management of cancer treatment (Jain et al. 2018). In the following sections, nanoparticles were discussed in detail concerning their inherent material.

### 2.6.1 Lipidic Nanoparticles

Lipidic drug delivery systems emerged as the most potent drug delivery system due to their higher stability and targeting efficacy. Till now, lipidic nanoparticles (NPs) are the most advanced and highly explored carrier system in the pharmaceutical research domain. Lipidic drug delivery system contains numerous types of NPs, i.e. Solid lipid nanoparticles, Liposomes, Lipid conjugate, etc. Many scientists explored the use of estrogen derivatives to be used as targeting moiety to target these tumours. Salkho et al. used an estrogen derivative, estrone, to develop a doxorubicin-loaded lipidic vesicular system for estrogen receptor targeting to estrogen receptor-positive cancer cells. The estrone-conjugated liposomes showed notably higher retention in MCF-7 (ER (+) cells) than the MDA-MB-231 cells (ER (−) cells), dictating the targeting potential of estrone (Salkho et al. 2018). Similarly, Paolino et al. demonstrated the synthesis and evaluation of nanoliposomes, which were covalently attached to the thyroid-stimulating hormone (TSH), having the ability to specifically bind to TSH receptors (TSHr) over-expressed on thyrocytes. The targeting using conjugated NPs leads to a significant reduction in the tumour volume and weight w.r.t. naïve counterparts. Similarly, He et al. synthesized stealth liposomes surface decorated with luteinizing hormone-releasing hormone (LHRH) receptor over-expressed on the majority of cancer cells, to accelerate the inflow of NPs to LRHH expressing tumour cells through receptor-mediated endocytosis (He et al. 2010). Group reported significantly higher tumour retention of drug encapsulated LHRH decorated liposomes in the tumour leading to reduced tumour

volume in the ligand-conjugated liposomes treated group as compared to naïve MTO ($P < 0.05$) or unconjugated drug encapsulated liposomes ($P < 0.05$) groups. The same group also presented another peptide ligand, gonadorelin, a peptide analog of LHRH, in dictating higher potential to bound vesicles to LHRH receptor over-expressing tumours. It was evident from their studies that gonadorelin bound liposomes displayed noteworthy stability and provided sustained release kinetics of loaded MTO, and thus showed improved in vitro efficacy. Several other studies employing in vivo xenograft model were also presented in the previous literature. In another experiment, LHRH analogue was employed to target docetaxel-loaded liposomes to ovary cancer, which delivered nine times more docetaxel at the ovarian tumour compared with the naïve drug or unconjugated liposomes with decreased exposure to the other peripheral organs like liver and spleen (Qin et al. 2008). Likewise, the transferrin receptor 1 (TfR1) aka CD71 is another marker for tumourigenesis. Bhagwat et al. documented the synthesis of transferrin-conjugated solid lipid nanoparticles (SLNs). These particles were consistent and effective enough to deliver loaded Tamoxifen citrate to breast cancer. Other scientific groups too presented work in the same field. Dai et al. developed transferrin-conjugated paclitaxel-loaded lipidic nanoparticles to target Tf receptors in leukaemia cells. The IC50 value of Tf-conjugated paclitaxel-loaded liposomes was 0.45 μg/ml as compared to 2.8 μg/ml for unconjugated nanoparticles. Overall, the outcomes clearly illustrated the targeting potential of the Tf-conjugated lipid nanoparticle system to the leukaemia cells. In another study, Radhakrishnan et al. reported the use of bombesin to target solid lipid nanoparticles encapsulating epigallocatechin gallate to breast cancer. Bombesin confers affinity towards gastrin-releasing peptide receptors (GRPR) over-expressed in breast cancer. In vitro and in vivo data showed the superiority of the developed formulations. The animals treated with the developed formulations illustrated greater survivability and reduction in tumour in case of bombesin-conjugated SLN viz. a viz. naïve drug. Similar outcomes were also corroborated by Kashanian et al., who presented a capable dual ligand-targeted system (folate-apoferritin) using cationic SLN. The dual-drug targeted delivery system in cancer therapy: nanocomplexes of conjugated cationic solid lipid nanoparticles. The cytotoxicity of the dual-targeted SLN (DTPLNs) was greater than individual-unconjugated SLNs (PLNs) in folate receptor-positive cells. The enhanced cytotoxicity of DTPLNs, as compared with PLNs, can be explained by the greater cellular uptake via FRs-mediated endocytosis in MCF-7 (breast cancer) and PC-3 (prostate cancer) cell lines as cancer cells need more FA than healthy cells for sustaining uncontrolled cell proliferation. These higher need was perfectly being utilized by the group to target the uncontrolled cancer cells (Amer Ridha et al. 2021). Multifunctional nanocarriers have been widely applied due to their enhanced effect on tumour therapeutics. Wang et al. constructed a highly effective dual targeting liposomal drug delivery system. They exploited immune targeting ligand (Self-Peptide (SP)) and tumour identification ligand (anti-ER (Estrogen Receptor) antibody) for effective breast cancer therapy. The anti-ER antibody assists in directing the liposomes ER-positive breast cancer. SP could help to inhibit the 'Reticulo Endothelial System (RES)' uptake that causes persistent circulation of NPs in

systemic circulation. Results clearly demonstrated successful attaining of the hypothesis. In vivo fluorescence images of the mice at different time points with different formulations illustrated the higher retention of the developed liposomal-targeted synergetic-conjugated liposome system in the tumour and reduction in the tumour volume to about 38.0%. In contrast, the tumour growth rates of the groups treated with different non-targeted NPs formulation and their naïve counterparts were 68.1%, 95.2%, 95.9%, 103.7%, 131.9%, and 158.4%, respectively (Wang et al. 2019b).

Apart from the higher potential of lipidic NPs in tumour research, they still have reduced pace. This lagging is due to their higher leaching of the drug content from the NPs. Moreover, lipids have an inherent characteristic of degradation due to oxidation. Furthermore, liposomes kind of nanoparticles cannot be explored for oral delivery due to their instability in GI fluids. For the mentioned reasons, polymeric nanoparticles were explored to find the probable alternative to the lipid NPs.

### 2.6.2 Polymeric Nanoparticles

With the emergence of newer, safer, and biocompatible polymers, a surge in research exploration, clinical trials, and approved drugs from polymeric nanoparticles is progressing. Hence, it becomes of utmost importance to mention the role of polymers in the mitigation or detection of hormonal tumours.

As already mentioned, estrogen derivatives have the potential to target hormonal cancers like breast cancer. By exploring the same hypothesis, Kurmi et al. also developed and evaluated estrone decorated doxorubicin-loaded chitosan NPs. Estrone conjugation reduced the secondary and adverse effects of the drug by actively transporting the drug to the tumour area. Moreover, those chitosan polymeric nanoparticles helped in increasing the bioavailability and half-life of the drug, which further passively increases the efficacy. In the literature, many scientists explored the use of estrogen derivatives to be used as targeting moiety to target these tumours. Polymeric nanoparticles can be used for encapsulation of hydrophobic drugs too. The polymeric architecture decides the encapsulation efficiency of the drug. Paclitaxel is the first line of treatment for many of the cancers; however, the clinical used formulation of paclitaxel contained a very high level of surfactants like Cremophor EL (CrEL) and tween 80 to solubilize a higher amount of hydrophobic drugs in hydroalcoholic base. However, these surfactants were known to produce remarkable hypersensitivity reactions, making drugs toxic for cancer patients, leading to a reduction in patient compliance. Thus, PEG (Hydrophilic polymer)-associated polymeric nanoparticles emerged as an excellent choice to passively target tumours by reducing the RES uptake and avoiding aggregation of NPs. In the past literature, other receptors like Follicle-stimulating hormone receptor (FSHR) is over-expressed on ovarian cancer cells. By using a peptide derived from FSH, the group developed peptide-conjugated nanoparticles to target ovary cancers to produce higher improved anti-proliferation and antitumour effects compared with naïve

drug or its unconjugated counterpart with 70% inhibition of tumour volume (3.5 folds higher than clinical paclitaxel formulation) (Zhang et al. 2009). Zang et al. presented hormone peptide-conjugated Poly lactic acid (PLA) nanoparticles, facilitating paclitaxel internalization to ovary tumours. The results remarkably demonstrate the significantly higher anticancer properties of the formulation. FSH33-NPs mediate NP to internalize in ovarian cancer at a higher rate as compared to naïve drugs (Zhang et al. 2009).

### 2.6.3 Inorganing Nanoparticle

Metallic and semi-metallic materials are profoundly used as contrast agents in cancer research. However, the ease in preparation and heat-absorbing/emitting properties of these substrate helps in designing the potential application of drug delivery for cancer. Moreover, recently ligand-conjugated inorganic nanoparticles were exploited for better properties such as higher contrasting power, multiple functionalities of the inorganic nanoparticles, and better imaging properties. Their unique material gives multiple characteristics to them which can be explored in multiple tumours for theranostic purposes. These nanoparticles include magnetic nanoparticles, silver nanoparticles, gold nanoparticles, silica nanoparticles, etc. Fluorescent nanoparticles are promising tools for living cancer cell imaging and cancer targeting. To explore the same fluorescent NPs, Chen et al. synthesized and characterized estrogen complexes dye-doped fluorescent nanoparticles (FNPs) as optical probes for breast cancer. The results were evident to show that estrogen binding can effectively enhance the accumulation of nanoparticles in the breast cancer itself, causing better contrast images (Chen et al. 2019). The targeting ability of the ER(+) ligands was further assessed by Xia et al. They presented an anti-ER probe in 99mTc-labeled estradiol with diethylenetriaminepentaacetic acid (DTPA) as a cheating agent. Tumour mice (MCF-7) significantly reduced tumour uptake of 99mTc-DTPA-estradiol ($2.24 \pm 0.28$%ID/g) when tumours were pre-injected with an excess of DTPA-estradiol, suggesting specificity of 99mTc-DTPA-estradiol against ERs in tumours (Xia et al. 2016).

Antiestrogen compound, such as tamoxifen (TAM), is a famous drug used in the treatment of ER (+) breast cancers. This antiestrogen compound was carefully explored to initiate dual targeting against breast cancer. Dreaden et al. prepared tamoxifen PEG decorated Gold NPs. Results showed enhanced higher efficacy of the TAM-conjugated Gold to ER(+) breast cancer cells. Particle uptake was found up to 2.7-fold versus the free drug. Combined targeting selectivity and enhanced potency provide opportunities for both multimodal endocrine treatment strategies and adjunctive laser photothermal therapy (Dreaden et al. 2009).

Just like lipidic nanoparticles, transferrin has been extensively used in metallic NPs to target them to hormonal cancers. Li et al. developed transferrin-conjugated gold NPs for targeting and imaging and therapy for breast tumours. Results show that the transferrin–transferrin receptor-mediated cellular uptake of gold nanoparticles is six times that in the absence of this interaction. As a consequence,

the laser power effective for photothermal therapy of the cancer cells was reduced to values of two orders of magnitude lower. Likewise, folic acid derivative, methotrexate (MTX), has also been used for targeting metallic nanoparticles. Chen et al. presented MTX surface-conjugated gold NPs for effective tumour targeting. Notably, MTX–gold nanoparticle illustrated potentiated cytotoxicity on numerous tumour cell lines compared with an equal dose of free MTX due to its "concentrated effect". Administration of conjugated gold nanoparticles suppressed the growth of Lewis lung carcinoma (LL2), whereas negligible activity was persistent with no antitumour effect (Chen et al. 2007). Ferro magnetic NPs were also explored by many scientific groups for the effective targeting of breast cancer cells. Through a published report, Meng et al. illustrated LHRH-conjugated superparamagnetic iron oxide nanoparticles (SPIONs) to specifically target breast cancer cells. A series of investigations involving contrast scanning studies proved the higher uptake of KHRH-SPIONs in the tumour tissue than their other non-conjugated counterparts (Meng et al. 2009).

### 2.6.4 Caron Nanotubes and Carbon Dots-Based Hormonal Cancer Therapies

Carbon nanotubes (CNTs) have emerged as the breakthrough in nanotherapeutics with multiple applications due to their remarkable unique structures and properties, including high aspect ratios, large surface areas, rich surface chemical functionalities, and size stability on the nanoscale. Through potential surface functionalization, the CNTs can be given better dispensability as well as selectivity towards cancer cells. CNTs' inner cavities and surface can be used to carry multiple drugs, biotherapeutic agents, etc. CNTs have been used for photodynamic therapy due to their capacity to provide space for inorganic metal ions encapsulation. On the similar verge of the utilization of the NPs, estradiol was appended for targeting the NPs to hormonal cancer cells via estrogen receptors targeting. Das et al. presented estradiol-based PEG-linked multi-walled CNTs (MWCNTs). The structures were intrinsically cells for intranuclear drug delivery of doxorubicin causing better anti-breast cancer potential. The anti-tumour efficacy of doxorubicin encapsulated estradiol-PEG bound MWCNTs produced 18, 17, and 5 higher compared to the groups exposed to saline, drug-deprived estradiol-PEGMWCNTs, and free DOX. respectively (Das et al. 2013). By continuing on the same line, Ghosh et al. work reported the synthesis of a 17β-estradiol-based amphiphiles comprising of polyethene glycol (PEG) moiety linked through succinic acid that non-covalently dispersed (76%) the single-walled carbon nanotubes (SWNTs) in water for the breast cancer targeting. The in vitro data illustrated promising ~three fold higher efficiency than that of ER-negative MDA-MB-231 (advanced breast cancer cell) and HeLa cells that are deprived of estrogen receptors (Ghosh et al. 2016). Hyaluronic acid (HA), a natural polysaccharide, has a higher affinity for CD44 receptors over-expressed on numerous tumour cells. Being hydrophilic, the HA proved stealth properties to the NPs. These HA-conjugated NPs can be used to deliver drugs in

the tumour tissue more than the unconjugated or naïve drugs. Li et al. developed an HA-modified carbon dots (HA-CDs) carrier by one-step hydrothermal treatment within 1 h with citric acid and branch-PEI as core carbon source. HA functionalization not only worked as the ligand, but also produced a hydrophilic character to the surface that helps in targeting as well as excusing RES uptake. These carbon dots were also conjugated with doxorubicin using pH cleavable bond (succinic acid) causing the release of doxorubicin in the acidic tumour microenvironment which resulted in superior anti-tumour efficacy of the carrier against heterotopic and orthotopic 4 T1 cell tumour models as compared to naïve drugs. Moreover, the hydrophilic HA also aids in elevating the carrier stability, dispersibility, and hemocompatibility (Li et al. 2020).

### 2.6.5 Miscellaneous

There are plenty of drug delivery systems that have been used for targeting hormonal cancer that does not come under certain of the above classes. However, they predominantly ascertain their existence in the therapeutic world due to their potential efficacy and stability. These moieties include dendrimers, drug ligand complexes, fullerenes, etc. Dendrimers are molecular (nano) architectures of well-defined size (depends on the generation) and several terminal groups with certain symmetrical and globular geometry, starting from a multifunctional core unit, structure branches in three dimensions from the inside outwards. These layers are designated as "Generations". With each generation, a fixed number of atoms are added into the structure with a definite rise in size and molecular weight. The exact information about the size and molecular weight of the dendrimer is helpful in the characterization of the conjugated carriers using Mass spectroscopy and Nuclear Magnetic Resonance (NMR). Each generation increases a set of flanking ends that can be used to attach ligand molecules to target-specific cancer cells (Swami et al. 2014). However, the cavities between the dendrimer generation and aliphatic chains can be utilized to encapsulate drugs (hydrophobic and hydrophilic). These advantages made dendrimers a self-reliant nanotherapeutic. There are multiple scientists who have worked over the same to fabricate targeted drug delivery systems for hormonal cancer. Modi. Et al. designed poly(amidoamine) (PAMAM) dendrimers to preferentially target the FSHR to ovarian cancer cells, but not to other non-tumourigenic cells. The higher targeting efficacy of dendrimers displayed resulted in a noteworthy internalization and downregulation of an anti-apoptotic protein called surviving. In vivo studies too illustrated higher retention of the fabricated ligand-conjugated dendrimer in the tumour vicinity (Modi et al. 2014). On the same line, Bi et al. synthesized based on PAMAM 5 Generation as a platform with LHRH peptide as a targeting moiety over the surface. Results were again encouraging with higher stability (Bi et al. 2008). The inner cavities of the dendrimers were also employed for the encapsulation of the paclitaxel (hydrophobic) drug. Bhatt et al. demonstrated that the newly developed dendrimers hold promise as an efficient delivery system for PTX or other hydrophobic chemotherapeutic agents for targeted delivery to tumours.

Results demonstrated increased cellular uptake, cytotoxicity, and apoptotic potential of PTX compared with free paclitaxel and G4-TOS-PEG-PTX. Tf conjugated G4-PTX-dendrimer assisted to inhibited growth of human cervical epithelial cells spheroids significantly (Bhatt et al. 2019). Zang et al. expanded the work by combining multiple approaches such as fullerenes nanocarriers, artesunate (iron-dependent drug), and hyaluronic acid (HA). In this approach, HA was grafted onto fullerene to get a water-soluble biomaterial (HA-C60) with excellent biocompatibility and then combined with transferrin (Tf) to obtain a multifunctional drug delivery system (HA-C60-Tf) with significant tumour-targeting efficacy and powerful photodynamic therapy capacity (Bhatt et al. 2019). In another approach, bevacizumab, a monoclonal antibody for ovary tumour targeting, was utilized as polymer-drug conjugate by Pham et al. The results were significantly high as compared to naïve drugs (Bhatt et al. 2019).

## 2.7 Peptide-Based Anti-Tumour Approaches for Hormonal Cancers

A large number of peptide-based drugs have been approved for their application in cancer therapy in general and hormonal cancers in particular. This application of peptides in the treatment of hormonal cancers is evident from several different paradigms that regulate tumour progression and disease progression (Thundimadathil 2012). Peptide-based nanotherapeutics are also significant in providing personalized medicine approach in a way that these are efficient in targeting some specific kinds of cancers in some particular patients by inhibiting and affecting only the cancer cells while sparing the healthy cells. Many peptide/protein-based receptors have been discovered which employ tumour targeting peptides and have led to the creation of a kind of a "recent wave" in occupying a larger share of the anti-cancer nano-therapeutics. Figure 2.3 represents several molecular mechanisms that exist, by which peptides are useful in targeting the hormonal cancers, viz. by directing the movement or pharmacokinetics of the anti-tumour drugs, by interaction with the peptide-based receptors, some peptide-based hormonal therapies of cancers, peptide-based anti-cancer vaccines, as radionuclide carriers, etc. (Ediriwickrema and Saltzman 2015; Tran et al. 2017).

Direct application of nano peptides in anti-tumour therapy in conjugation with other therapeutic regimens has acquired momentum in the recent past. The anti-tumour activities of peptide-based nanoformulation are dependent on their inhibition of the oncogene expressing capabilities, prevention of the angiogenesis pathways, regulation of the various signal transduction pathways, and inhibition of the protein-protein interactions involved in the pathophysiology of the tumour cells uncontrolled and unregulated proliferation (Bertrand et al. 2014). In this regard, the anti-PD-L1 peptide was formulated for paclitaxel (PTX) pro-drug and photosensitizer pheophorbide-A co-loaded cationized gold nanoclusters for multimodal therapy of breast cancer. This nanomedicine was composed of RBCs membrane-coating; hyaluronic acid (HA) outer covering with cationic gold nanoparticles as the inner

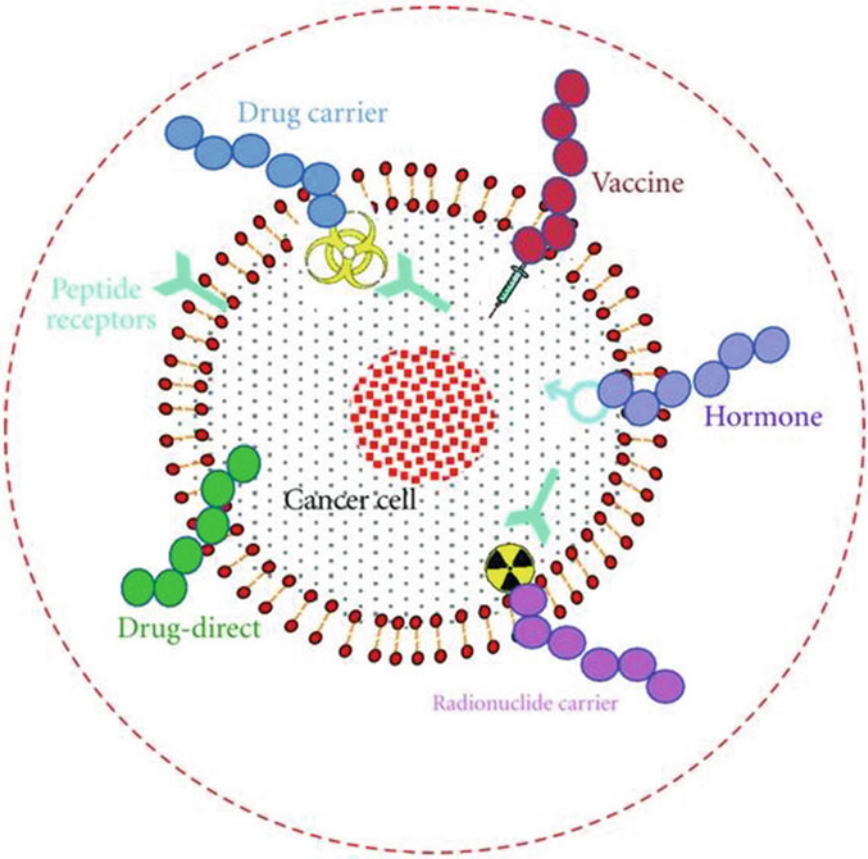



**Fig. 2.3** Different possible treatment options of cancer using peptides. Peptides can be used as anti-cancer drug, cytotoxic drug carrier, vaccine, hormones, and radionuclide carrier

core materials. The outer membrane from RBCs aided in prolonged blood circulation and improved cancer cell uptake and the HA portion allowed for the tumour microenvironment (TME) stimulus-dependent drug release from the nanoformulation. This combinatorial approach of ROS production by photosensitizer components, accompanied with the cancer cell inhibiting effect of PTX as well as immune activation of anti-PD-L1 peptide, culminated into the appreciable cancer cell inhibiting and tumour-metastasis prevention efficacy (Yu et al. 2019). Another approach talks about the formulation of polylactic acid and polyethene glycol polypropylene-glycol polyethene glycol-based multi-block copolymers-based nanocarriers for simultaneous delivering of anti-tumour peptide NuBCP9 with PTX for the treatment of breast. BCP-9 aids in the inhibition of BCL-2, an anti-apoptosis proteinaceous molecule that enhances the surviving capacities of drug-resistant cancer cells. This synergistic combinatorial approach of PTC with

NuBCP-9 peptide has resulted in almost 40 times decrease in $IC_{50}$ values in vitro and an appreciably enhanced cancer inhibition in the in vivo model of xenograft tumours (Gupta et al. 2018b).

In the context of peptide-based nano-drugs in ovarian cancer, a dendrimer-based arginine-rich peptide has been worked upon as a doxorubicin prodrug nanocarrier for tumour microenvironment stimuli-responsive drug delivery across cancer cell hurdles. Doxorubicin was combined with dendrimer-based peptides by acid-dependent cleaving of the hydrazine bondings and circumferential arginine-based residues got blocked out by applying 2,3-dimethyl maleic anhydride to mask the tumour microenvironment acidified pH responses. Finalized prodrug formulation prepared by this approach can self-assemble in a hydrous milieu and make virus-like nanocarriers having a particle size of 136 nm. Nanocarriers exhibited considerable penetration in cancer tissues and cells after being activated by a weakly acidic environment in cancer tissues. I.V. administration of this virus-like peptide-based prodrug nanocarriers in SKOV3/R anti-cancer drug-resistant ovarian cancer model of nude mice delivered considerably significant cellular and tumour-tissue double penetrabilities, enhanced the blood residence time, and effective tumour inhibitory capabilities (Zhang et al. 2018). Another report elaborates on the combination of follicle-stimulating hormone (FSH) and peptide-based polyethyleneimine–polyethene glycol nanocarriers containing growth-regulating small hairpin RNA molecules for silencing the proto-oncogene α to culminate into its increased anti-tumour effectiveness in the ovarian tumour xenograft model (Fan et al. 2014).

Combinatorial therapeutic strategies of employing peptide-based and hormonal agents with radio-nuclides or cancer management drugs have been researched but have got mixed outcomes. Lutathera, which is an example of lutetium 177 coupled with the somatostatin-based analogue has been approved by FDA for gastroenteropancreatic's cancers of neuroendocrine nature, whereas another peptide hormone, zoptarelin coupled with doxorubicin, has failed the clinical studies in Phase III stage for the treatment of advanced-stage uterine cancer. Many of the reports of polymer-based and lipid nanocarriers for peptide hormones as well as several of their combinations, however, have not yet been researched to their full potential (Mehrotra et al. 2020).

The initial example of peptide hormone employed for anti-tumour therapeutics was the luteinizing hormone-releasing hormone (LHRH) agonist as researched by Schally et al. for the management of prostate cancers. After this event, various other agonists of LHRH have been formulated, while some of these including goserelin, buserelin, leuprolide, and triptorelin got approval from US-FDA for the management of cancers. Many others of these LHRH antagonists including abarelix, cetrorelix, degarelix, etc. are now US-FDA-approved to be employed for advanced stages of prostate cancer (Engel et al. 2007). Another polypeptide-based nanoparticle was formulated for prostate cancer which employed the combination of proteasomal inhibiting agents and histone deacetylation inhibitor is taken concomitantly in zein-based nanoparticles. This is because prostate cancer develops resistance, and followed by its progressive metastasis, causes increased incidences of cancer-related deaths in men. In this approach, vorinostat, as well as bortezomib, was loaded into

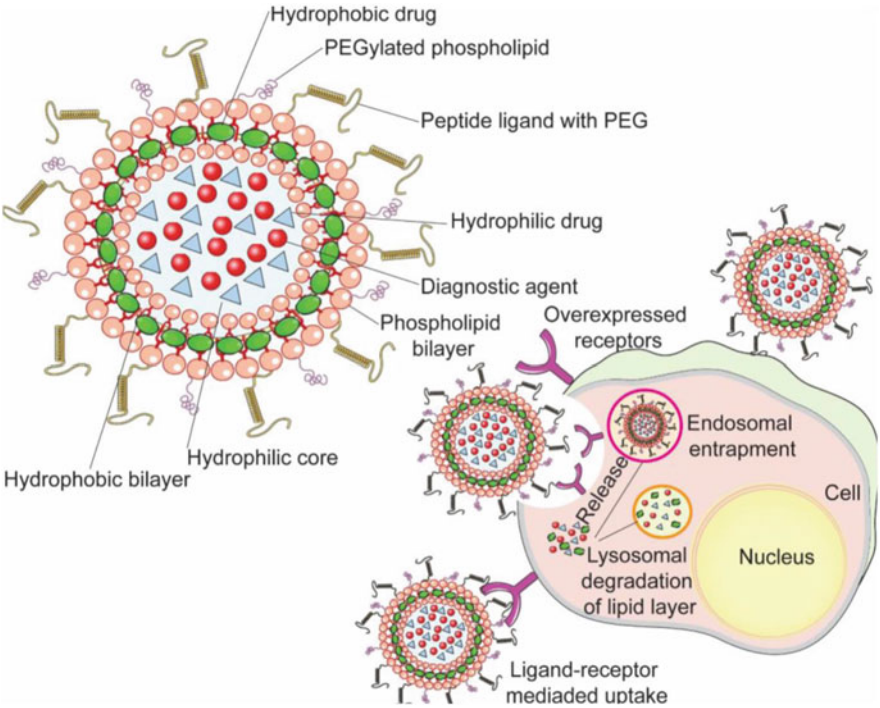



**Fig. 2.4** Schematic illustration showing the peptide-functionalized liposomal NDS acting on receptors overexpressed on the surface of cancer cells via targeted delivery. The peptide conjugated to the NDS binds specifically to the receptors upregulated on the surface of cancer cells, which is followed by its uptake by the cancer cells through receptor-mediated endocytosis. Subsequently, the payload of the NDS is released by the degradation of the lipid bilayer via the endosomal–lysosomal pathway

zein-based nanocarriers for combination therapy of metastasizing prostate cancer. The nanocarriers possessed the advantages of pH-dependent and precisely controlled release of drug from drug-loaded nanoformulation. These nanocarriers also demonstrated higher cellular uptakes in various prostate cancer cell lines and caused higher cytotoxic and apoptotic effects. Furthermore, the higher anti-metastatic efficacy and better induction of pro-apoptotic factors by these nanocarriers establish their potential applicability in the management of prostate cancers. This peptide-based nanoformulation further demonstrated higher in vivo anti-cancer efficacy in comparison to naïve or free form of drugs as well as their combinatorial approach and caused the least toxic effects to normal cells and tissues as depicted in Fig. 2.4. Taken together, these vorinostat and bortezomib combinations containing zein nanocarriers can prove to be of potential applicability in the efficient management of advanced-stage prostate tumours with high metastatic potential (Thapa et al. 2017).

## 2.8 Nucleic Acid-Based Nanomedicine Targeting Hormonal Cancers

### 2.8.1 DNA-Based Nano-Therapeutics Targeting Hormonal Cancers

Recent DNA nanostructures and smart stimuli-responsive nanomaterials can effectively present the entrapped drug cargo in the presence of several drivable forces. The regulated and controlled drug release from these DNA-based nanocarriers by alteration of dynamic aspects of DNA nanostructure and sometimes in response to micro-environment of tumour tissues leads to highly specific delivering of the entrapped active pharmaceutical ingredients to cancer cells and tissues. Therefore, specified pharmaceutical delivery appreciably increases the pharmacological effectiveness and significantly lowers down the undesired and adverse and toxic reactions on the non-cancerous normal and healthy cellular environments. Despite significant pharmacological applicabilities of these DNA-based and pH-responsive smart nanomaterials, several limitations need to be taken care of before these paradigms can be employed in clinical settings. Since therapeutic and pharmacological effectiveness of these DNA nanostructures has been researched in some proof of concept fashion, detailed in vivo properties of these must be looked into and explained, including the colloidal stabilities, their half-lives in the blood circulation, their immunogenic potential, dose-dependent cytocompatibility and biocompatibility, various pharmacokinetic parameters, and their various body clearance routes as well as their mechanism.

One approach for the formulation of DNA nanostructures is the hybridization process through which hairpin conjugation-based catalysis can form DNA-based nano-ribbons. Their rigidity and highly regulated DNA components are employed to formulate significant one-dimensional nano-ribbon and various other two-dimensional and even three-dimensional nano-crystals. Several distinct technologies have been undertaken by many researchers to design and formulate various nano-ribbons for the delivery of siRNAs, drugs like doxorubicin, many photosensitizer molecules, etc. (Ouyang et al. 2020). DNA-based nano-ribbons were developed by some altered DNA-origami-based technologies and several reports have suggested that these DNA-based nano-ribbons can efficiently deliver the encapsulated or loaded siRNAs into human cancer cell lines. These multifunctionalized DNA nanostructures have depicted appreciable operations in the cancer diagnostic as well as therapeutic strategies due to smaller size, optimized and desirable morphological features, and high cyto- and biocompatibilities. Many researchers have put collective efforts into developing several kinds of these DNA nano-ribbons, viz. Liang et al. formulated the DNA-based nano-ribbon by two compartmental techniques, where one compartment gets loaded with the -GC- base pairings for the delivery of doxorubicin drug, while the other compartment was made up of AS1411 aptamers, which serves as the DNA-based aptamer. This formulation aided in the increment of tolerabilities against the drug doxorubicin in the human breast cancer cell lines and also led to the inhibition of cancer progression and metastasis (Liang et al. 2004). In some other paradigms, DNA-based

nanoformulations have been prepared by employing circular DNA nanotechnology-based techniques for various ligand-mediated functionalization approaches which include neuregulin-1 and their biomedical applicabilities. Researchers have also formulated DNA-based nano-spindals for the efficient loading of daunorubicin drug molecules for the targeting of human epidermal growth factor receptor-2 located on cellular membranes of the MCF-7 breast cancer cell lines which have shown drug resistance. The drug loading capacities and encapsulation efficiencies of daunorubicin were established by analyzing the UV-Visible spectroscopic shift. Thereafter, the MTT assay exhibited the decreased cell viabilities of breast cancer cells once the treatment of daunorubicin-loaded DNA-based nanostructures was given. Furthermore, outcomes of the cell proliferation and apoptosis as obtained from flow cytometry studies demonstrated that these DNA-based nanostructures caused higher apoptosis, i.e. up to 65% in comparison to free or naïve forms of the drug. Hence, it can be suggested that most kinds of DNA nanoformulation result in stiffer, consistent, and highly biocompatible kinds of targeted anti-tumour therapies (Baig et al. 2021).

In the context of another hormonal cancer, viz., prostate cancer, researchers have formulated a DNA aptamer for the prostate-specific antigen (PSA) which possessed a constant number of sequencing for the facilitation of doxorubicin attachments and formation of the dimerized complexes of DNA-based aptamers. The nanostructure got immediately taken by prostate-specific membrane antigen (PSMA) possessing tumour cells. These dimerized DNA-based aptameric complexes represent complexes that can deliver doxorubicin to these PSMA-positive tumour cells. Under normal physiological circumstances, doxorubicin gets released from these within the initial 8–10 h. In this way, doxorubicin could be delivered to C4–2 cell lines by employing the nanostructures which could get localized in the nuclear region. Thus, it could be suggested that the nanostructures have quite higher specificities and enhanced durabilities, which can aid in the delivery of doxorubicin to cancer cells as well as in vivo (Boyacioglu et al. 2013).

### 2.8.2 RNA-Based Anti-Cancer Nano-Therapeutics for Hormonal Cancers

Ribonucleic acids (RNAs) present a larger portion of the human transcriptional machinery and perform quite important parts in cellular and molecular pathophysiology as well as the pathogenesis of various disorders. Amounting evidence has established the functional plays of RNAs in tumour pathology and cancer progression which establishes their potential applicabilities in the treatment of various tumours. Various roles of RNAs in the exogenous silencing or inhibition of the various genes and/or proteins that have their erroneous over-expression in tumours can exhibit high capabilities in the therapeutic paradigms of various kinds of cancers. However, specific targeting capabilities, stabilities of siRNAs, and their effective and efficient delivery still possess a large number of limitations in their clinical progress (Wang et al. 2019a). Nanotechnology highlights a hopeful advancement for

entrapment and delivery of these RNAs and can offer higher pharmacological activity and safety indices with lower toxicity portfolios. Advancements in the pre-clinical, as well as clinical, reports about the utilization of engineered nanocarriers loaded with many types of RNAs to manage various cancer types have been elaborated. Quite selective and specific approaches have been followed in the context of RNAs for regulating several signalling cascades and molecular events and pathways at cellular and molecular levels in cancer cell proliferation and controlling of various check-points in the cell cycle. Invasiveness of cancer cells and the tumour metastasis, control of the angiogenetic events with the tumour microenvironment, and chemotherapy of resistant cancer types are the other aspects where RNAs have demonstrated higher efficiencies by nanotechnology-based tumour targeting paradigms (Hattab and Bakhtiar 2020).

Silencing or knocking down various genes with RNA-based interference approaches for the treatment of breast cancers is one of the therapeutic paradigms. For example, BCL-2 gene expression higher than normal leads to enhancement of cancer cell survivability via inhibition of the apoptotic pathways. Therefore, a recent and novel method for suppression of the anti-apoptotic genes undertakes the employment of small-interfering RNAs in tumour cell lines. However, some challenges persist in applying these siRNAs which include the necessity of vector molecules to permeate across cellular membranes and deposit the desired nucleic acid into the target cell or nucleus. For this purpose, hybrid nanoparticles containing RNAs have been formulated for the promotion of the delivery of siRNAs into the cultured human MCF-7 cell lines of breast cancer. For the evaluation of silencing capacities of anti-apoptotic genes, BCL-2, by these siRNA-loaded nanocarriers would enhance the cancer cell inhibition. After 48 h incubating time, expression levels of these genes lowered down from 49% to 23%; siRNA sequences loaded in nanocarriers led to the death of cancer cells in the concentrations of as low as 200 nM after an incubation time point of 72 h. Since the proteins under target can be linked to the resistance of cancer cells against anti-cancer chemotherapeutics, the nanocarriers got also evaluated with doxorubicin drugs. Outcomes of this approach demonstrated an appreciable decrease in $IC_{50}$ values of doxorubicin molecules, once the silencing of anti-apoptotic genes was completed. Additionally, the upregulation of cancer cell apoptosis was also observed for both of the incubation time points as well (de Mello Jr et al. 2017).

Likewise in another report, it is stated that MDM2 being an oncogene is responsible for the inactivation of p53, the tumour suppressor gene, and its expression is appreciably higher in triple-negative breast cancer cells. If MDM2 gets overexpressed, it causes p53 suppression, thereby affecting the proliferation and apoptosis of breast cancer cells cell. In this regard, effective delivery of siRNAs has been undertaken by using carbon nanotubes which got functionalized with polyethene glycol. The encapsulated siRNAs then targeted and caused the silencing of the MDM2 genes. It has therefore been suggested that these polyethene glycol-modified single-walled carbon nanostructures can effectively deliver siRNAs and aid in the promotion of the silencing of MDM2, leading to a suppressed and lowered cancer cell proliferation and induction of the apoptosis of breast cancer (Chen et al. 2012).

Cyclin-dependent kinases as well as some casein kinases are some of the significant protein kinases that regulate surviving capability of the cancer cells. These kinases are significantly over-expressed in triple-negative breast cancer cells and tissues. A novel kind of tenfibgen-coated nanocarriers were formulated which contained some RNA molecules against those over-expressed CDKs like CDK11 and were meant to target tenascin kid of receptors which are abundant in the stroma of breast cancer cells. I.V. injection of these nanocarriers were meant for genetic suppression of the target mRNAs and their resultant proteins, thereby finally leading to the appreciable lowering down of the cancer cell proliferation and tumour growth and metastasis (Xu et al. 2019).

In the context of the nanotherapeutics ovarian cancer, Kenny and co-workers formulated siRNA containing PEGylated liposomal theranostic nanocarriers which also possessed the capability to decrease the expression levels of survivin molecules and inhibited the growth of cancerous tissues and enabled the real-time monitoring of siRNA payload delivery by magnetic resonance imaging combined with florescence. The mean particle size of the nanocarriers was around 90 nm. In vivo imaging demonstrated that after the i.v. administration of nanocarriers in mice via tail vein, various signal intensities of fast-spin echo imaging got enhanced because of the availability of gadolinium carrying lipids of these nanocarriers. Magnetic resonance relaxivity of this siRNA containing nanocarriers got comparatively enhanced as compared to that of the clinical magnetic resonance imaging contrast media. Additionally, apart from their efficacy in bio-imaging, siRNA containing nanocarriers also led to an appreciable lowering of OVCAR-3 cancer xenograft growth, 48 and 72 h after the administration of siRNA-loaded nanocarriers. Protein expression also demonstrated that survivin expression gets considerably lowered down after the nanocarrier administration. Fluorescent microscopic analysis of sections of tissues exhibited co-localization of rhodamine-loaded bimodal PEGylated liposomal nanocarriers and Alexa-Fluor tagged siRNA inside the tumour cells. Thus, along with the delivery of therapeutic siRNAs, concomitant supervision of the delivery can also be done (Kenny et al. 2011).

Ramos and co-researchers prepared gold nanorods by employing the layer by layer method for the investigation of RNA-mediated lowering down of the expression of protein luciferase by shRNA in human prostate cancer cell lines (22Rv1-Luc) (Ramos and Rege 2013). Further for prostate cancer targeting, chitosan containing hybrid nanocomplexes was formulated which possessed considerably higher physical stabilities in physiological medium. These hybrid nanoformulations had a particle size of 200 nm and were synthesized by the combination of siRNA against survivin, for effective systemic delivery. Tumour targeting capabilities, as well as anti-cancer efficiency of this siRNA containing nanoformulations, were assessed in the prostate cancer model of mice xenografts with NIR-fluorescence-based bio-imaging as well as cancer growth and metastasis supervision. The chitosan containing nanocarriers had lower cytotoxic effects, and comparatively higher biocompatibilities, enhanced mucoadhesive properties, and increased cellular permeabilities; however, these also possess few lacunae, which include decreased gene delivering efficacies in vivo (Ki et al. 2014).

## 2.9 Translational Potential and Clinical Advances in Innovative Treatment of Hormonal Cancers

Cancer research has put huge efforts in discovering novel and effective therapeutic paradigms for the alleviation of unwanted and harmful adverse effects of the presently available traditional therapies. Various technological advances have presently been under critical assessments for clinical trial studies or are being undertaken in inpatient settings. While nanotherapeutics has contributed hugely to developing the various biocompatible and biodegradable materials for diagnosis and treatment approaches for hormonal cancers, biomedical engineering of cellular and extracellular components obtained from cancer patients paves the way for the formulation of additional advanced systems and unequivocal directional and cancer-specific therapeutic schemes (Ahmad and Ahsan 2020; Ahmad et al. 2019a; Pucci et al. 2019). Various efforts have been put into developing the various newer and recent prognosis, diagnosis, and therapeutic nanoformulations that have led to tremendous translational potential in hormonal cancer-related research activities for their flourishment. Fig. 2.5 depicts the various genome and proteome-based technical advancements that have resulted in the tremendous quantity of data crucial for the expansion of in-depth realization of hormonal-cancer pathophysiology. Newer and

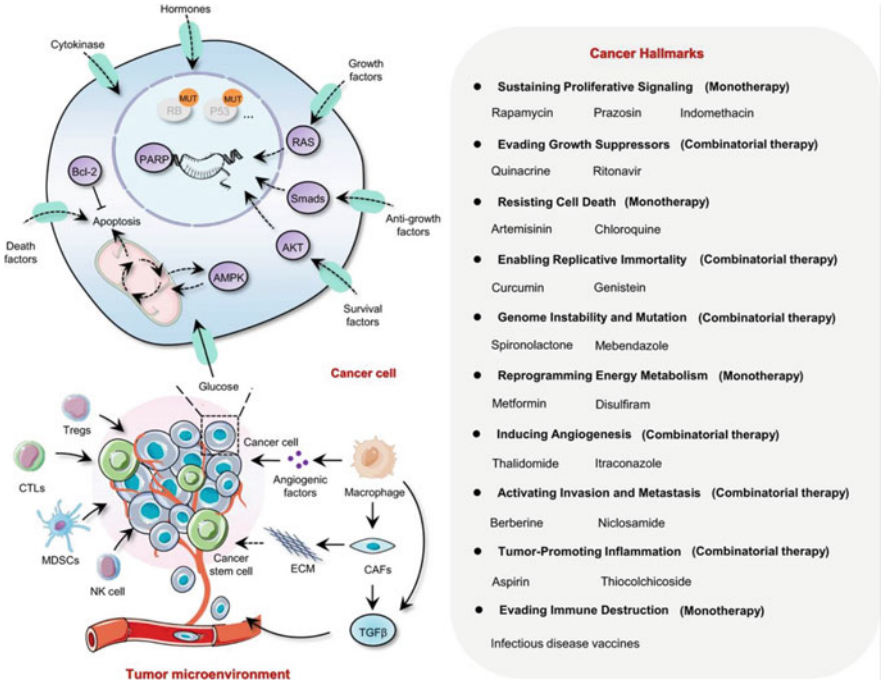



**Fig. 2.5** Recent cellular and molecular paradigms employed for realization of clinical translations potential of anti-cancer nanomedicine

recent research paradigms focusing on distinctions among healthy and cancerous cellular pathology caused the drug development targeting specialized biomolecules and nanocarriers, appreciably improving anti-cancer activity and additionally reducing the toxicological profiles of drugs in hormonal cancer patients which are observed in the case of present-day conventional anti-cancer tenement regimen. Recent novel anti-cancer drug targets undertake cell-cycle regulation, angiogenesis in tumour tissues, cancer cell apoptosis, targeting the DNA damage and DNA repair, and regulation of the growth factors including their growth factor-receptors. High-level collaborative efforts among various clinical researchers, physicians, surgeons, and pharmaceutical industry experts have proven critical for the conduction of pre-clinical and clinical evaluations of nanomedicine for hormonal cancers for their translation originating from the laboratory determinations and then into clinical therapies (Ahmad et al. 2019b; Goldblatt and Lee 2010; Gupta et al. 2018a).

In the context of clinical advancements and translational potential of breast cancer nano-therapeutics, gold NPs exhibited various interesting characteristic features due to their electrical as well as optical features and lower toxic profiles and higher biocompatibilities. These Au-NPs have been exploited in various contrast media in X-ray bio-imaging, CT-scans, photo-acoustic image acquisition, and photodynamic treatment of cancer cells and tissues. A kind of core-shell nanocarriers having gold and silica nano-shell and polyethene glycol coatings has been approved by the United States Food and Drug Administration (US-FDA) and thus has then been commercialized by the brand name of AuroShell by Nanospectra for nano-therapy of breast cancers with the photodynamic therapeutic regimen (Kim and Jeong 2017). Polymeric nanocarriers are composed of highly biocompatible and biodegradable or natural as well as synthetic polymers, including but not limited to poly-lactide-co-glycolide (PLGA), poly-ε-caprolactone (PCL), alginate-based polymers and co-polymers, albumin and its other heterogenous polymers, etc. Few of these nano-formulations are already approved by US-FDA, viz. albumin-based paclitaxel containing nanoparticles by the name of "Abraxane" metastatic breast cancer therapy and also for the treatment of pancreatic ductal adenocarcinoma. It also includes a bioengineered protein and interleukin-2 combination with the diphtheria toxins by the brand name of "Ontake" employed for therapy of non-Hodgkin's T-cell lymphomas of peripheral origin (Nasir et al. 2015).

Three studies in the clinical settings have undergone trials implicating that the application of drug-carrying exosomal nanoformulations has undergone evaluation for therapy of various cancers, viz. phase one clinical study evaluated the efficacy of this exosomal formulation for delivering the curcumin and compared the effects on normal healthy tumour tissues. Another phase two clinical study investigated the in vivo effects of autologous cancer tissue descended microparticulate formulation loaded with the anti-cancer drug methotrexate (MTX) in human subjects of cancers, while the final clinical study focused on another microparticulate formulation derived from erythrocytes which were prepared for delivering the methotrexate and applied for treatment of ovarian cancers (G.W.D. J 2021; Jin 2021; Xu and Ting 2021). In other aspects of ovarian cancer, it is suggested that neutral liposomal nanoformulations prepared from DOPAC (1,2-dioleoyl-sn-glycerol-3-

phosphatidylcholine) exhibit higher efficiencies in the in vivo model of ovarian cancers in mice. This is in this context that a phase one clinical study has recruited the human subjects for the evaluation of the safe and effective nature of siRNA containing DOPAC-based formulations for the treatment of advanced stages of ovarian cancers (M.D. Anderson Cancer Center 2021).

In the recent past, exosomal carrier detection capabilities have been exploited as an authentic and dependable method in pre-clinical practices to be employed in various tumour types, due to their ability to identify their inclusions: some cancer-related double-stranded DNAs, tumour-based messenger RNAs, as well as cancer, linked micro RNAs, long non-coding RNAs, protein molecules, and lipid-based agents. Double-stranded DNAs have also been observed in exosomal carriers which are sequestered out of the serum or plasma of various kinds of tumour cells, some of the highly mutated genes which include mutated forms of TP53 genes and KRAS. These genetic abnormalities have been recognized as cancer disease prediction markers. Likewise, AR-V7 messenger RNAs from these exosomal carriers have been applied as the prognosis marker for the development of resistance against the hormonal therapies in patients having metastasizing prostate cancers (Del Re et al. 2017). Antibodies have also been employed as immune-conjugating agents when linked to some drugs or drug-loaded nanocarriers or in naïve or free forms as well. In the initial cases, these are employed for the targeting of some specific cancer antigens which have been expressed on the tumour tissues. Antibodies applied in this technique also consider those antibodies which can be bonded to human epidermal growth factor receptors-2, epidermal growth factor receptors, transferrin receptors, and prostate-specific membrane antigens (Bazak et al. 2015).

## 2.10 Future Perspectives and Conclusion

Nano-therapeutical paradigms against hormonal cancers have already produced quite appreciable findings in their diagnosis and detection, as well as therapy, and possess the significant capabilities to yield much more, with in-depth and focalized pathways of research and development which bear many advantages for the future industries and the opportunities for nanocarrier-based hormonal cancer management. But it has also been absolved that nanotechnological advances need to be interpreted for their in-depth understanding and risk associated with the use of nanocarriers should also be evaluated for its safe development, and the scientific expertise needs to be relished in various arenas to bring closed different discoveries from the laboratory scale to the clinical translational scenario where patients can get the maximum benefits. As a fast-improving interdisciplinary science, nanotechnology for hormonal cancers can bring several views for improving the traditional treatments of hormonal cancers and offer appreciably safer and more effective therapeutic paradigms. Various nanoparticles-based approaches have already been FDA-approved or are under clinical studies for delivering the chemotherapies against hormonal cancers (including liposomal formulations, polymeric nanocarriers, and nanoforms of paclitaxel). For the promotion of hormonal cancer

treatment nanocarriers, still, a large number of scientific efforts can be undertaken. For example, there is still a requirement for much improvement of the therapeutic strategies of hormonal cancers types, which possess high recurrence chances and the mortality is comparatively higher which includes the examples of triple-negative as well as HER2-positive breast cancers. Nanocarriers for HER-2 breast cancers for the HER2 targeting ligand like lapatinib, trastuzumab, or pertuzumab appreciably enhance the effectiveness of HER2 receptor-positive cancer therapies. While the nanocarriers-induced necrotic damage of cancer cells provides some newer solutions for the treatment of triple-negative breast cancers, the safety issues concerned with the application of viral vectors are often the chief concern in the advancements of clinical studies. Various anti-cancer mechanisms have been emerging and attracting a lot of enthusiasm concerning RNA-based nanotherapeutics for hormonal cancers. RNA-associated interferences are focusing on the nano-therapy of hormonal cancers at genetic stages by degradation of messenger RNAs or suppression of the RNA translational processes in cancer cells and tissues when other antibody or inhibitor-based therapies cannot target the hormonal cancers appreciably. Immunological adverse reactions are the qualifications of RNA-interference-based therapy research since hormonal receptors are implicated. With nano carrier-based therapy against hormonal cancers, cancer tissues are treated with non-invasive treatment paradigms, which become advantageous for patients who respond to chemotherapy or radiotherapies. Finally, the invention of the newer nanomaterials-based medicinal approaches will be an alternative pathway for the creation of biomolecular targets which fall under the broad perspectives of nanotherapeutics for hormonal cancers. Chemotherapy, various hormone-based approaches, RNA moieties, or virus-based paradigms can also be formulated with non-toxic nanocarriers and can release the drugs into cancer tissues and can respond to pH-based triggers. Ligand targeting, viz. by various aptamer-based techniques, antibody conjugations, and peptide- and protein-dependent therapies can be undertaken through their bioconjugation and regulated their accumulation of nanocarriers within hormonal cancer cells. The entrapment of NIR-based fluorophores in nanocarriers also extends their applications against hormonal cancers. Overall, the nanocarriers will prove to be versatile platforms for most types of drugs to encompass various types of therapeutics strategies mentioned above, making nanocarrier-based therapy and other personalized nanomedicine for generalized promises in hormonal cancer treatments. In conclusion, the versatile nature of nanocarriers will allow delivering the multiple drugs and pharmacological agents which possess the capability for targeting the various hormonal cancer kinds. This will also lead to the improvement in the efficacy and advances in both hormonal cancer diagnostic procedures and therapeutic paradigms. These distinct features make nanomedicine interesting for research studies, and simultaneously, appealing for the research and scientific communities with an aim for the improvement of patient outcomes. In this review, we have presented our views about the various recent advancements of various nanoparticles and also exemplified their applicability in hormonal cancer therapies, particularly in breast cancers, ovarian cancers, endometrial cancers, prostate cancers, etc. These nano-systems would also be assessed or evaluated for various other kinds of cancers.

The selection of nanoparticle materials will also be critical for the achievement of a more effective hormonal tumour targeting. Unique characteristics (escape from the immune mechanism, achieving the longer blood circulation times, inherent biocompatibilities and biodegradabilities of nanocarriers, avoidance of the use of pharmaceutical additives or excipients) prolong their lifetime, adhesion capabilities, and targeting of the hormonal cancer cells and tissues.

## References

Ahmad A, Ahsan H (2020) Ras-mediated activation of NF-κB and DNA damage response in carcinogenesis. Cancer Investig 38(4):185–208

Ahmad A, Gupta A, Ansari MM, Vyawahare A, Jayamurugan G, Khan R (2019b) Hyperbranched polymer-functionalized magnetic nanoparticle-mediated hyperthermia and niclosamide bimodal therapy of colorectal cancer cells. ACS Biomater Sci Eng 6(2):1102–1111

Ahmad A, Khan F, Mishra RK, Khan R (2019a) Precision cancer nanotherapy: evolving role of multifunctional nanoparticles for cancer active targeting. J Med Chem 62(23):10475–10496

Ali I, Wani WA, Saleem K (2011) Cancer scenario in India with future perspectives. Cancer Ther 8: 56–70

Ameer MA, Fagan SE, Sosa-Stanley JN, Peterson DC (2020) Anatomy, abdomen and pelvis, uterus. StatPearls Publishing

Amer Ridha A, Kashanian S, Rafipour R, Hemati Azandaryani A, Zhaleh H, Mahdavian EJP et al (2021) A promising dual-drug targeted delivery system in cancer therapy: nanocomplexes of folate–apoferritin-conjugated cationic solid lipid nanoparticles. Pharm Dev Technol 26(6): 673–681

American Cancer Society (2019a) Breast Cancer

American Cancer Society (2019b) What is breast cancer. https://www.cancer.org/cancer/breast-cancer/about/what-is-breast-cancer.html

Anand P, Kunnumakkara AB, Sundaram C, Harikumar KB, Tharakan ST, Lai OS et al (2008) Cancer is a preventable disease that requires major lifestyle changes. Pharm Res 25(9): 2097–2116

Baba AI, Câtoi C (2007) Principles of anticancer thearapy. Comparative Oncol

Bae YH, Park K (2011) Targeted drug delivery to tumors: myths, reality and possibility. J Control Release 153(3):198

Baig MMFA, Zou T, Neelakantan P, Zhang C (2021) Development and functionalization of DNA nanostructures for biomedical applications. J Chin Chem Soc 68(2):228–238

Bazak R, Houri M, El Achy S, Kamel S, Refaat T (2015) Cancer active targeting by nanoparticles: a comprehensive review of literature. J Cancer Res Clin Oncol 141(5):769–784

Bertrand N, Wu J, Xu X, Kamaly N, Farokhzad OC (2014) Cancer nanotechnology: the impact of passive and active targeting in the era of modern cancer biology. Adv Drug Deliv Rev 66:2–25

Bhatt H, Kiran Rompicharla SV, Ghosh B, Torchilin V, Biswas SJN (2019) Transferrin/α-tocopherol modified poly (amidoamine) dendrimers for improved tumor targeting and anticancer activity of paclitaxel. Nanomedicine 14(24):3159–3176

Bi X, Shi X, Baker JR (2008) Polymer Edition Synthesis, characterization and stability of a luteinizing hormone-releasing hormone (LHRH)-functionalized poly (amidoamine) dendrimer conjugate. J Biomater Sci Polym Ed 19(1):131–142

Biggers A (2020) Everything you should know about hormonal imbalance: healthline media. https://www.healthline.com/nutrition/balance–hormones#TOC_TITLE_HDR_4

Boyacioglu O, Stuart CH, Kulik G, Gmeiner WH (2013) Dimeric DNA aptamer complexes for high-capacity–targeted drug delivery using pH-sensitive covalent linkages. Mol Ther Nucleic Acids 2:e107

Brown CR, Hambleton IR, Hercules SM, Alvarado M, Unwin N, Murphy MM et al (2017) Social determinants of breast cancer in the Caribbean: a systematic review. Int J Equity Health 16(1):60
Byrne JD, Betancourt T, Brannon-Peppas L (2008) Active targeting schemes for nanoparticle systems in cancer therapeutics. Adv Drug Deliv Rev 60(15):1615–1626
Caban S, Aytekin E, Sahin A, Capan Y (2014) Nanosystems for drug delivery. OA Drug Des Deliv 2(1):2
Casey SC, Vaccari M, Al-Mulla F, Al-Temaimi R, Amedei A, Barcellos-Hoff MH et al (2015) The effect of environmental chemicals on the tumor microenvironment. Carcinogenesis 36 (Suppl_1):S160–SS83
Castle PE, Einstein MH, Sahasrabuddhe VV (2021) Cervical cancer prevention and control in women living with human immunodeficiency virus. Cancer J Clin 71(6):505–526
Cavanagh J, Fairbrother WJ, Palmer AG III, Skelton NJ (1995) Protein NMR spectroscopy: principles and practice. Academic Press
Chen H, Ma X, Li Z, Shi Q, Zheng W, Liu Y et al (2012) Functionalization of single-walled carbon nanotubes enables efficient intracellular delivery of siRNA targeting MDM2 to inhibit breast cancer cells growth. Biomed Pharmacother 66(5):334–338
Chen Y-H, Tsai C-Y, Huang P-Y, Chang M-Y, Cheng P-C, Chou C-H et al (2007) Methotrexate conjugated to gold nanoparticles inhibits tumor growth in a syngeneic lung tumor model. Mol Pharm 4(5):713–722
Chen Y, Yang L, Liu J, Chen ZJB (2019) Estrogen conjugated fluorescent silica nanoparticles as optical probes for breast cancer cells imaging. Biomicrofluidics 13(4):044113
Cooper GM (2000) The development and causes of cancer. 2nd
Das M, Singh RP, Datir SR, Jain SJ (2013) Intranuclear drug delivery and effective in vivo cancer therapy via Estradiol–PEG-appended multiwalled carbon nanotubes. Mol Pharm 10(9): 3404–3416
Davis JD, Lin S-Y (2011) DNA damage and breast cancer. World J Clin Oncol 2(9):329–338
de Mello Jr LJ, Souza GRR, Winter E, Silva AH, Pittella F, Creczynski-Pasa TB (2017) Knock-down of antiapoptotic genes in breast cancer cells by siRNA loaded into hybrid nanoparticles. Nanotechnology 28(17):175101
Del Re M, Biasco E, Crucitta S, Derosa L, Rofi E, Orlandini C et al (2017) The detection of androgen receptor splice variant 7 in plasma-derived exosomal RNA strongly predicts resistance to hormonal therapy in metastatic prostate cancer patients. Eur Urol 71(4):680–687
Dreaden EC, Mwakwari SC, Sodji QH, Oyelere AK, El-Sayed MAJBc. (2009) Tamoxifen–poly (ethylene glycol)–thiol gold nanoparticle conjugates: enhanced potency and selective delivery for breast cancer treatment. Bioconjug Chem 20(12):2247–2253
Ediriwickrema A, Saltzman WM (2015) Nanotherapy for cancer: targeting and multifunctionality in the future of cancer therapies. ACS Biomater Sci Eng 1(2):64–78
Engel JB, Schally AV, Dietl J, Rieger L, Hönig A (2007) Targeted therapy of breast and gynecological cancers with cytotoxic analogues of peptide hormones. Mol Pharm 4(5):652–658
Fan L, Chen J, Zhang X, Liu Y, Xu C (2014) Follicle-stimulating hormone polypeptide modified nanoparticle drug delivery system in the treatment of lymphatic metastasis during ovarian carcinoma therapy. Gynecol Oncol 135(1):125–132
Garg N, Baishnab S, Das R, Kaur K, Gupta S, Arora S (2019) Research, management, insights of breast cancer and barriers to its therapy. 7(2):73–86
G.W.D. J. (2021) Phase I clinical trial investigating the ability of plant exosomes to deliver curcumin to Normal and malignant colon tissue 2021 Nov 22. Report No.: NCT01294072
Ghosh M, Das PKJC, Biointerfaces SB (2016) Doxorubicin loaded 17β-estradiol based SWNT dispersions for target specific killing of cancer cells. Colloids Surf B Biointerfaces 142:367–376
Goldblatt EM, Lee W-H (2010) From bench to bedside: the growing use of translational research in cancer medicine. Am J Transl Res 2(1):1
Golombek SK, May J-N, Theek B, Appold L, Drude N, Kiessling F et al (2018) Tumor targeting via EPR: strategies to enhance patient responses. Adv Drug Deliv Rev 130:17–38

Gonzalez-Angulo AM, Morales-Vasquez F, Hortobagyi GN (2007) Overview of resistance to systemic therapy in patients with breast cancer. Adv Exp Med Biol 608:1–22

Gupta A, Ahmad A, Singh H, Kaur S, Neethu KM, Ansari MM et al (2018a) Nanocarrier composed of magnetite core coated with three polymeric shells mediates LCS-1 delivery for synthetic lethal therapy of BLM-defective colorectal cancer cells. Biomacromolecules 19(3):803–815

Gupta D, Kumar M, Tyagi P, Kapoor S, Tyagi A, Barman TK et al (2018b) Concomitant delivery of paclitaxel and NuBCP-9 peptide for synergistic enhancement of cancer therapy. Nanomedicine 14(4):1301–1313

Haley B, Frenkel E (2008) Nanoparticles for drug delivery in cancer treatment. In: Urologic oncology: seminars and original investigations. Elsevier

Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144(5):646–674

Hattab D, Bakhtiar A (2020) Bioengineered siRNA-based Nanoplatforms targeting molecular Signaling pathways for the treatment of triple negative breast cancer: preclinical and clinical advancements. Pharmaceutics 12(10):929

He Y, Zhang L, Song C (2010) Luteinizing hormone-releasing hormone receptor-mediated delivery of mitoxantrone using LHRH analogs modified with PEGylated liposomes. Int J Nanomedicine 5:697

Henderson BE, Feigelson HS (2000) Hormonal carcinogenesis. Carcinogenesis 21(3):427–433

Howlader N, Noone AM, Krapcho M, Miller D, Brest A, Yu M, et al. (2021) SEER cancer statistics review, 1975–2018. national cancer institute Bethesda: national cancer institute. https://seer.cancer.gov/csr/1975_2018/

Jain A, Jain A, Parajuli P, Mishra V, Ghoshal G, Singh B et al (2018) Recent advances in galactose-engineered nanocarriers for the site-specific delivery of siRNA and anticancer drugs. Drug Disco Today 23(5):960–973

Jain A, Sharma T, Saini S, Katare OP, Soni V, Singh B (2020) Nanocargos: a burgeoning quest in cancer management. Current Nanomedicine 10(2):149–163

Jin Y (2021) Clinical trial of tumor cell-derived microparticles packaging chemotherapeutic drugs to treat malignant pleural effusion 2019 Nov 22. Report No.: NCT02657460

Kapse-Mistry S, Govender T, Srivastava R, Yergeri M (2014) Nanodrug delivery in reversing multidrug resistance in cancer cells. Front Pharmacol 5(doi):159–180

Kenny GD, Kamaly N, Kalber TL, Brody LP, Sahuri M, Shamsaei E et al (2011) Novel multifunctional nanoparticle mediates siRNA tumour delivery, visualisation and therapeutic tumour reduction in vivo. J Control Release 149(2):111–116

Khurana RK, Jain A, Jain A, Sharma T, Singh B, Kesharwani P (2018) Administration of antioxidants in cancer: debate of the decade. Drug Discov Today 23(4):763–770

Ki M-H, Kim J-E, Lee Y-N, Noh SM, An S-W, Cho H-J et al (2014) Chitosan-based hybrid nanocomplex for siRNA delivery and its application for cancer therapy. Pharm Res 31(12):3323–3334

Kim E-M, Jeong H-J (2017) Current status and future direction of nanomedicine: focus on advanced biological and medical applications. Nuclear medicine molecular imaging 51(2):106–117

Kim JJ, Kurita T, Bulun SE (2013) Progesterone action in endometrial cancer, endometriosis, uterine fibroids, and breast cancer. Endocr Rev 34(1):130–162

Learner A (2019) Cell biology and cancer. https://www.learner.org/courses/biology/support/8_cancer.pdf

Leyrer CM, Berriochoa CA, Agrawal S, Donaldson A, Calhoun BC, Shah C et al (2017) Predictive factors on outcomes in metaplastic breast cancer. Breast Cancer Res Treat 165(3):499–504

Li J, Li M, Tian L, Qiu Y, Yu Q, Wang X et al (2020) Facile strategy by hyaluronic acid functional carbon dot-doxorubicin nanoparticles for CD44 targeted drug delivery and enhanced breast cancer therapy. Int J Pharm 578:119122

Liang H-F, Hong M-H, Ho R-M, Chung C-K, Lin Y-H, Chen C-H et al (2004) Novel method using a temperature-sensitive polymer (methylcellulose) to thermally gel aqueous alginate as a pH-sensitive hydrogel. Biomacromolecules 5(5):1917–1925

M.D. Anderson Cancer Center (2021) EphA2 gene targeting using neutral liposomal small interfering RNA delivery: a phase I clinical trial 2021 Nov 22. Report No.: NCT01591356
Maeda H, Bharate G, Daruwalla J (2009) Polymeric drugs for efficient tumor-targeted drug delivery based on EPR-effect. Eur J Pharm Biopharm 71(3):409–419
Maeda H, Sawa T, Konno T (2001) Mechanism of tumor-targeted delivery of macromolecular drugs, including the EPR effect in solid tumor and clinical overview of the prototype polymeric drug SMANCS. J Control Release 74(1–3):47–61
Maeda H, Wu J, Sawa T, Matsumura Y, Hori K (2000) Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. J Control Release 65(1):271–284
Maskarinec G, Sen C, Koga K, Conroy SM (2011) Ethnic differences in breast cancer survival: status and determinants. Women's Health (Lond Engl) 7(6):677–687
Mehrotra N, Kharbanda S, Singh H (2020) Peptide-based combination nanoformulations for cancer therapy. Nanomedicine 15(22):2201–2217
Meier W (2000) Polymer nanocapsules. Chem Soc Rev 29(5):295–303
Meng J, Fan J, Galiana G, Branca R, Clasen P, Ma S et al (2009) LHRH-functionalized superparamagnetic iron oxide nanoparticles for breast cancer targeting and contrast enhancement in MRI. Mater Sci Eng 29(4):1467–1479
Mettler L, Eckmann-Scholz C, Semm I, Alkatout I (2014) Factors to consider in gynecological surgery. Womens Health 10(3):323–338
Modi DA, Sunoqrot S, Bugno J, Lantvit DD, Hong S, Burdette JEJN (2014) Targeting of follicle stimulating hormone peptide-conjugated dendrimers to ovarian cancer cells. Nanoscale 6(5): 2812–2820
Mussi SV, Silva RC, de Oliveira MC, Lucci CM, de Azevedo RB, Miranda Ferreira LA (2013) New approach to improve encapsulation and antitumor activity of doxorubicin loaded in solid lipid nanoparticles. Eur J Pharm Sci 48(1–2):282–290
Nakamura T, Akita H, Yamada Y, Hatakeyama H, Harashima H (2012) A multifunctional envelope-type nanodevice for use in nanomedicine: concept and applications. Acc Chem Res 45(7):1113–1121
Nasir A, Kausar A, Younus AJ (2015) A review on preparation, properties and applications of polymeric nanoparticle-based materials. Polymer-Plastics Technol Eng 54(4):325–341
National Institute of Cancer Prevention and Research (NICPR) (2020) Cancer Statistics India. http://cancerindia.org.in/cancer-statistics/. Accessed 24 Nov 2021
Nwadike VR (2018) Can a hormone imbalance affect your menstrual cycle?: Healthline media. https://www.healthline.com/health/hormonal-imbalance-menstrual-cycle#symptoms
Ouyang X, Wang M, Guo L, Cui C, Liu T, Ren Y et al (2020) DNA nanoribbon-templated self-assembly of Ultrasmall fluorescent copper nanoclusters with enhanced luminescence. Angew Chem 132(29):11934–11942
Pucci C, Martinelli C, Ciofani G (2019) Innovative approaches for cancer treatment. In: Current perspectives and new challenges. Ecancermedicalscience, p 13
Qiao R, Liu C, Liu M, Hu H, Liu C, Hou Y et al (2015) Ultrasensitive in vivo detection of primary gastric tumor and lymphatic metastasis using upconversion nanoparticles. ACS Nano 9(2): 2120–2129
Qin Y, Song Q-G, Zhang Z-R, Liu J, Fu Y, He Q et al (2008) Ovarian tumor targeting of docetaxel-loaded liposomes mediated by luteinizing hormone-releasing hormone analogues. Arzneimittelforschung 58(10):529–534
Ramos J, Rege K (2013) Poly (aminoether)–gold Nanorod assemblies for shRNA plasmid-induced gene silencing. Mol Pharm 10(11):4107–4119
Rawla P (2019) Epidemiology of prostate cancer. World J Oncol 10(2):63
Rous P, Kidd JG (1941) Conditional neoplasms and subthreshold neoplastic states: a study of the tar tumors of rabbits. J Exp Med 73(3):365–390
Salkho NM, Paul V, Kawak P, Vitor RF, Martins AM, Al Sayah M et al (2018) Ultrasonically controlled estrone-modified liposomes for estrogen-positive breast cancer therapy. Artif Cells Nanomed Biotechnol 46(sup2):462–472

Sandhu PS, Kumar R, Beg S, Jain S, Kushwah V, Katare OP et al (2017) Natural lipids enriched self-nano-emulsifying systems for effective co-delivery of tamoxifen and naringenin: systematic approach for improved breast cancer therapeutics. Nanomedicine 13(5):1703–1713
Selimah Fauzee NJ, Dong Z, Wang Y (2011) Taxanes: promising anti-cancer drugs. Asian Pac J Cancer Prev 12(4):837–851
Senapati S, Mahanta AK, Kumar S, Maiti P (2018) Controlled drug delivery vehicles for cancer treatment and their performance. Signal Transduct Target Ther 3(1):1–19
Seyfried TN, Huysentruyt LC (2013) On the origin of cancer metastasis. Crit Rev Oncog 18(1–2): 43
Shigdar S, Li Y, Bhattacharya S, O'Connor M, Pu C, Lin J et al (2014) Inflammation and cancer stem cells. Cancer Lett 345(2):271–278
Singh J, Komal T, Arora S, Kaur A, Singh TG (2017) Research, management, Ebola hemorrhagic fever: recent update on disease status, current therapies and advances in treatment. 5(2):217–234
Singh R, James W, Lillard J (2009) Nanoparticle-based targeted drug delivery. Exp Mol Pathol 86(3):215–223
Sledge GW, Mamounas EP, Hortobagyi GN, Burstein HJ, Goodwin PJ, Wolff AC (2014) Past, present, and future challenges in breast cancer treatment. J Clin Oncol 32(19):1979–1986
Society AC (2019) What is breast cancer. https://www.cancer.org/cancer/breast-cancer/about/what-is-breast-cancer.html
Society AC (2021) Hormone therapy for prostate cancer. https://www.cancer.org/cancer/prostate-cancer/treating/hormone-therapy.html
Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A et al (2020) Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA-Cancer J Clin 68(6):394–424
Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A et al (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
Swami R, Kumar D, Khan W, Sistla R, Shastri N (2014) Polymer–drug conjugate in focal drug delivery. Springer, Focal Controlled Drug Delivery, pp 117–147
Tannock IF (1998) Conventional cancer therapy: promise broken or promise delayed? Lancet 351: SII9–SII16
Thapa RK, Nguyen HT, Jeong J-H, Shin BS, Ku SK, Choi H-G et al (2017) Synergistic anticancer activity of combined histone deacetylase and proteasomal inhibitor-loaded zein nanoparticles in metastatic prostate cancers. Nanomedicine 13(3):885–896
Thundimadathil J (2012) Cancer treatment using peptides: current therapies and future prospects. J Amino Acids 2012
Tran S, DeGiovanni P-J, Piel B, Rai P (2017) Cancer nanomedicine: a review of recent success in drug delivery. Clin Transl Med 6(1):1–21
Wang W-T, Han C, Sun Y-M, Chen T-Q, Chen Y-Q (2019a) Noncoding RNAs in cancer therapy resistance and targeted drug development. J Hematol Oncol 12(1):1–15
Wang Y, Wang Z, Qian Y, Fan L, Yue C, Jia F et al (2019b) Synergetic estrogen receptor-targeting liposome nanocarriers with anti-phagocytic properties for enhanced tumor theranostics. J Mater Chem B 7(7):1056–1063
WHO (2018) Latest global cancer data: cancer burden rises to 18.1 million new cases and 9.6 million cancer deaths in 2018;2019
Xia X, Feng H, Li C, Qin C, Song Y, Zhang Y et al (2016) 99mTc-labeled estradiol as an estrogen receptor probe: preparation and preclinical evaluation. Nucl Med Biol 43(1):89–96
Xu J, Liu Y, Li Y, Wang H, Stewart S, Van der Jeught K et al (2019) Precise targeting of POLR2A as a therapeutic strategy for human triple negative breast cancer. Nat Nanotechnol 14(4): 388–397

Xu MD, Ting H (2021) A phase I/II clinical trial study on autologous erythrocytes derived microparticles packaging methotrexate peritoneal perfusion in the treatment of malignant ascites 2017 Nov 22. Report No.: NCT03230708

Yu W, He X, Yang Z, Yang X, Xiao W, Liu R et al (2019) Sequentially responsive biomimetic nanoparticles with optimal size in combination with checkpoint blockade for cascade synergetic treatment of breast cancer and lung metastasis. Biomaterials 217:119309

Zhang XY, Chen J, Zheng YF, Gao XL, Kang Y, Liu JC et al (2009) Follicle-stimulating hormone peptide can facilitate paclitaxel nanoparticles to target ovarian carcinoma in vivo. Cancer Res 69(16):6506–6514

Zhang X, Xu X, Li Y, Hu C, Zhang Z, Gu Z (2018) Virion-like membrane-breaking nanoparticles with tumor-activated cell-and-tissue dual-penetration conquer impermeable cancer. Adv Mater 30(27):1707240

# Progress of Cancer Nano Medicine, Clinical Hurdles, and Opportunities

3

Priti Tagde, Anshul Sharma, Kamya Goyal, and Shammy Jindal

**Abstract**

Cancer is the second leading cause of mortality in the globe. One of the most severe flaws in most anticancer medicines is their lack of tumor selectivity. Nanomedicine for cancer treatment given intravenously to avoid renal clearance cannot pass through tight endothelial junctions in normal blood arteries and accumulate in plasma at high levels. In this chapter, we will look at the advancements of nanomedicine in all areas of pharmaceuticals. We will also discuss the challenges encountered during clinical testing and development of cancer medicines, as well as future opportunities.

**Keywords**

Cancer · Nanomedicine · Clinical hurdles · Chemotherapy · EPR effect

P. Tagde
Amity Institute of Pharmacy, Amity University Uttar Pradesh, Noida, India

A. Sharma
Department of Pharmaceutics, Laureate Institute of Pharmacy, Kathog, India

Department of Pharmaceutical Sciences, Laureate Institute of Pharmacy, Kathog, India

K. Goyal
Department of Pharmaceutical Chemistry, GHG Khalsa College of Pharmacy, Gurusar Sadhar, Ludhiana, India

S. Jindal (✉)
Department of Pharmaceutics, Laureate Institute of Pharmacy, Kathog, India



M. Rahman et al. (eds.), *Hormone Related Cancer Mechanistic and Nanomedicines*,
https://doi.org/10.1007/978-981-19-5558-7_3

## 3.1 Introduction

Cancer nanomedicine is the use of nanotechnology in cancer treatment, and although the area has progressed significantly, there is still much more to be done. The EPR effect, tumor targeting, and deposition, and the role of "nano" in cancer treatment are all highly contested subjects in the area. This chapter indicates cancer nanomedicine and discusses challenges, concepts, and prospects (Tseng et al. 2020). With a projected rise in the number of patients diagnosed worldwide from 18.1 million in 2018 to 23.6 million in 2030, cancer is one of the most severe disease burdens in contemporary times. Traditional treatments have made tremendous progress, but they still have limits and are far from optimal (Haider et al. 2020). As a result, safe, effective, and broadly applicable therapies are urgently required (Ouyang et al. 2020). The development of new delivery methods based on membrane-core (MC) nanoparticles for transferring chemotherapeutics, nucleic acids, and immunological modulation techniques has increased anticancer effectiveness and decreased side effects considerably in recent decades (Ferraro et al. 2020).

Cancer is a disease that is very diverse and complex. The treatment strategy is curative or noncurative, depending on the severity of the illness and the patient's overall clinical condition. It may use both imaging and direct vision techniques to determine the degree of disease ("staging") (e.g., visualization at the time of surgery). The goal of the curative ("radical") operation, or surgery coupled with radiotherapy and chemotherapy, is to eliminate the whole tumor (von Roemeling et al. 2017).

Many primary tumors (tumors at the site of cancer development) may be cured using the best treatment methods, but metastatic tumors cause the overwhelming majority of cancer-related fatalities. When a cure isn't possible, the emphasis shifts to improving the overall survival of patients, such as with chemotherapy and targeted treatment (e.g., with molecular-targeted agents and antibodies) (Tong and Kohane 2016).

As a result, metastatic illness is an appealing target for the treatment of novel therapies to improve patient survival. Thousands of cancer nanomedicines have been created too far, with just a few dozen having been authorized for clinical use. The primary benefit for most of them is a lowering in adverse effects, such as nausea/vomiting, hair loss, anemia, and cardiotoxicity. While several recent clinical studies have shown promise, the survival benefits of nanomedicine over conventional therapy are usually small (Balasubramanian et al. 2018a).

For example, no difference in survival was observed in a recent meta-analysis comparing liposomal vs. non-liposomal chemotherapy (Balasubramanian et al. 2018b) clinical studies, which included a total of 2589 individuals. These findings contrast sharply with preclinical mouse studies, which showed a significant improvement in survival. The discrepancies are attributable to variances in human and rat tumor microenvironments, dosage regimens, pharmacodynamics, and a lack of uniformity in preclinical anticancer efficacy studies' execution and presentation. Although many of the drug delivery methods used in these clinical trials were established many years ago, the basic ideas and reasoning that underlie most cancer

nanomedicine fields have stayed relatively unaltered (van der Meel et al. 2017). This involves, for instance, the use of nanomaterials to directly destroy tumors through cytotoxic drug delivery that has been refined using animal models. Moving ahead, we should think about how we might enhance and expand on existing methods to both optimize our fundamental understanding of nanomaterial behavior in cancer nanomedicine and make it easier to translate that knowledge into better patient outcomes. Human cancers are complex and diverse, with differences across patients, between several tumors in the same patient (e.g., original tumor and metastatic, and between metastases), and even within the same tumor microenvironment (Bae et al. 2013).

This has significant consequences for therapeutic responsiveness and resistant strains. Nonetheless, malignancies have many characteristics, such as unabated proliferation and development, immune system alterations, and angiogenesis activation (i.e., new blood vessels). The aberrant tissue environment related to cancer presents difficulties as well as possibilities for therapy development. Obstacles to the delivery of medicines to tumors include vascular and interstitial barriers (Anchordoquy et al. n.d.).

This has huge consequences for anticancer drugs, antibodies, and nanomedicines penetrating tumors. Unique characteristics associated with tumors, on the other hand, present opportunities because they may allow for selective detection and treatment. Dyes inoculated into the blood of mice and rats (with implanted tumors) or cats and dogs (with spontaneous tumors) have been recognized to extravasate out of the blood circulation and accumulate in tumors since the late 1950s (Shi et al. 2020; Xie et al. 2020).

## 3.2 Using the Next-Generation of Cancer Nanomedicine

Most malignancies need combinational treatments, like surgery, radiation therapy, and chemotherapy, often coupled with biological treatment, like antibodies therapy, immunomodulation, (Weiner 2015; Åstrand et al. 2016), and T cell engineering. Nanomedicine may help with these methods by directing tumor removal surgery, (ii) improving radiation, (iii) co-delivering medicines to decrease the risk of acquiring multidrug-resistant malignancies, and (iv) activating the immune system to generate or maintain antitumor activities.

The potential of utilizing cancer nanomedicine to categorize treatment based on imaging and response complements the methods described above. Early work on antibody imaging aided clinical translation and approval processes (knowing where the substance is and what will happen to it helps with development and regulatory decisions, and molecular imaging is now an effective tool in pharmaceutical research and trial design (Sandström et al. 2016).

Nanomedicine is becoming more reliant on imaging methods. While it's essential to keep in mind the expenses of increasing imaging capabilities, there are methods to cut down. The employment of "companion particles" with good imaging capabilities in conjunction with therapeutic particles is an intriguing recent example. Another

example is the use of nanomaterials or medicines such as quantum dots or doxorubicin, which are both fluorescent and therapeutic (e.g., cytotoxic). In nanomedicine, imaging may assist with dosage modifications and give feedback on the therapy (Keyaerts et al. 2016).

Nanoparticles for tumor therapy and imaging that can remove rapidly and safely have recently improved (Thomas et al. 2016). Also, employing carriers that are strong enough that any excess is eliminated from the body (e.g., via the kidneys and urine) since before the medication is released, reducing off-target toxicity, may open up new options for drug administration (In't Veld et al. 2020).

Cancer is the leading cause of mortality worldwide. The current treatments are insufficient, prompting a need for more advanced technology. The rapid advancement of nanotechnology in creating nanomedicine products offers tremendous potential for improving cancer treatment methods. Nanomedicine products provide the possibility of developing advanced targeting methods as well as multifunctionality. They may enhance traditional treatments' pharmacokinetics and pharmacodynamics, potentially increasing the effectiveness of already available anticancer drugs (Wicki et al. 2015a). Advancements in medical biology have only slowly translated into significant progress in cancer treatment during the last two decades. According to the World Health Organization (WHO), cancer was responsible for 8.2 million fatalities in 2012, accounting for 13% of all deaths, according to the World Health Organization (WHO). Global cancer occurrences are projected to rise from 14 million in 2012 to 22 million in the next two decades (World Health Organization 2013). The lack of targeted delivery of anticancer chemicals to neoplastic tissue is one of the major causes. Dose-limiting toxicity is often seen after high systemic exposure to antineoplastic drugs. As a result, to overcome existing limits in cancer treatment, tailored delivery is critical. Recent advances in nanotechnology are anticipated to enhance medication delivery, boosting effectiveness while reducing anticancer treatment adverse effects (Song et al. 2021).

Nanocarriers are distinguished by their nanoscale size, high surface-to-volume ratio, and favorable physicochemical properties. They can change a drug's pharmacokinetics and pharmacodynamic characteristics, thus improving its therapeutic index. Drug loading onto nanocarriers may improve in vivo stability, prolong the duration a chemical spends in the bloodstream, and permit controlled drug release (Ahmad et al. 2021). As a result, nanomedicine substances may change medication biodistribution by enabling them to concentrate near the tumor site. The increased permeability and retention effects are the name for this phenomenon (Riaz et al. 2021).

Nanomaterials based on organic, inorganic lipids, proteins, or glycan compounds, as well as synthetic polymers, have been used to create new cancer therapies. According to clinicaltrials.gov, 1575 nanomedicine formulations have been filed for clinical trials as of December 2014 (Manoharan et al. 2021). On the other hand, most clinical trials concentrate on commercially accessible medicines such as liposomal doxorubicin and albumin-bound paclitaxel (Kirn and Thorne 2009). New indications or therapies in combination with other anticancer drugs are currently being studied.

In light of this, we highlight current advances in nanomedicine treatments in early and late clinical trials in this analysis. We deliberate the possibilities for developing sophisticated clinical nanomedicine treatments in the future. We also handle issues that arise during medication development and regulatory approval (Andtbacka et al. n.d.; Chung et al. 2020).

## 3.3 Viral Nanoparticles for Cancer Therapy

An innovative approach for creating nanoparticles for cancer therapy is to utilize tumor-homing viruses that have been engineered to generate therapeutic proteins. Myxoma and vaccinia viruses, for example, prefer to reproduce in tumor cells. Blocking apoptotic pathways, deregulating cell reproduction, and immune evasion are all favorable characteristics of cancer cells for effective pox virus replication (Shahgolzari et al. 2020).

JX-594 is a poxvirus that multiplies in tumor cells and kills them via the EGFR-Ras-MAPK signaling pathway. JX-594 also codes for granulocyte colony-stimulating factor (G-CSF), a protein that may help the immune system fight cancer. Intramuscular injection of this oncolytic virus resulted in three partial remissions and six cases of stable disease in 10 patients with primary or metastatic liver cancer. Flu-like symptoms and hyperbilirubinemia were the most frequent side effects (Parato et al. 2012).

In a second phase one trial, JX-594 was given intravenously (i.v.) to 23 patients with advanced solid tumors. After successfully homing to tumor tissue, JX-594 showed dose-dependent antitumor effectiveness. Normal tissue did not show any signs of viral replication (Park et al. 2015). Although the virus was picked up by the epithelium adjacent to it, it did not reproduce. When an oncolytic virus was administered intravenously, this solid evidence study was the first to show dose-dependent viral multiplication and tumor response (Heo et al. 2013).

The OPTiM trial (Kaufman and Bines 2010) was the first significant phase 3 study employing oncolytic viruses in individuals with advanced melanoma (stages IIIB–IV). The study's main aim was to get a long-term response. 16.3% of patients infected with the virus had a long-term response, compared to just 2.1% in the control group (Harrington et al. 2015).

Surprisingly, non-injected metastases showed responses, indicating that the virus may move to non-injected tumor locations. Cellulitis was the most common grade 3/4 hazard, affecting 2.1% of study participants. Although there was a long-term survival tendency, this has yet to be verified. T-Vec may be the first oncolytic virus to get FDA approval for cancer treatment (Tam et al. 2019).

Various medication delivery methods have been developed as a significant advancement in nanotechnology and chemical/pharmaceutical engineering. These systems aim to enhance chemotherapeutic medication bioavailability of drugs and target site deposition (Westphal et al. 2013).

Several additional oncolytic viruses have been tried in clinical trials in recent years, but nobody has yet made it to market. Concerns regarding biosafety and

cytocompatibility are two of its main disadvantages. Many reviews have addressed the clinical outcomes and difficulties (Bottagisio et al. 2019).

## 3.4 Nanocarrier's Properties

### 3.4.1 Physicochemical Properties

Nanomaterials for cancer research may be customized in size, shape, and surface properties to treat particular cancers. The size of the nanocarriers is essential for their passage through the circulation and subsequent distribution to tumor tissue. While smaller nanoparticles are more readily accumulated in tumors' leaky blood arteries than bigger ones, they may also extravasate into normal tissue. On the other hand, large nanoparticles cannot extravasate as readily and therefore have a very varied distribution in circulation and may optimize the size of nanoparticles to enhance selective absorption into tumor tissue. The form of the nanocarriers may affect fluid dynamics, affecting absorption. Due to synthesis and testing difficulties, spherical nanocarriers seem to be more prevalent than nonspherical nanocarriers at the moment (Larsen et al. 2020).

Nanocarriers' charge may have an impact on their stability and dispersion in the blood. Positively charged nanoparticles were previously found to be the most successful in targeting tumor vasculature; however, switching to a neutral charge after extravasation allowed faster nanoparticle diffusion into tumor tissue. The surfaces of the nanocarriers may also be changed with ligands that stimulate particular kinds of endocytosis and cellular absorption into tumor tissue, extending blood circulation and promoting specific types of endocytosis (Ryu et al. 2020).

### 3.4.2 Solubility, Degradation, and Clearance Are all Factors to Consider

Drugs that are poorly soluble in water may be removed from the circulation before reaching tumor tissue. Using hydrophilic nanoparticles to enclose these medicines may enhance their solubility, increasing their bioavailability in vivo and allowing for more effective administration. It has been shown that coating nanoparticles with polyethylene glycol (PEG), a hydrophilic and nonionic polymer, increases their solubility and stability (Tran et al. 2017).

PEG is uncharged. Therefore, it doesn't interfere with the operation of charged molecules like DNA. Monocytes and macrophages are more likely to detect foreign objects coated with opsonin proteins (Siegel et al. 2017). Opsonizing hydrophobic molecules may decrease their capacity to penetrate tumor tissue and lead to inflammation due to phagocytic cells secreting cytokines. PEGylated nanoparticles hide their hydrophobicity, allowing them to spend more time in the bloodstream before reaching tumor tissue (Siegel et al. 2016).

This decrease in clearance not only extends the nanoparticle's half-life, but also enhances its bioavailability. This increased bioavailability enables the medication to circulate in the bloodstream for more extended periods, avoiding breakdown before reaching the target region. Additional surface changes, such as ligands for overexpressed receptors, may aid in the selective absorption of medicines in the tumor. Controlled release systems may also help to avoid nonspecific harmful medication delivery to healthy tissue (Hanahan and Weinberg 2011).

## 3.5 Targeting

To reach tumor tissue, nanocarriers may be tailored to use passive and active targeting methods. The increased permeability and retention (EPR) effect enables nanoparticles to collect leaky blood vessels in tumors without surface modification passively. On the other hand, passive targeting cannot completely prevent the possibility of nanocarriers accumulating in organs with fenestrated blood arteries, such as the liver or spleen (Tahmasbi Rad et al. 2019). Moreover, the microenvironments of different cancers differ, which may be a stumbling block for nanomedicine production (Bregoli et al. 2016). The attaching of ligands to the surface of nanocarriers is used for active targeting. These ligands are highly selective for receptors and other cancer-specific targets, such as glycans, which are overexpressed on the surface of tumor cells. Conjugation of these ligands may prevent nanocarriers from being taken up by tissue other than tumor tissue. Examples of ligands include transferrin, folic acid, enzymes, modified antibodies, and macromolecules like proteins and polysaccharides (Truong et al. 2015). The density of these ligands should be adjusted to prevent detection by the RES and interaction with serum proteins, allowing nanoparticles to circulate for longer (von Roemeling et al. 2017; Wang et al. 2017).

### 3.5.1 Tumor Targeting

Tumor targeting is one of the possible basic benefits of nanotechnology for cancer therapy. Nanotechnology's goal in cancer therapy is to distinguish malignant cells from nonmalignant cells and selectively destroy malignant cells. Passive and active targeting are two basic mechanisms involved in the differentiation of malignant and nonmalignant cells.

To raise the concentration of nanoparticles (NPs) in the tumor, passive targeting uses the increased permeability and retention (EPR) effect (Shi et al. 2020). To localize NPs to malignant cells, active targeting may encompass selective intermolecular interactions of antigens, most commonly proteins, expressed on the surfaces of cancer cells, or conversely, exploiting biochemical properties linked with malignancy and matrix metalloproteinase secretion (Kang et al. 2020).

Both passive and active targeting techniques may be used alone or in combination. Both methods benefit from NP surface changes that reduce absorption by the

macrophage phagocytic system (MPS) (Huang et al. 2020), increasing circulation time.

### 3.5.2 Targeting via the EPR

The cancerous vascular is well-accepted to be leaking, contrary to the structured society of regular vascular, since cancerous cells do not respond to intercellular communication essential for efficient cellular proliferation (Subhan et al. 2021). Polymers may reach the disease through a reticuloendothelial system and remain due to decreased lymph drainage in cancers due to higher porosity and persistence (EPR). The EPR's effectiveness is determined by many therapeutic variables, such as clinical outcome, kind, and diversity. The amount of treatment being addressed influences the EPR's effectiveness. Isolation of materials by the EPR is effective in the MW range 40 kDa–800 kDa, which correlates to glycosylated minimal radii of 2.3–6.1 nm, as per Maeda (Gmeiner and Ghosh 2014). Interestingly, the EPR showed that a minimal of 25 kDa was needed for enzyme localization in cancer cells, with different size proteins having better uptake—however, small compounds needed documenting progress for storage. On the other hand, lipids cannot revert to their original state after being continuously attacked (Bertrand et al. 2014).

Extensive nanomaterials with an aerodynamic radius more significant than the kidney clearance limit, which may also physically contact from leaky tumor vasculature, cause Porphyrin molten rock. Recent research has looked at ways to broaden the traditional notion of Porphyrin cancer therapy. Bort et al. talk about their research into using superparamagnetic iron particles to target tumors (Liyanage et al. 2019).

Xu et al. compare the tumor aiming effectiveness of 3 and 30 nm receptor particles in a research method. Their findings indicate that adding a focusing ligand to 3 nm nanoparticles improved tumor-targeted point and tumor penetration, but not for 30 nm nanoparticles (Gurunathan et al. 2018). Pharmaceutical and muscle ache have been used to stimulate the cancer cell to enhance the EPR effect and Nanoscience efficacy. Biological and behavioral methods to alter the cancer cell are also suggested as priming tactics to enhance EPR impact (Li et al. 2021a).

### 3.5.3 Active Targeting

Systemic toxicity may be utilized in conjunction with EPR-based targeted delivery to enhance biomedicine molten rock and stability. Antibodies, fractions (e.g., nanobodies), and acids are examples of tumor protein targets. The uses of chemotherapy drugs in cellular diagnostics of cancer, inflammatory illnesses, and heart disease are summarized (Belhadj et al. 2020). The use of polyclonal immunoglobulin genomic imaging techniques in theranostics and treatment plans is also emphasized. Hypermethylation (EGFR) targeted nanobodies to deliver photocatalysts to tumors for photothermal treatment. Photocatalysts are coupled to both tetravalent and biparatopic changes in cognition. While bioavailability patterns

of these two kinds of agonists vary, they both cause comparable amounts of necrotic following photothermal treatment, ending in tumor shrinkage (Liang et al. 2021).

Another scholar describes the utilization of a synthesized vocabulary learning estrogen protein decapeptide to transport docetaxel and siRNAs to breast cancer cells through nano-sized solid lipids. The nanotechnology was inhaled, and it was shown to be effective in sticking to the tumor site (Stack et al. 2021). To enhance the distribution of glucan chlorine polyols to B16 cancer, Zhong and colleagues use cyclic RGD to help refine. The dynamically focused polymeric micelles had a 6.5 h in vivo clearance half-life in the bloodstream, resulting in effective cancer treatment (6.7% ID/g) and potential therapeutic efficacy (Zhang et al. 2015). Nanotechnology combination therapies are another way to enhance chemotherapy treatment. Zhao et al. (Zhou et al. 2015) highlight the possibility of this strategy for the treatment of glioblastoma, which makes use of synergistic psychoactive drugs. The authors go through the rationale for nanotechnology medication combos as well as recent clinical advances in grain size distribution therapeutic compounds and describe a kind of biomedicine that causes cancer starving by inhibiting proliferation and circulatory blockage. To obtain synergies for clinical trials, thermo have been coupled with other methods such as chemotherapeutic, regenerative medicine, and photothermal therapy. To enhance cyclophosphamide treatment, Zhu et al. report on a pH-sensitive nanoscience composition comprising an enzyme, targeted ultrasonic tumor elimination, and hypoxic relief (Zhou et al. 2015). Their telomerase nanoparticle were capable of converting $H_2O_2$ to $O_2$, increasing oxygen content in tumors, and reducing tumor hypoxia, all of which enhanced cisplatin effectiveness.

New planes built on genomics manufacturing processes that enable cancer dosage forms have been explored compared to existing nanomedicines utilized for EPR-based targeted therapy. (Miller-Kleinhenz et al. 2018). Intracellularly vesicles' intrinsic tissue virulence factors are very intriguing for targeted treatment, and the authors discuss techniques for loading bioactive drugs into exosomes and modifying tactics to enhance tumor persecuting capabilities. Nanocarriers that respond to both internal and external stimuli are addressed, as well, as their help improves therapeutic effectiveness. To improve treatment results, cancer nanomaterials have been widely coupled with chemotherapy. Huang et al. review current advances in combined graphene oxide, emphasizing nanocarriers that enhance chemotherapeutic effectiveness by modifying the tumor immune ecology (TIME) (Huang et al. 2016).

Immunosuppressive nanotechnology based on exosomes co-loaded with an activated receptor beta-blocker and an immunologic cell death initiator was described by Panagi et al. in an observational investigation. The combined therapy based on liposomes increases the immunology of quadruple breast cancers, while also increasing the effectiveness of circuit blocking antibodies. Imaging can assist capture tumor targeting efficiency and the diversity of the EPR effect in tumors, which is essential for tumor targeting and integrative cancer nanotechnology. The use of cognitive computing to comprehend and guide the trickery of the EPR effect and tumor cells for improving biomedicine therapy is mentioned, focusing on using the latest events in fermenting techniques and approaches in systems toxicology of biomedicine (Panagi et al. 2020).

### 3.5.4 Enhanced Permeability and Retention (EPR) Imaging

The use of imaging methods such as partner analysis of the proposed, nano theragnostic, traditional imaging technology, and histopathology to ultimately allow client classification is addressed. De Maar et al. published a thorough study on parametric imaging methods for evaluating the diversity of nanomedicines' geographic variation in tumors, which is a significant—and frequently ignored—cause of ineffective nanotherapy (De Maar et al. 2020). Quasi clinical screening tests (nuclear imaging, radiography, ct scans, and ultrasonic), optical imaging, and electron microscopy imaging are the three classes of diagnostic devices discussed by the authors for evaluating the intra-tumoral allocation of nanocarriers. A new information on the use of elevated ex vivo segments and sub-ultrasonography to investigate the geographic variation of exosomes in four distinct malignant tumors were carried out. Their findings show that vascular dispersion and vessel assistance are important factors in effective lipid formation and dispersion in malignancies. In circumstances that allow for surgery, Qi et al. report on the use of salicylic acid coupled with gold nanoparticles for cellular diagnostics of pancreatic cancer (Qi et al. 1993). Their findings show that alginate particle size and the biological activities of attached dyes have an impact on the effectiveness of tumor cells' detection. Lastly, using EPR-based molten rock, Li et al. reported on means maximizing coagulated with Pharmaceutical excipients and radionuclide for cellular diagnostics and endoradiotherapy of malignant tumors. The star polyethylene particles showed a very high tumor targeting effectiveness (15–22%ID/g) in CT26 tumor cells mice, which led to the animals' increased survival after endoradiotherapy treatment (Li et al. 2021b).

## 3.6 Challenges and Barrier in Success of Nanomedicine for Cancer

After a nanomedicine is injected into the body, it is subjected to various barriers, which can limit the total amount of nanomedicine that reaches the tumor, influencing the outcome. For example, macrophages found in the liver, spleen, lungs, lymph nodes, and skin can phagocytoze and degrade nanoparticles. If the nanoparticles are opsonized with serum proteins, aggregate, are large in size, or have a cationic surface charge, phagocytosis is enhanced (Sengupta 2017; Kievit and Zhang 2011).

To address the aforementioned challenges and avoid macrophage capture, nanoparticles (ideally less than 100 nm in diameter) are traditionally coated with polyethyleneglycol (PEG), which reduces opsonization and acts as a 'stealth' cloak around the nanoparticles (Sun et al. 2014).

Despite the aforementioned challenge, a recent analysis of data from 117 reports published over the last decade revealed that only 0.7% (median) of the injected nanomedicine dose reaches the tumor (Wilhelm et al. 2016; Rosenblum et al. 2018). It is possible that only a portion of this dose reaches the cancer cells, with the remainder trapped in the stromal compartment. The limited success of cancer

nanomedicine clinical translation, according to this provocative analysis, is due to a small fraction of the administered drug reaching the desired site of action. Attempts have been made to improve tumor targeting by incorporating homing mechanisms, such as tumor-binding antibodies, aptamers, and peptides, on the surface of nanomedicines. However, such approaches have been shown to increase intratumoral delivery to 0.9% of the injected dose (Sun et al. 2020).

Another reason for nanomedicine's failure is our lack of understanding of disease heterogeneity in the patient population, inability to fine-tune the system based on disease biology or stage of the target patients, and failure to build an evidence platform supporting a specific end clinical application (Hare et al. 2017).

In a recent study, gamma-scintigraphy/SPECT imaging of indium/technetium-labeled liposomes and detection of drug fluorescence in patient biopsies were used to document nanomedicine biodistribution and accumulation in human tumors in a small number of patients. Tumor accumulation of indium-labeled liposomes differed by tumor type, ranging from 53% of the injected dose/kg in breast cancers to $33 \pm 16\%$ of the injected dose/kg in head and neck cancers. These findings highlight the concern that nanomedicine access and/or accumulation may be disease-dependent and vary from tumor to tumor. The significance of this concept should not be underestimated. The inter-tumor variability in nanomedicine delivery is confirmed by a recent analysis of the EPR effect in spontaneous canine carcinomas and sarcomas that showed substantial heterogeneity in the level of liposome uptake, as measured by CT/PET scanning (Hare et al. 2016; Jang and Sengupta 2019).

## 3.7 Progress in Nanomedicines

The progress in nanomedicine is presented in Tables 3.1 and 3.2.

## 3.8 Summary and Future Perspective

In the diagnosis and treatment of cancer, nanotechnology is becoming increasingly important. Because NPS is so small compared to cells and organelles, they may interact with specific cell features, allowing for active tumor cell targeting. The size regime of NPs is also appropriate for EPR-based passive tumor tissue targeting. As a consequence, as compared to low molecular weight medicines, nano-sized materials provide substantial chemotherapeutic benefits. These properties are being effectively exploited for improved chemotherapeutic drug delivery, resulting in enhanced anticancer effectiveness and reduced systemic toxicity (Wiwatchaitawee et al. 2021; Pei et al. 2020; Cheng et al. 2021).

NPs may engage with magnetic fields, NIR irradiation, and other external fields due to their chemical diversity, enabling for exact interactions between exterior areas and tumor tissue and potentially with specific malignant cells in vivo. The chemical composition of NPs varies, which allows for external field disruption and enhanced contrast in imaging applications (Rommasi and Esfandiari 2021; Zhang et al. 2021).

**Table 3.1** Progress of nanomedicine in chronological order

| Generic name or proprietary name | Nanotechnology type | Active pharmaceutical ingredient | Cancer type | Type of therapy | Status | Reference |
|---|---|---|---|---|---|---|
| Liposomal doxorubicin (Doxil) | Pegylated liposome | Doxorubicin | HIV-related Kaposi sarcoma, ovarian cancer, and multiple myeloma | Nontargeted chemotherapy | Approved by FDA (1995) | Shi et al. (2017) |
| Liposomal daunorubicin (DaunoXome) | Liposome | Daunorubicin | HIV-related Kaposi sarcoma | Nontargeted chemotherapy | Approved by FDA (1996) | Shi et al. (2017) |
| Liposomal doxorubicin (Myocet) | Liposome | Doxorubicin | Metastatic breast cancer | Nontargeted chemotherapy | Approved in Canada and Europe (2001) | Shi et al. (2017) |
| Nab-paclitaxel (Abraxane) | Albumin nanoparticle | Paclitaxel | Breast, lung, and pancreatic cancer | Nontargeted chemotherapy | Approved by FDA (2004) | Chaturvedi et al. (2019) |
| Polymeric micelle paclitaxel (Genexol-PM) | Polymeric micelle | Paclitaxel | Breast cancer | Nontargeted chemotherapy | Approved in Korea (2007) | Hare et al. (2017) |
| Liposomal vincristine (Marqibo) | Liposome | Vincristine | Acute lymphoblastic leukaemia | Nontargeted chemother-apy | Approved by FDA (2012) | Chaturvedi et al. (2019) |
| SMANCS | Polymer conjugate | Neocarzinostatin | Liver and renal cancer | Nontargeted chemotherapy | Approved in Japan | Hare et al. (2017) |
| Liposomal cisplastin (Lipoplatin) | Pegylated liposome | Cisplastin | Non-small cell lung cancer (NSCLC) | Nontargeted chemotherapy | Phase III trial | Stathopoulos et al. (2010) |
| Nab-rapamycin (ABI-009) | Albumin nanoparticle | Rapamycin | Advanced malignant pecoma | Nontargeted chemotherapy | Phase II trail | https://clinicaltrials.gov/ct2/show/NCT03657420 (n. d.) |

| | | | | | | |
|---|---|---|---|---|---|---|
| CRLX-101 | Polymeric nanoparticle | Camptothecin | Metastatic renal cell carcinoma and recurrent ovarian cancer | Nontargeted chemotherapy | Phase II trail | Lazarus et al. (2012) |
| NK-105 | Polymeric micelle | Paclitaxel | Metastatic and recurrent breast cancer | Nontargeted chemotherapy | Phase III trail | Lazarus et al. (2012) |
| Liposomal paclitaxel (EndoTAG-1) | Liposome | Paclitaxel | Pancreatic cancer, liver metastases, and triple-negative breast cancer | Nontargeted chemotherapy | Phase II trail | Schuch (2005) |
| MM-302 | HER2- targeting liposome | Doxorubicin | HER2-positive breast cancer | Targeted chemotherapy | Phase II/III | Park et al. (2001) |
| MBP-426 | TFR targeting liposomes | Oxaliplatin | Gastric and gastro-oesophageal adeno carcinoma | Targeted chemotherapy | Phase I/II trail | Johnsen et al. (2019) |
| BIND-014 | PSMA-targeting polymeric nanoparticle | Docetaxel | NSCLC | Targeted chemotherapy | Phase II trail | Hare et al. (2017) |
| Liposomal cytarabine-daunorubicin (CPX-351 or Vyxeos) | Liposome | Cytarabine and daunorubicin (5:1) | High-risk acute myeloid leukaemia | Combinational chemotherapy | Phase III trail | Lancet et al. (2018) |
| CPX-1 | Liposome | Lrinotecan and floxuridine (1:1) | Advanced colorectal cancer | Combinational chemotherapy | Phase II trail | Shi et al. (2017) |
| ThermoDox | Liposome | Doxorubicin | Hepatocellular carcinoma | Stimuli-responsive chemotherapy | Phase III trail | Chaturvedi et al. (2019) |
| Atu027 | Liposome | SiRNA against protein kinase A3 | Advanced or metastatic pancreatic cancer | Gene therapy | Phase I/II trail | Gomes-da-Silva et al. (2012) |
| PNT2258 | Liposome | DNA oligonucleotide against BCL-2 | Relapsed or refractory non- Hodgkin and diffuse large B cell lymphoma | Gene therapy | Phase I/II trail | Shi et al. (2017) |

(continued)

**Table 3.1** (continued)

| Generic name or proprietary name | Nanotechnology type | Active pharmaceutical ingredient | Cancer type | Type of therapy | Status | Reference |
|---|---|---|---|---|---|---|
| CALAA-01 | TfR targeting polymeric nanoparticle | siRNA against Ribonuleotide reductase M2 | Solid tumors | Gene therapy | Phase I | Chaturvedi et al. (2019) |
| SGT53 | TfR targeting liposome | Plasmid encoding normal human wild-type p53DNA | Recurrent glioblastoma and metastatic pancreatic cancer | Gene therapy | Phase II trail | Hare et al. (2017) |
| Tecemotide | Liposome | MUC1 antigen | NSCLC | Immunotherapy | Phase III trail | Shi et al. (2017) |
| Lipovaxin-MM | Liposome | Melanoma antigen | Malignant melanoma | Immunotherapy | Phase I trail | Hare et al. (2017) |
| CYT-6091 | Colloidal gold nanoparticle | TNF | Advanced solid tumor | Immunotherapy | Phase I trail | Shi et al. (2017) |
| JVRS-100 | Lipod nanoparticle | Plasmid DNA | Relapsed or refractory leukaemia | Immunotherapy | Phase I trail | Hare et al. (2017) |

**Table 3.2** Antibody drug conjugates that have been approved (ADCs). There are now three ADCs on the market (two out of five approved ADCs have been withdrawn from market). They either contain a radionuclide payload or a cytotoxic drug payload

| Product [company] | ADC | Target | Drug | Indication | Approval | References |
|---|---|---|---|---|---|---|
| Mylotarg® [Pfizer/ Wyeth] | Gemtuzumabozogamicin | CD33 | Calicheamicin | Acute myeloid leukemia | 2001 (withdrawal 2010) | Zhu et al. (2017); Núñez et al. (2018) |
| Adcetris® [Seattle genetics] | Brentuximabvedotin | CD30 | MMAE | Non-Hodgkin lymphoma | 2011 | Younes et al. (2012); Berger et al. (2017); Foyil and Bartlett (2012) |
| Kadcyla® [Roche/ Genentech/ ImmunoGen] | Trastuzumabemtansine | HER2 | DM1 | Breast cancer | 2013 | Chari et al. (2014); Kim and Kim (2015) |
| Zevalin® [IDEC/ Spectrum] | Ibritumomabtiuxetan | CD20 | Yttrium-90 or Indium-111 | Non-Hodgkin lymphoma | 2002 | Deshantri et al. (2018); Wicki et al. (2015b) |
| Bexxar® [Corixa/ GlaxoSmithKline] | Tositumomab | CD20 | Iodine-131 | Non-Hodgkin lymphoma | 2003 (withdrawal 2014) | Wicki et al. (2015b); Zhou et al. (2014); Ghazal (n.d.) |

The exceptional sensitivity of the link between external fields and malignant cells in the presence of normal tissue provided by appropriate NPs is expected to lead to more accurate and quicker diagnoses, as well as improved treatment outcomes. One problem that has to be studied further is the toxicity of nanoparticles, which may limit the use of particular NPs for cancer treatment. (Yang et al. 2014) Nonetheless, cancer treatments based on nanotechnology will keep evolving, resulting in improved treatment outcomes.

## References

Ahmad MZ, Rizwanullah M, Ahmad J, Alasmary MY, Akhter MH, Abdel-Wahab BA, Warsi MH, Haque A (2021) Progress in nanomedicine-based drug delivery in designing of chitosan nanoparticles for cancer therapy. Int J Polym Mater Polym Biomater 8:1–22

Anchordoquy TJ, Barenholz Y, Boraschi D, Chorny M, Decuzzi P, Dobrovolskaia MA, Farhangrazi ZS, Farrell D, Gabizon A, Ghandehari H, Godin B. Mechanisms and barriers in cancer nanomedicine: addressing challenges, looking for solutions

Andtbacka RH, Collichio FA, Amatruda T, Senzer NN, Chesney J, Delman KA, Spitler LE, Puzanov I, Doleman S, Ye Y, Vanderwalde AM (n.d.) OPTiM: A randomized phase III trial of talimogenelaherparepvec (T-VEC) versus subcutaneous (SC) granulocyte-macrophage colony-stimulating factor (GM-CSF) for the treatment (tx) of unresected stage IIIB/C and IV melanoma

Åstrand M, Nilvebrant J, Björnmalm M, Lindbo S, Hober S, Löfblom J (2016) Investigating affinity-maturation strategies and reproducibility of fluorescence-activated cell sorting using a recombinant ADAPT library displayed on staphylococci. Protein Eng Des Sel 29(5):187–195

Bae YH, Mrsny RJ, Park K (2013) Cancer targeted drug delivery. Springer, New York

Balasubramanian V, Liu Z, Hirvonen J, Santos HA (2018a) Bridging the knowledge of different worlds to understand the big picture of cancer nanomedicines. Adv Healthc Mater 7(1):1700432

Balasubramanian V, Liu Z, Hirvonen J, Santos HA (2018b) Nanomedicine: bridging the knowledge of different worlds to understand the big picture of cancer nanomedicines (Adv. Healthcare mater. 1/2018). Adv Healthc Mater 7(1):1870005

Belhadj Z, He B, Deng H, Song S, Zhang H, Wang X, Dai W, Zhang Q (2020) A combined "eat me/don't eat me" strategy based on extracellular vesicles for anticancer nanomedicine. J Extracell Vesicles 9(1):1806444

Berger GK, McBride A, Lawson S, Royball K, Yun S, Gee K, Riaz IB, Saleh AA, Puvvada S, Anwer F (2017) Brentuximabvedotin for treatment of non-Hodgkin lymphomas: a systematic review. Crit Rev Oncol Hematol 109:42–50

Bertrand N, Wu J, Xu X, Kamaly N, Farokhzad OC (2014) Cancer nanotechnology: the impact of passive and active targeting in the era of modern cancer biology. Adv Drug Deliv Rev 66:2–5

Bottagisio M, Lovati AB, Galbusera F, Drago L, Banfi G (2019) A precautionary approach to guide the use of transition metal-based nanotechnology to prevent orthopedic infections. Materials 12(2):314

Bregoli L, Movia D, Gavigan-Imedio JD, Lysaght J, Reynolds J, Prina-Mello A (2016) Nanomedicine applied to translational oncology: a future perspective on cancer treatment. Nanomedicine 12(1):81–103

Chari RV, Miller ML, Widdison WC (2014) Antibody–drug conjugates: an emerging concept in cancer therapy. Angew Chem Int Ed Engl 53(15):3796–3827

Chaturvedi VK, Singh A, Singh VK, Singh MP (2019) Cancer nanotechnology: a new revolution for cancer diagnosis and therapy. Curr Drug Metab 20(6):416–429

Cheng Z, Li M, Dey R, Chen Y (2021) Nanomaterials for cancer therapy: current progress and perspectives. J Hematol Oncol 14(1):1–27

Chung YH, Cai H, Steinmetz NF (2020) Viral nanoparticles for drug delivery, imaging, immunotherapy, and theranostic applications. Adv Drug Deliv Rev 156:214–235

De Maar JS, Sofias AM, Siegel TP, Vreeken RJ, Moonen C, Bos C, Deckers R (2020) Spatial heterogeneity of nanomedicine investigated by multiscale imaging of the drug, the nanoparticle and the tumour environment. Theranostics 10(4):1884

Deshantri AK, Moreira AV, Ecker V, Mandhane SN, Schiffelers RM, Buchner M, Fens MH (2018) Nanomedicines for the treatment of hematological malignancies. J Control Release 287:194–215

Ferraro MG, Piccolo M, Misso G, Maione F, Montesarchio D, Caraglia M, Paduano L, Santamaria R, Irace C (2020) Breast cancer chemotherapeutic options: a general overview on the preclinical validation of a multi-target ruthenium (III) complex lodged in NucleolipidNanosystems. Cell 9(6):1412

Foyil KV, Bartlett NL (2012) Brentuximabvedotin and crizotinib in anaplastic large-cell lymphoma. Cancer J 18(5):450–456

Ghazal D (n.d.) Examining the therapeutic potential of hJAA-F11 in breast and lung cancers in murine models (Doctoral dissertation, State University of New York at Buffalo)

Gmeiner WH, Ghosh S (2014) Nanotechnology for cancer treatment. Nanotechnol Rev 3(2): 111–122

Gomes-da-Silva LC, Santos AO, Bimbo LM, Moura V, Ramalho JS, de Lima MCP, Simões S, Moreira JN (2012) Toward a siRNA-containing nanoparticle targeted to breast cancer cells and the tumor microenvironment. Int J Pharm 434(1–2):9–19

Gurunathan S, Kang MH, Qasim M, Kim JH (2018) Nanoparticle-mediated combination therapy: two-in-one approach for cancer. Int J Mol Sci 19(10):3264

Haider M, Abdin SM, Kamal L, Orive G (2020) Nanostructured lipid carriers for delivery of chemotherapeutics: a review. Pharmaceutics 12(3):288

Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144(5):646–674

Hare JI, Lammers T, Ashford MB, Puri S, Storm G, Barry ST (2016) Challenges and strategies in anti-cancer nanomedicine development: An industry perspective. Adv Drug Deliv Rev 108. https://doi.org/10.1016/j.addr.2016.04.025

Hare JI, Lammers T, Ashford MB, Puri S, Storm G, Barry ST (2017) Challenges and strategies in anti-cancer nanomedicine development: An industry perspective. Adv Drug Deliv Rev 108:25–38

Harrington KJ, Puzanov I, Hecht JR, Hodi FS, Szabo Z, Murugappan S, Kaufman HL (2015) Clinical development of talimogenelaherparepvec (T-VEC): a modified herpes simplex virus type-1–derived oncolytic immunotherapy. Expert Rev Anticancer Ther 15(12):1389–1403

Heo J, Reid T, Ruo L, Breitbach CJ, Rose S, Bloomston M, Cho M, Lim HY, Chung HC, Kim CW, Burke J (2013) Randomized dose-finding clinical trial of oncolytic immunotherapeutic vaccinia JX-594 in liver cancer. Nat Med 19(3):329–336

https://clinicaltrials.gov/ct2/show/NCT03657420. (n.d.) Accessed on 28, Nov,2021

Huang J, Li Y, Orza A, Lu Q, Guo P, Wang L, Yang L, Mao H (2016) Magnetic nanoparticle facilitated drug delivery for cancer therapy with targeted and image-guided approaches. Adv Funct Mater 26(22):3818–3836

Huang Y, Zhang Y, Huo F, Chao J, Cheng F, Yin C (2020) A new strategy: distinguishable multi-substance detection, multiple pathway tracing based on a new site constructed by the reaction process and its tumor targeting. J Am Chem Soc 142(43):18706–18714

In't Veld RV, Ritsma L, Kleinovink JW, Que I, Ossendorp F, Cruz LJ (2020) Photodynamic cancer therapy enhances accumulation of nanoparticles in tumor-associated myeloid cells. J Control Release 10(320):19–31

Jang HL, Sengupta S (2019) Transcellular transfer of nanomedicine. Nat Nanotechnol 14(8): 731–732

Johnsen KB, Bak M, Melander F, Thomsen MS, Burkhart A, Kempen PJ, Andresen TL, Moos T (2019) Modulating the antibody density changes the uptake and transport at the blood-brain

barrier of both transferrin receptor-targeted gold nanoparticles and liposomal cargo. J Control Release 295:237–249
Kang H, Rho S, Stiles WR, Hu S, Baek Y, Hwang DW, Kashiwagi S, Kim MS, Choi HS (2020) Size-dependent EPR effect of polymeric nanoparticles on tumor targeting. Adv Healthc Mater 9(1):1901223
Kaufman HL, Bines SD (2010) OPTIM trial: a phase III trial of an oncolytic herpes virus encoding GM-CSF for unresectable stage III or IV melanoma. Future Oncol 6(6):941–949
Keyaerts M, Xavier C, Heemskerk J, Devoogdt N, Everaert H, Ackaert C, Vanhoeij M, Duhoux FP, Gevaert T, Simon P, Schallier D (2016) Phase I study of 68Ga-HER2-nanobody for PET/CT assessment of HER2 expression in breast carcinoma. J Nucl Med 57(1):27–33
Kievit FM, Zhang M (2011) Cancer nanotheranostics: improving imaging and therapy by targeted delivery across biological barriers. Adv Mater 23(36):H217–H247
Kim EG, Kim KM (2015) Strategies and advancement in antibody-drug conjugate optimization for targeted cancer therapeutics. Biomol Ther 23(6):493
Kirn DH, Thorne SH (2009) Targeted and armed oncolytic poxviruses: a novel multi-mechanistic therapeutic class for cancer. Nat Rev Cancer 9(1):64–71
Lancet JE, Uy GL, Cortes JE, Newell LF, Lin TL, Ritchie EK, Stuart RK, Strickland SA, Hogge D, Solomon SR, Stone RM (2018) CPX-351 (cytarabine and daunorubicin) liposome for injection versus conventional cytarabine plus daunorubicin in older patients with newly diagnosed secondary acute myeloid leukemia. J Clin Oncol 36(26):2684
Larsen SR, Michels L, dos Santos ÉC, Berg MC, Gates WP, Aldridge LP, Seydel T, Ollivier J, Telling MT, Fossum JO, Bordallo HN (2020) Physicochemical characterisation of fluorohectorite: water dynamics and nanocarrier properties. Microporous Mesoporous Mater 306:110512
Lazarus, D., Kabir, S. and Eliasof, S., 2012. CRLX301, a novel tumor-targeted taxane nanopharmaceutical
Li B, Chu F, Lu Q, Wang Y, Lane LA (2021a) Alternating stealth polymer coatings between administrations minimizes toxic and antibody immune responses towards nanomedicine treatment regimens. Acta Biomaterialia 121:527–540
Li Y, Wang S, Jiang X, Wang X, Zhou X, Wan L, He X (2021b) Preparation and validation of cyclodextrin-based excipients for radioiodinatedhypericin applied in a targeted cancer radiotherapy. Int J Pharm 599:120393
Liang DS, Wen ZJ, Wang JH, Zhu FF, Guo F, Zhou JL, Xu JJ, Zhong HJ (2021) Legumain protease-sheddablePEGylated, tuftsin-modified nanoparticles for selective targeting to tumor-associated macrophages. J Drug Target:1–25
Liyanage PY, Hettiarachchi SD, Zhou Y, Ouhtit A, Seven ES, Oztan CY, Celik E, Leblanc RM (2019) Nanoparticle-mediated targeted drug delivery for breast cancer treatment. BiochimicaetBiophysicaActa (BBA)-reviews on. Cancer 1871(2):419–433
Manoharan D, Chang LC, Wang LC, Shan YS, Lin FC, Wu LC, Sheu HS, Su WP, Yeh CS (2021) Synchronization of nanoparticle sensitization and Radiosensitizing chemotherapy through cell cycle arrest achieving ultralow X-ray dose delivery to pancreatic Tumors. ACS Nano
Miller-Kleinhenz J, Guo X, Qian W, Zhou H, Bozeman EN, Zhu L, Ji X, Wang YA, Styblo T, O'Regan R, Mao H (2018) Dual-targeting Wnt and uPA receptors using peptide conjugated ultra-small nanoparticle drug carriers inhibited cancer stem-cell phenotype in chemo-resistant breast cancer. Biomaterials 152:47–62
Núñez C, Estévez SV, del PilarChantada M (2018) Inorganic nanoparticles in diagnosis and treatment of breast cancer. J Biol Inorg Chem 23(3):331–345
Ouyang C, Zhang S, Xue C, Yu X, Xu H, Wang Z, Lu Y, Wu ZS (2020) Precision-guided missile-like DNA nanostructure containing warhead and guidance control for aptamer-based targeted drug delivery into cancer cells in vitro and in vivo. J Am Chem Soc 142(3):1265–1277
Panagi M, Voutouri C, Mpekris F, Papageorgis P, Martin MR, Martin JD et al (2020) TGF-β inhibition combined with cytotoxic nanomedicine normalizes triple negative breast cancer microenvironment towards anti-tumor immunity. Theranostics 10(4):1910

Parato KA, Breitbach CJ, Le Boeuf F, Wang J, Storbeck C, Ilkow C, Diallo JS, Falls T, Burns J, Garcia V, Kanji F (2012) The oncolytic poxvirus JX-594 selectively replicates in and destroys cancer cells driven by genetic pathways commonly activated in cancers. Mol Ther 20(4): 749–758

Park SH, Breitbach CJ, Lee J, Park JO, Lim HY, Kang WK, Moon A, Mun JH, Sommermann EM, Avidal LM, Patt R (2015) Phase 1b trial of biweekly intravenous Pexa-Vec (JX-594), an oncolytic and immunotherapeutic vaccinia virus in colorectal cancer. Mol Ther 23(9): 1532–1540

Park JW, Kirpotin DB, Hong K, Shalaby R, Shao Y, Nielsen UB, Marks JD, Papahadjopoulos D, Benz CC (2001) Tumor targeting using anti-her2 immunoliposomes. J Control Release 74(1–3): 95–113

Pei X, Zhu Z, Gan Z, Chen J, Zhang X, Cheng X, Wan Q, Wang J (2020) PEGylated nano-graphene oxide as a nanocarrier for delivering mixed anticancer drugs to improve anticancer activity. Sci Rep 10(1):1–5

Qi AS, Zheng X, Du CY, An BS (1993) A cellular automaton model of cancerous growth. J Theor Biol 161(1):1–2

Riaz U, Mehmood T, Iqbal S, Asad M, Iqbal R, Nisar U, Akhtar MM (2021) Historical background, development and preparation of nanomaterials. In: Nanotechnology. Springer, Singapore, pp 1–13

Rommasi F, Esfandiari N (2021) Liposomal nanomedicine: applications for drug delivery in cancer therapy. Nanoscale Res Lett 16(1):1–20

Rosenblum D, Joshi N, Tao W, Karp JM, Peer D (2018) Progress and challenges towards targeted delivery of cancer therapeutics. Nat Commun 9(1):1–12

Ryu JH, Yoon HY, Sun IC, Kwon IC, Kim K (2020) Tumor-targeting glycol chitosan nanoparticles for cancer heterogeneity. Adv Mater 32(51):2002197

Sandström M, Lindskog K, Velikyan I, Wennborg A, Feldwisch J, Sandberg D, Tolmachev V, Orlova A, Sörensen J, Carlsson J, Lindman H (2016) Biodistribution and radiation dosimetry of the anti-HER2 affibody molecule 68Ga-ABY-025 in breast cancer patients. J Nucl Med 57(6): 867–871

Schuch G (2005) EndoTAG-1. MediGene. Curr Opin Investig Drugs 6(12):1259–1265

Sengupta S (2017) Cancer nanomedicine: lessons for immuno-oncology. Trends Cancer 3(8): 551–560

Shahgolzari M, Pazhouhandeh M, Milani M, YariKhosroushahi A, Fiering S (2020) Plant viral nanoparticles for packaging and in vivo delivery of bioactive cargos. Wiley Interdiscip Rev Nanomed Nanobiotechnol 12(5):e1629

Shi J, Kantoff PW, Wooster R, Farokhzad OC (2017) Cancer nanomedicine: progress, challenges and opportunities. Nat Rev Cancer 17(1):20–37

Shi Y, Van der Meel R, Chen X, Lammers T (2020) The EPR effect and beyond: strategies to improve tumor targeting and cancer nanomedicine treatment efficacy. Theranostics. 10(17): 7921

Siegel RL, Miller KD, Fedewa SA, Ahnen DJ, Meester RG, Barzi A, Jemal A (2017) Colorectal cancer statistics, 2017. CA Cancer J Clin 67(3):177–193

Siegel RL, Miller KD, Jemal A (2016) Cancer statistics, 2016. CA Cancer J Clin 66(1):7–30

Song M, Liu C, Chen S, Zhang W (2021) Nanocarrier-based drug delivery for melanoma therapeutics. Int J Mol Sci 22(4):1873

Stack T, Liu Y, Frey M, Bobbala S, Vincent M, Scott E (2021) Enhancing subcutaneous injection and target tissue accumulation of nanoparticles via co-administration with macropinocytosis inhibitory nanoparticles (MiNP). Nanoscale Horiz 6(5):393–400

Stathopoulos GP, Antoniou D, Dimitroulis J, Michalopoulou P, Bastas A, Marosis K, Stathopoulos J, Provata A, Yiamboudakis P, Veldekis D, Lolis N (2010) Liposomal cisplatin combined with paclitaxel versus cisplatin and paclitaxel in non-small-cell lung cancer: a randomized phase III multicenter trial. Ann Oncol 21(11):2227–2232

Subhan MA, Yalamarty SS, Filipczak N, Parveen F, Torchilin VP (2021) Recent advances in tumor targeting via EPR effect for cancer treatment. J Pers Med 11(6):571
Sun X, Ni N, Ma Y, Wang Y, Leong DT (2020) Retooling cancer nanotherapeutics' entry into tumors to alleviate tumoral hypoxia. Small 16(41):2003000
Sun T, Zhang YS, Pang B, Hyun DC, Yang M, Xia Y (2014) Engineered nanoparticles for drug delivery in cancer therapy. Angew Chem Int Ed 53(46):12320–12364
Tahmasbi Rad A, Chen CW, Aresh W, Xia Y, Lai PS, Nieh MP (2019) Combinational effects of active targeting, shape, and enhanced permeability and retention for cancer theranostic nanocarriers. ACS Appl Mater Interfaces 11(11):10505–10519
Tam CS, Trotman J, Opat S, Burger JA, Cull G, Gottlieb D, Harrup R, Johnston PB, Marlton P, Munoz J, Seymour JF (2019) Phase 1 study of the selective BTK inhibitor zanubrutinib in B-cell malignancies and safety and efficacy evaluation in CLL. Blood 134(11):851–859
Thomas A, Teicher BA, Hassan R (2016) Antibody–drug conjugates for cancer therapy. Lancet Oncol 17(6):e254–e262
Tong R, Kohane DS (2016) New strategies in cancer nanomedicine. Annu Rev Pharmacol Toxicol 6(56):41–57
Tran S, DeGiovanni PJ, Piel B, Rai P (2017) Cancer nanomedicine: a review of recent success in drug delivery. Clin Transl Med 6(1):1–21
Truong NP, Whittaker MR, Mak CW, Davis TP (2015) The importance of nanoparticle shape in cancer drug delivery. Expert Opin Drug Deliv 12(1):129–142
Tseng TW, Rajaji U, Chen TW, Chen SM, Huang YC, Mani V, Jothi AI (2020) Sonochemical synthesis and fabrication of perovskite type calcium titanate interfacial nanostructure supported on graphene oxide sheets as a highly efficient electrocatalyst for electrochemical detection of chemotherapeutic drug. Ultrason Sonochem 1(69):105242
van der Meel R, Lammers T, Hennink WE (2017) Cancer nanomedicines: oversold or underappreciated? Expert Opin Drug Deliv 14(1):1–5
von Roemeling C, Jiang W, Chan CK, Weissman IL, Kim BY (2017) Breaking down the barriers to precision cancer nanomedicine. Trends Biotechnol 35(2):159–171
Wang L, Shi C, Wright FA, Guo D, Wang X, Wang D, Wojcikiewicz RJ, Luo J (2017) Multifunctional telodendrimer nanocarriers restore synergy of bortezomib and doxorubicin in ovarian cancer treatment. Cancer Res 77(12):3293–3305
Weiner GJ (2015) Building better monoclonal antibody-based therapeutics. Nat Rev Cancer 15(6): 361–370
Westphal M, Ylä-Herttuala S, Martin J, Warnke P, Menei P, Eckland D, Kinley J, Kay R, Ram Z (2013) ASPECT study group. Adenovirus-mediated gene therapy with sitimageneceradenovec followed by intravenous ganciclovir for patients with operable high-grade glioma (ASPECT): a randomized, open-label, phase 3 trial. Lancet Oncol 14(9):823–833
Wicki A, Witzigmann D, Balasubramanian V, Huwyler J (2015a) Nanomedicine in cancer therapy: challenges, opportunities, and clinical applications. J Control Release 28(200):138–157
Wicki A, Witzigmann D, Balasubramanian V, Huwyler J (2015b) Nanomedicine in cancer therapy: challenges, opportunities, and clinical applications. J Control Release 200:138–157
Wilhelm S, Tavares AJ, Dai Q, Ohta S, Audet J, Dvorak HF, Chan WC (2016) Analysis of nanoparticle delivery to tumours. Nat Rev Mat 1(5):1–12
Wiwatchaitawee K, Quarterman JC, Geary SM, Salem AK (2021) Enhancement of therapies for glioblastoma (GBM) using nanoparticle-based delivery systems. AAPS PharmSciTech 22(2): 1–6
World Health Organization (2013) Global tuberculosis report 2013. World Health Organization
Xie Z, Fan T, An J, Choi W, Duo Y, Ge Y, Zhang B, Nie G, Xie N, Zheng T, Chen Y (2020) Emerging combination strategies with phototherapy in cancer nanomedicine. Chem Soc Rev 49(22):8065–8087
Yang L, Sajja HK, Cao Z, Qian W, Bender L, Marcus AI, Lipowska M, Wood WC, Wang YA (2014) uPAR-targeted optical imaging contrasts as theranostic agents for tumor margin detection. Theranostics 4(1):106

Younes A, Yasothan U, Kirkpatrick P (2012) Brentuximabvedotin. Nat Rev Drug Discov 11(1):19
Zhang F, Cao J, Chen X, Yang K, Zhu L, Fu G, Huang X, Chen X (2015) Noninvasive dynamic imaging of tumor early response to nanoparticle-mediated photothermal therapy. Theranostics 5(12):1444
Zhang M, Gao S, Yang D, Fang Y, Lin X, Jin X, Liu Y, Liu X, Su K, Shi K (2021) Influencing factors and strategies of enhancing nanoparticles into tumors in vivo. Acta Pharmaceutica Sinica B
Zhou W, Ke SQ, Huang Z, Flavahan W, Fang X, Paul J, Wu L, Sloan AE, McLendon RE, Li X, Rich JN (2015) Periostin secreted by glioblastoma stem cells recruits M2 tumour-associated macrophages and promotes malignant growth. Nat Cell Biol 17(2):170–182
Zhou L, Xu N, Sun Y, Liu XM (2014) Targeted biopharmaceuticals for cancer treatment. Cancer Lett 352(2):145–151
Zhu L, Zhou Z, Mao H, Yang L (2017) Magnetic nanoparticles for precision oncology: theranostic magnetic iron oxide nanoparticles for image-guided and targeted cancer therapy. Nanomedicine 12(1):73–87

# Emergence of Nanohybrids in Hormonal Cancer-Targeted Therapy

4

Hasan Ali, Md Akbar, Kainat Alam, Shipra Daniel, Mohammad Javed Ansari, Deepak Bedanand Jha, Faraat Ali, and Shweta Srivastava

**Abstract**

Chemotherapeutic agent's toxicity issues can be addressed by using nano-drug carriers, which have been shown to be more effective at treating cancer than conventional chemotherapeutics while also being less toxic to healthy cells. This is due to the carriers' improved enhanced permeability and retention effect (EPR), as well as their active cellular uptake. Nano-carriers containing chemotherapeutic drugs can be conjugated with molecules that bind to overexpressed receptors to boost therapeutic efficacy and limit the risk of probable harm. Some intriguing

H. Ali
Department of Pharmacy, Meerut Institute of Technology, Meerut, India

M. Akbar (✉)
Department Pharmaceutical Medicine, Jamia Hamdard university, New Delhi, India

K. Alam
KKECS College of Nursing, Bengaluru, Karnataka, India

S. Daniel
Christian College of Nursing, Faculty of Health Sciences, Sam Higginbottom University of Agriculture, Technology & Sciences, Allahabad, Uttar Pradesh, India

M. J. Ansari
Department of Pharmaceutics, College of Pharmacy, Prince Sattam Bin Abdulaziz University, Al-kharj, Saudi Arabia

D. B. Jha
Oriental University, Indore, India

F. Ali
Department of Licensing and Enforcement, Laboratory Services, Botswana Medicines, Regulatory Autority, Gaborone, Botswana

S. Srivastava
Kanpur Institute of Technology, Kanpur, India



M. Rahman et al. (eds.), *Hormone Related Cancer Mechanistic and Nanomedicines*,
https://doi.org/10.1007/978-981-19-5558-7_4

clinical uses have been demonstrated for polymeric nanoparticles, liposomes, and micelles. Nano-carrier chemotherapeutic drugs have recently been authorized for clinical use, while others are either in preclinical or clinical development phases. As drug delivery systems, however, nano-drug carriers offer several benefits; yet, their poor biodegradability, low bioavailability, poor tissue distribution, and possible toxicity raise concerns about their safety especially in long-term usage. Most cancer treatments fail because of drug resistance as one of the major contributing factors. Consequently, new nano-drug carrier systems for cancer treatment are needed in order to reduce chemotherapy-related adverse events and better treat cancer.

**Keywords**

Cancer · Nano-carriers · Polymeric nanoparticles · Bioavailability · Enhanced permeability · Retention effect

## 4.1 Introduction

Cancer is a term that denotes to group of ailments initiated by an unrestrained abnormal division of cells that can further penetrate or expand to other normal tissues/organs of the body. Cancer treatment modalities include generally chemotherapy (drug-based), radiotherapy, surgery, or a combination thereof. Treatment of cancers by employing chemotherapeutic agents is a conventional treatment modality, which typically exhibits its action by disrupting the synthesis of DNA and arrest of uncontrolled cell division, consequently resulting into the death of speedily multiplying malignant cells or arrest of uncontrolled cell division.

Chemotherapeutic agents are usually nonspecific and able to destruct normal healthy and unaffected cells, which often result in critical, unavoidable, unanticipated, and painful side effects, like nausea, sometime vomiting, lack of appetite, cachexia, loss of hair, gastric irritation, etc. Unrestrained critical adverse effects of chemo agents on normal cells are one of the noteworthy factors behind the increased death rate of patients with malignancy. Additionally, the availability of chemotherapeutic agents to cancerous cells is comparatively meagre, generally higher strengths are needed in order to treat the cancer, which consequently lead to increased toxicity in healthy tissues, which may further increase the chances of drug resistance. Hence, it is necessary to improve treatment modality based on chemotherapeutic agents that can target malignant cells specifically and decrease the chances of severe adverse side events with the improvement in therapeutic effectiveness. Recently an improved knowledge of cancer biology, better accessibility and availability of multipurpose smart material comprising of lipids, polymers inorganic carriers, biomacromolecular frameworks and hydrogels have led to good safety and efficacy. Consequently, it has steered the improvement and development of drug carrier system that could carry the chemo agents to target cells with enhanced efficacy and improved safety with reduction in chances of adverse effects (Jin et al. 2020; Neidle and Thurston 2005).

In general, evolution of nanotechnological tools has deeply impacted the applicability of clinical therapeutics. Nano-drug carriers have established the potential to overcome the challenges associated with toxicity of chemotherapeutic agents by improving the treatment efficacy as compared to conventional chemotherapeutic drugs, while avoiding toxicity in normal cells because of characteristics like better accumulation in cancerous cells, generally, by improved enhanced permeability and retention effect (EPR) and active cellular uptake. Active targeting strategies, improving the therapeutic efficacy and minimizing the chances of possible toxicity, may be established by conjugating nano-carriers with chemotherapeutic agents to molecules that bind to overexpressed receptors. Polymeric nanoparticles, liposomes, and micelles have shown promising clinical applications among evolving nano-drug carriers. Numerous nano-carrier-based chemotherapeutic agents are recently approved clinically and some are either in preclinical or clinical stages of development. However, nano-drug carriers cater significant advantages as the drug delivery systems, but instability in the blood circulation, poor biodegradation, reduced bioavailability, inadequate tissue distribution, and potential toxicity risks increase the apprehensions over their safety, particularly for long-term use. Drug resistance is one of the important causes for the failure of most of the cases in cancer treatment. Because of this reason, nano-drug carrier systems with improved targeting capacity are required for better treatment of cancer, with reduction of adverse side effects related with chemotherapy (Jin et al. 2020; Neidle and Thurston 2005; Senapati et al. 2018).

## 4.2 Hormone-Related Cancer

Hormones perform a critical role in the etiology of various types of cancer worldwide, encompassing women-specific malignancies like breast, ovary, and endometrium cancers and man-specific cancers like prostate malignancies. The pathways by which hormones influence the risk of malignancy are likely to be controlling the speed of division of cells, cell differentiation, and count of vulnerable cells. Hormones have a substantial influence on cell division in the endometrial cells; estrogens promote mitotic cell division, while progesterone inhibit it. Delayed menopause, treatment with estrogen, and obesity increase the risk of endometrial cancer, but usage of oral contraceptive decreases the risk. Therefore, risk grows in percentage to the time period of contact (exposure) to the estrogen generally unrestricted by progesterone, possibly due to unopposed estrogens initiating uncontrolled endometrial cell division. Generally, hormones exhibit a considerable impact on cell division of endometrium as compared to breast epithelium in nonpregnant women. However, both progestins and estrogens seem to excite mitotic cell division. Premature and delayed onset of menstruation and hormone replacement therapy (estrogen in this case) escalate the chances of breast cancer possibly due to elevated concentration of progesterone and/or estrogen in the plasma and hence consequently more exposure to the breast. Chances of breast cancer risk are diminished substantially by early first parity and multiple births (multiparity) possibly due to

differentiation of breast cells because of hormones and hence resultant decrease in the susceptible cells.

Generally, hormones indirectly influence the epithelial cells, lining the ovaries, rather they instigate ovulation, followed by the process of cell division at the time repair of the epithelium. Usually, the chances of ovarian malignancy rise with delayed menopause and reduce after the intake of oral contraceptive and pregnancy, signifying that the number of ovulations in the life may be an important factor. For the hormone-related malignancies, chances of risk change in some years of contact to sex hormones and some changes in risk continued for many years, demonstrating that hormone may influence both early and late phases of tumorigenesis. The implication of sex hormones in the etiology of prostate malignancies and other infrequent malignancies is less known. The development of the prostate gland is relied on the testosterone, which is eventually changed into the dihydrotestosterone within the organ. There have been many studies suggesting that prostate malignancies are developing after androgen therapy (Key 1995).

### 4.2.1 Breast Cancer

The cancers associated with breast are one of the most common malignancies among women around the globe. Approximately, 70–80% breast cancer patients of early-stage are generally nonmetastatic. Advanced malignancies of breast with metastases are generally considered as incurable with existing therapy. At the molecular level, malignancies of breast are considered as heterogeneous disease. Molecular physiognomies include stimulation of hormone receptors (progesterone/estrogen receptor) or onset of BRCA mutations and stimulation of human epidermal growth factor receptor 2 (HER2). Therefore, treatment modality varies according to the molecular subtype. Treatment modalities for these malignancies are of various types; it encompasses generally two approaches, viz., systemic therapeutic treatment strategies (employing antineoplastic agents, hormone replacement therapy, and monoclonal antibodies) and localized treatment modalities which include surgical procedures and radiotherapy. Futuristic therapeutic modalities in breast cancer are aimed to personalize therapy and treatment escalation and/or de-escalation according to the early therapeutic response and cancer biology (Harbeck et al. 2019).

### 4.2.2 Ovarian Cancer

Ovarian cancer is one of the important causes of mortalities because of gynecological cancers. Generally, ovarian carcinomas are originated from the surface epithelium of ovary and its cystic remnants. It is evidenced that hormones such as progesterone and estrogens are involved in the carcinogenesis of ovaries. Nevertheless, it is quite difficult to comprehend the mechanisms of action on the carcinogenic process. Recent data have showed that estrogens favor carcinogenesis of the ovarian surface epithelium, whereas progesterone protects against ovarian carcinogenesis.

Precisely, estrogens available in ovulatory follicles exhibit both mitogenic and genotoxic to ovarian surface epithelium. However, pregnancy equivalent progesterone concentrations are very much impactful as apoptotic agents for ovarian surface epithelium and malignant cells. Hence, high dose progesterone may be utilized as an exfoliation agent for shedding the aged ovarian surface epithelium of precancerous cells. A few clinical studies have showed effectiveness of progestins, aromatase inhibitors, and antiestrogens, alone or in combination with chemo agents in treating ovarian cancer (Ho 2003). It has been observed through many epidemiological studies that reproductive and hormonal variables are the key factors which are involved in the pathophysiology of ovarian cancer. Direct effects exerted by progestin and estrogen, incessant periodic ovulation, and gonadotropin signaling are key hormonal concepts that are suggested to elucidate the etiology of ovarian cancer. The gonadotropin hypothesis is supposed to be the first theory of sex hormones as a likely mechanism causing ovarian cancer. According to this theory, OC spreads because of unwarranted excitation of ovarian tissue by luteinizing hormone and follicle-stimulating hormone (Cramer and Welch 1983). Contact to the excess gonadotropins, related to the menopause, infertility treatment, or ovulation, has been recognized as an imperative cause of ovarian cancer (Cramer and Welch 1983; Riman et al. 2002; Rodriguez et al. 2001).

### 4.2.3 Prostate Cancer

Prostate cancer develops in reaction to the testosterone. When testosterone reaches to the prostate cell, it assists to select genes that are switched on or off. Antiandrogen agents can help to slow down the growth of cancer by switching genes on and off. However, as the time passes, sometimes these malignancies become drug resistant to antiandrogens and consequently relapse (American Cancer Society 2019). How testosterone controls the complex process of gene activity is of utmost importance to understand the etiology of prostate cancer. Initially, a human cell copies the related gene, cutting and pasting and then modifying different parts of the sequence by the process known as gene splicing before the synthesis of corresponding protein. Specifically, splicing regulator proteins regulate the patterns of splicing that a cell exhibits. Hence, in prostate cancer cells, testosterone can change the splicing patterns, but exact molecular mechanism is not yet known clearly. It has been studied that a set of genes turn off in reaction to the antiandrogen agents in the patients with prostate cancer. It is also reported that testosterone controls epithelial splicing regulatory protein 2 (ESRP2), a master splicing regulator, which is exists in epithelial cells. Extra ESRP2 slowed down the prostate cancer growth found in mice. Nevertheless, ESRP2 concentrations are elevated in prostate cancer cells, and generally level of the regulator protein reduced in the presence of antiandrogen agents (Munkley et al. 2019; Munkley et al. 2017).

### 4.2.4 Endometrial Carcinoma (EC)

It one of the most widespread gynecologic malignancies and is generally faced by every gynecologist. A detailed knowledge of anatomy, pathophysiology, extent of spread in women populace, and treatment strategies for malignancy associated with endometrium permits the gynecologists and obstetricians to categorize the women at high risk, aid timely diagnosis of cancer, and impart their role to risk reduction measurements. The most common symptoms of endometrial cancer are unusual bleeding from uterus and vaginal secretions. Patients with progressive cancer may exhibit the symptoms like those with advanced ovarian cancer, such as pelvic or abdominal pain, distension, early satiety, and alteration in bowel or bladder function (Mayo Clinic 2021).

The uterus is a hollow organ that resembles a medium-sized pear in size and form. The fetus grows in the uterus and develops during pregnancy. It has two parts:

- the upper part, which is also known as the *corpus* (Latin word for the body), and,
- the lower part of the uterus is known as cervix, as it joins with the vagina.

Endometrial cancer starts to grow when the endometrial cells initiated to divide uncontrollably. Malignant cells can grow virtually in any part/organ of the body and metastasize (spread to other parts). Malignancy associated with endometrium starts in the cells of the inner facing of the uterus. Endometrial cancer is one of the common categories of malignancy in the uterus. (American Cancer Society 2020).

## 4.3 Treatment Modalities of Hormone-Related Cancer

There are a number of cancer treatments strategies, the kind of treatment modality that the patient will receive generally depends on the type of malignancy the patient has and in which stage it is present. A few patients with malignancy receive only one type of treatment. Nonetheless, most of the patient population receive a combination of treatments, like chemotherapy, surgical procedures, radiotherapy, immunotherapy, targeted therapy, and/or hormone replacement therapy (Cramer and Welch 1983; Riman et al. 2002). Here some treatment modalities for hormone-related cancers are discussed.

### 4.3.1 Breast Cancer

The stage of breast malignancy is a vital factor selection of treatment choices. Usually, the more the spread of breast cancer, the more treatment is possibly to provide.

Stage 0 cancers are noninvasive and restricted to the interior of the milk duct (does not invade nearby tissues). The ductal carcinoma in situ and lobular carcinoma in situ are also represented as stage 0; however, these are not cancers. Though, it may

lead to an augmented risk of breast cancer. Hence, LCIS poses the threat of evolving invasive breast malignancy in the later stage. Because LCIS is neither a precancer nor a cancer, generally no therapy is required as such, after the biopsy. Occasionally, LCIS is detected by employing a needle biopsy. In that case, the oncologist may advise to eradicate LCIS using surgical procedure or other type of breast conservation procedure (lumpectomy) in order to ensure that LCIS was the only abnormality. Generally, a patient with DCIS can undergo either breast-conserving surgery (BCS) or simple mastectomy. Mastectomy could be a good choice, if DCIS is spread widely in the breast.

Majority of patients with breast cancer in I, II, or III stage undergo surgery, frequently followed by radiotherapy. Some of the patients also receive chemotherapy therapy. Treatment strategy basically depends on the extent of spread of cancer. Hence, if the extent of spread of malignancy is more, the patient will likely to take more treatment. Maximum patients having breast cancer in I, II, or III stage get systemic drug therapy as a part of the treatment plan. Drug therapy may encompass hormone replacement therapy, chemotherapy, monoclonal antibodies like pertuzumab, trastuzumab, or abemaciclib immunotherapy, and combinations thereof.

Stage IV malignancies have extension outside the breast including lymph nodes to other organs like liver, bones, lungs, brain, etc. Key treatment plan for stage IV breast malignancy is chemotherapy which includes chemotherapy, hormone therapy, and immunotherapy, and a combination thereof. Radiotherapy and surgical procedures may be employed as alternative in certain conditions. Though cancer in this stage is considered incurable, treatment can generally retard the growth of cancer, for symptomatic relief, and support some patients to live longer.

Breast cancer might recur in some patients after the treatment. Recurrence could be local (in the same breast), regional (in neighboring lymph nodes), or distant. A local recurrence in the breast after breast-conserving surgery (lumpectomy) is generally treated by mastectomy. The problems of relapses around the mastectomy area are addressed by eradicating the tumor whenever required if the first therapy was mastectomy. If radiation therapy has not been employed previously, it is usually provided after this procedure. After radiotherapy/surgery or/and hormone replacement therapy, targeted immunotherapy (such as trastuzumab), chemotherapy, or a combination thereof may be employed. If surgery or radiotherapy is not a possibility, these medications may be utilized.(American Cancer Society 2019).

### 4.3.2 Ovarian Cancer

Surgical procedure is the initial treatment strategy for stage I ovarian cancer. Generally, both ovaries, both fallopian tubes and the uterus, are removed. The treatment strategy after surgical procedure is mainly dependent on the substage of malignancy.

For stages, namely IA and IB, treatment after surgical procedures is mainly governed by the tumor grade. Majority of the patients do not require any treatment

after surgical procedures for low grade tumors (grade 1). For high grade (grade 2) tumors, patients are either treated with chemotherapeutic agents or kept under observation after surgical procedures without further treatment. The chemotherapeutic agents employed generally are cisplatin/carboplatin and docetaxel/paclitaxel for 3–6 cycles. For high grade (grade 3) tumors, treatment plan usually covers the equivalent chemotherapy for grade 2 stage IA and IB malignancies. Surgical procedures to eliminate the tumor for stage IC is, however, the first-line treatment. After surgical procedures, chemotherapy is preferred, typically with 3–6 cycles of treatment with paclitaxel and carboplatin. Primary peritoneal malignancies and stage I fallopian tube cancer patients undergo the same treatment as ovarian cancer of stage I. For stage IIA and IIB malignancies, treatment initiates with surgery for staging and debulking, which includes a hysterectomy and bilateral salpingo-oophorectomy. Chemotherapy is generally advised for at least 6 cycles, after the surgical procedures. Usually, carboplatin in combination with paclitaxel is employed in the treatment plan.

Stage IIIA1, IIIA2, IIIB, and IIIC are usually treated in the similar way as to the stage II malignancies. In this case, initially, cancer is debulked and surgically staged, similar to the stage II. The ovaries, fallopian tubes, uterus, and omentum are removed by surgical procedures. The objective of surgery is to leave behind no tumor larger than 1 cm, or essentially no visible tumor. Once this objective is achieved, tumor is considered to be *optimally debulked*. Occasionally, the malignancy may reach to the intestines; in order to eliminate tumor, intestine must have to be incised, in part.

After undergoing surgical procedures, a combination chemotherapy is required. Cisplatin/Carboplatin in combination with paclitaxel, intravenous for 6 cycles, is generally recommended. Monoclonal antibodies like bevacizumab might be administered in combination with a chemotherapeutic agent. Alternative choice is to give in intraperitoneal (IP) and intravenous (IV) chemotherapeutic agent after surgical procedures. Intraperitoneal chemotherapy is generally considered only if the tumor was optimally debulked − it may also not work if much tumor is left behind in the abdomen.

In advanced stage that is stage IV, cancer has grown to distant organs like lungs, liver, bones, etc. Advanced stage malignancies are very difficult to treat with current therapeutic regimen; however, stage IV malignancy can still be managed. Treatment of stage IV can be carried out like stage III, with debulking of malignancy, followed by chemotherapeutic agents monoclonal antibodies, e.g., bevacizumab. Bevacizumab is given either alone or in combination with chemotherapeutic agents olaparib; otherwise treat with chemotherapeutic agents first. If the malignancy shrinks from the chemotherapy, surgical procedures may be employed; subsequently additional chemotherapy is required. Generally, three cycles of chemotherapy are employed before surgical procedures (American Cancer Society 2019).

### 4.3.3 Prostate Cancer

In prostate cancer, stages are key factors in selecting the paramount treatment modality. Staging of prostate cancer is mainly based on the magnitude of spread of malignancy and Gleason score based on the initial diagnosis, the prostate-specific antigen (PSA) level. Prostate malignancies that have not entered into the stages (I to III), oncologist identify risk groups based on how much the prostate cancer has spread, Gleason score, PSA level and prostate biopsy results in order to assist to govern treatment choices. Malignancies with lower risk groups have lower chances of spreading than those in higher-risk groups. Overall health, age, personal preferences, and life expectancy are the factors which are considered at the time of treatment. After the preliminary treatment and prostate cancer patient's life expectancy, oncologists help to determine potential treatment choices for the patients, based on the stages and the threat of malignancy relapses.

Prostate malignancies have not spread outside the prostate and are small in size in very-low-risk groups. These patients have Gleason score of six or less (grade group 1) and low PSA levels (less than 10). Observations are generally advised for the patients not having any other serious health complications. Brachytherapy or EBRT (radiotherapy) or surgical procedures like radical prostatectomy may be employed for men who wish to start treatment. Treatment modality for prostate malignancy with low-risk groups includes EBRT or brachytherapy (radiation therapy) or surgical procedures (radical prostatectomy), which mainly depend on probability of cancer growth, personal (patients) preferences, and risks and benefit of the treatment. If the observations after surgery exhibit that malignancy has characteristics of relapses, then the EBRT with or without androgen deprivation therapy (ADT) or observations with PSA levels with the plan to start radiotherapy as early as the PSA level starts to rise.

Prostate malignancy of intermediate-risk groups can be diagnosed on the examination or observed on an imaging test. EBRT or brachytherapy (radiotherapy) is an important choice for the patients of intermediate-risk group. It is generally recommended in combination with androgen deprivation therapy (ADT). Surgical procedure (radical prostatectomy) with pelvic lymph node removal is an alternate choice of treatment.

High-risk group prostate cancer patients may be treated with radiotherapy (EBRT alone in combination with brachytherapy) and ADT for about 1–3 years. Surgical procedures (such as radical prostatectomy) with pelvic lymph node dissection (PLND) might be advised. If malignancy is detected in the lymph nodes during surgery, ADT alone or in combination with EBRT may be recommended. Generally, radiotherapy alone or in combination with ADT is recommended, in case it is not detected in the lymph nodes, but may exhibit characteristics of recurrences. Radiotherapy or ADT is generally recommended, when the PSA level rises after surgical procedures. Additionally, in some cases, the chemotherapeutic agents like docetaxel and/or abiraterone, as an ADT agent, might be given in combination with radiotherapy.

### 4.3.4 Endometrial Cancer

The most crucial and important elements in considering the treatment modality are the stages of cervical cancer. However, additional causes can also influence the treatment choices, encompassing the precise situation (locus) of malignancy within the cervix, age of the patient, the category of cancer (whether adenocarcinoma or squamous cell), and whether the subject wants to have pregnancy, above all general health.

Basically, the treatment modality for stage IA1 is based on whether the subject (s) want to sustain fertility in order to bear children and whether or not the malignancy has spread into the lymph and blood vessels (also known as lymphovascular spread of cancer). For the subjects who want to maintain fertility in order to have children conization, cone biopsy is one of the most preferred treatment modalities. If the cone boundary does not comprise cancerous cells, also called negative margins, the subject can be kept under observations without further treatment, until the malignancy relapses. In case the boundary of the cone has cancerous cells also known as positive margins, then it means malignancy is left behind. Hence, it can be managed with the help of repeated conization or complete surgical removal of cervix, a radical trachelectomy. In case the malignancy has spread into lymph or blood vessels, treatment modality is the conization to eradicate lymph nodes located in the pelvis region. Alternate option to cone biopsy (conization) is the complete surgical removal of the pelvic lymph nodes (radical trachelectomy).

For women who do not want to have fertility, treatment choices are hysterectomy, if malignancy does not exhibit lymphovascular spread and the boundary of the biopsy has no malignant cells. A radical hysterectomy or repeat conization with the eradication of the lymph nodes present in the pelvis might be a choice, if the malignant cells are present in the boundary of the biopsy. If malignancy has spread into the lymph and blood vascular system, the patient may require a radical hysterectomy and removal of the pelvic lymph nodes. Usually, surgical procedures are not required, generally in such cases an external beam radiation (EBR) to the pelvis is employed after brachytherapy. If lymph nodes are not affected with the malignancy, still radiation may be a choice if the cancer is large in size, and the malignancy has spread into lymph and blood vessels, or invading the nearby connective tissue that braces the organs like vagina, bladder, and uterus. If the malignancy has spread to the tissues next to the uterus (parametrium) or to any nearby lymph nodes, or the tissue incised has positive boundary, EBRT along with chemotherapy is generally advised. The oncologist may also recommend brachytherapy followed by combined chemo therapy and radiation therapy.

Treatment strategy for stage IA2 is partly dependent on whether the patient wishes to maintain the fertility in order to have children. Conization with the surgical removal of pelvic lymph nodes and radical trachelectomy with pelvic lymph nodes surgical procedure are the choices of treatment for patients who want to bear children.

Pelvis EBRT with brachytherapy and radical hysterectomy along with pelvic lymph nodes are treatment choices for women who do not wish to maintain fertility. If the cancer is large and has spread into the lymph and blood vessels, and is grown into the surrounding tissue that braces organs like urinary bladder, uterus, and vagina, and lymph nodes having no cancer cells, radiotherapy might be a choice. If the malignancy has expanded to the parametrium and lymph nodes tissue isolate has positive margins, EBRT in combination with chemotherapy is generally recommended. The oncologist may recommend brachytherapy after the combination of radiation and chemotherapy.

In the stages IB and IIA, radical hysterectomy eradicates pelvic lymph nodes, and occasionally lymph nodes from the para-aortic region, if lymph nodes are not found to have malignancy. If the cancer is large and has spread into the lymph and blood vessels, and is grown into the surrounding tissue that braces organs like urinary bladder, uterus, and vagina, and lymph nodes having no cancer cells, radiotherapy might be a choice. If the malignancy has expanded to the parametrium and lymph nodes and tissue isolate has positive margins, EBRT in combination with chemotherapy is generally recommended. The oncologist may recommend brachytherapy after the combination of radiation and chemotherapy. If a woman is not healthy enough to tolerate surgery and determine not to undergo surgery, in that case radiation employing EBRT and brachytherapy might be a good choice. Concurrent chemoradiation (chemotherapy in combination with radiation) is another treatment strategy. In this case, chemotherapeutic agent may be carboplatin, cisplatin, or cisplatin in combination with fluorouracil.

In stages IIB, III, and IVA, chemotherapeutic agents may be carboplatin, cisplatin, or cisplatin in combination with fluorouracil. Radiotherapy encompasses both brachytherapy and EBRT. In stage IVB, where malignancy has spread out of the pelvic region to other regions, in that case cervical cancer is generally considered as incurable. In order to retard the growth of malignancy or for symptomatic relief only, treatment choices are radiotherapy with or without chemotherapeutic agents. Usually, cisplatin or carboplatin (platinum drugs) in combination with other drug(s), like, gemcitabine, topotecan, paclitaxel, etc., is one of the most standard regimens. Immunotherapeutic agents (monoclonal antibodies) like bevacizumab in combination with chemotherapeutic agents, pembrolizumab alone, the targeted drug tisotumabvedotin-tftv (Tivdak), or pembrolizumab (Keytruda) in combination with chemotherapeutic agents (with or without bevacizumab), are also good treatment choice.

In case of recurrence in the center of the pelvic region, widespread surgical procedures (like pelvic exenteration) are required for some patients, which proposes the higher probability to cure malignancy; however, it may show major side effects. Radiotherapy, sometimes in combination with chemotherapeutic agents, may be an alternative choice. Chemotherapeutic agents, immunotherapeutics, may be employed to retard the further growth of tumor or for symptomatic relief, but are not likely to cure tumor.

Stage I cervical malignancies are also diagnosed in pregnant women, though small in number. Hence, at the time of pregnancy, the treatment strategy is planned

according to size of tumor, spread of cancer nearby lymph nodes, and how far along the pregnancy is the specific type of cervical cancer. In stage IA, most of the oncologists consider it is safe to carry on the pregnancy and recommend treatment after a few weeks of birth. Surgical procedure is the treatment choice after birth for stage IA, which include radical trachelectomy, hysterectomy, or cone biopsy. In stage IB or higher, the patient and the oncologist must resolve whether or not to carry on the pregnancy. Treatment strategy is invasive if the patient decided not to continue the pregnancy which might be radiation/hysterectomy. Occasionally, chemotherapeutic agents can be employed at the time of pregnancy in the first 12 weeks or the third trimester in order to shrink the malignancy. If the patient chooses to carry on the pregnancy, the neonate must be delivered by cesarean method so that delivered baby can survive outside the womb (American Cancer Society 2019, 2020).

## 4.4 What Is Nanohybrid?

Vagueness in definition of nanohybrid literature is similar to the discussion available for single nanomaterials. Here, it was attempted to elucidate the literature's nuance-related nanohybrid and define it according to various regulatory agencies' perspective. In the literature available in material science, a normal surface engineering or mixing– with organic soft material and robust inorganic materials– is considered as hybridization. Anchoring a monomeric or polymeric material onto an inorganic, generally metallic nanomaterial, is claimed to form nanohybrid. Although slight surface engineering can alter the performance of nanomaterial, it is possible that the inherent physicochemical properties may remain unaltered. Hence, nanohybrid definition can be framed as: when more than one nanomaterial of the distinctive chemical properties or varied dimensions and measurement, anchored by weak or strong forces, or when one material coats another having an altered chemical features or when the complex soft organic molecules are altered to chemically link to nanomaterial surfaces, all are to improve the inherent functional properties in order to achieve multifunctional applications (Aich et al. 2014).

### 4.4.1 Nanohybrids in Cancer

Tumor microenvironment consists of fast-growing, hyperproliferative cancer cells having a demand for the generation of new vessels (neovascularization) and high metabolic rate to supply the nutrients to the rapidly dividing malignant cells (Carmeliet and Jain 2000). Microenvironment of cancerous cells is generally acidic because of utilization of the glycolysis pathway in order to obtain the required additional energy due to high metabolic activity (Pelicano et al. 2006). Tumor cells release growth factors and enzymes such as matrix metalloproteinases and it leads to an imbalance of angiogenic regulators dilating the tumor vessels, resulting in large gap junctions ranging from 200 to 2000 nm in size between endothelial cells

(Jain 2001). Additionally, the interstitial pressure elevated by the reduced lymphatic drainage added by a lower intravascular pressure generally limits the movement of large molecules/nanoparticles out of the malignant tissue blood vessels into the extravascular compartment; this phenomenon is also called enhanced permeability and retention effect (EPR) (Jain 2001). Hence, greater vascular permeability and deficient lymphatic drainage (EPR) in the cancerous tissue enable passive targeting by utilizing polymeric nanomaterials (Maeda 2001; Maeda and Matsumura 1989; Matsumura and Maeda 1986). The retention effect is further augmented by the chemical nature, molecular weight, and surface charge of nanomaterial. Drug loading to the polymers reduces the chances of abrupt variability of bioavailability and allows the drug molecule to target specificity. Nevertheless, the molecular weight of the nanomaterials as well exhibits a significant role in the drug delivery. Nanomaterials of a molecular weight of $<50$ kD or of a size $<6$ nm are generally cleared rapidly by the kidney after the systemic administration (Brenner et al. 1978; Maack et al. 1979; Mihara et al. 1993). For nonbiodegradable polymers-based nanocarriers, size is important so as to be cleared from the systemic circulation, after the administration of drug-loaded nano-carriers. Nano-carriers with cationic surface charge are, generally, more subject to glomerular filtration than their anionic counterparts, because of the negative charge on the glomerular capillaries, which results in a charge selective retention and the nano-carriers of larger molecular weight amass in the kidneys, liver, and lungs (Fujita et al. 1992; Mahato et al. 2003; Ma et al. 2005). Therefore, the perfect molecular weight of the nanomaterial generally depends on the specific functional application; it ranges from 30 to 100 kD. Nano-carriers that follow the above said conditions of size and surface properties, generally, escape the Reticulo-Endothelial System (RES) and are capable to circulate in the systemic circulation for a longer period of time with a higher probability to reach to the specific tissue (cancerous cells). Therapeutic molecules which are anchored to nanomaterial through ester or amide bonds are useful for precise drug release. In another example, the therapeutic molecule is released via thiol-dependent cleavage by protease cathepsin B. A pH-triggered drug release model involving hydrazine, cis-aconityl, acetyl linkages are studied (Duncan 2006; Ulbrich and Šubr 2004).

### 4.4.2 Advantages of Nanohybrids

- hybridization enhances the biocompatibility and photostability of materials employed in the synthesis of quantum dots.
- if molecules fail to exhibit a specific required purpose for a specific use, generally it is recommended to associate them with an appropriate molecule which can further improve or impart a new feature to the nanohybrids.
- various properties can be combined in a single structure that is nanohybrid.
- Physicochemical stability, biodegradability, biocompatibility and in vivo clearance can be improved (Choi et al. 2021).

### 4.4.3 Disadvantages of Nanohybrids

- lack of toxicity evaluation,
- lack of pharmacokinetic data,
- limited preclinical and clinical data (Choi et al. 2021).

## 4.5 Nanohybrids in Hormone-Related Cancer

### 4.5.1 Nanohybrids in Breast Cancer

Deficiency of cutting-edge approaches to inhibit the growth of breast cancer is one of the challenging issues for scientific community. Because of the vague tracing, low transfection proficiency, and poor therapeutic effects, nonviral gene delivery carriers for the treatment of cancer still face challenges. It is recently investigated that reduced graphene oxide (GO) can instigate apoptotic cell death, hence can be employed as a new treatment modality in cancer therapy. Organic–inorganic nano hybrids exhibit synergistic and balancing effects in nano-drug delivery system, hence can be employed as carrier in gene delivery. Interestingly, MXenes, a newly explored two-dimensional and conductive nanomaterial, has acknowledged an increasing attention in biosensing because of its large surface area and distinctive surface and interfacial properties. New folate-anchored chitosan-grafted disulfide-containing polyethylenimine copolymer-based silica nanohybrids were synthesized for the delivery of P-shRNA and taxol. Such nano-carriers can proficiently safeguard P-shRNA against degradation and showed good pH-responsive drug release and oxidation-reduction-triggered P-shRNA release behaviors. Folate can increase the cellular uptake of nano-medicines by multidrug-resistant breast cancer cells (Maheswari et al. 2021; Hossieny IbrahimIbrahim et al. 2019; Cheng et al. 2021; Luo et al. 2021; Vajhadin et al. 2022; Nabih and Hassn 2021; Jia et al. 2021).

### 4.5.2 Prostate Cancer

High-risk prostate cancer individuals (approximately 30–50%), who experience radiotherapy, may exhibit biochemical recurrence or biochemical failure (increase in prostate-specific antigen). The chances of biochemical recurrence are either due to distant disease or because of a poor local control. In this case, chemotherapy relied on Taxanes has been evidenced to be beneficial in prostate cancer. Combining chemotherapy (Taxanes based) with radiotherapy can improve the efficacy, though about 95% drug reaches to the healthy tissues, if administered in the form of conventional injectables, whereas approximately 2–5% drug only reaches to malignant tissue; adverse effects associated with taxanes in particular and anticancer agents in general are another critical issues. Additionally, drug resistance mechanisms associated with intracellular concentration of drugs generally reduce the efficacy of drug by reducing the drug concentration inside the tumor cell. Drug

resistance issues can be resolved after converting conventional anticancer formulations to nano-drug carriers by employing cutting edge nano technological tools, in order to maintain the concentration and residence time of drug in the affected organ or inside the malignant cell, eventually improving the effectiveness of drug. It has been investigated, in vitro, that a needle-shaped carrier can penetrate inside malignant cells and stay there for 10 days without the induction of cytotoxic effects.

A unique treatment modality, viz. boron neutron capture therapy (BNCT), has been employed for the management of malignancies, encompassing head and neck and brain cancers. For effective better treatment with BNCT technique, proficient and targeted delivery of a high boron dose to malignant cells is required. Basically, prostate-specific membrane antigen (PSMA) is the target for drug delivery and diagnostic purposes. The development of a sensitive, fast, and simple detection method of PSA is necessary for the timely diagnosis of prostate malignancies. Nanohybrids based on titanate nanotubes are fabricated by critical step by step synthesis procedure to cure prostate malignancies by intra-tumoral administration. The conjugation of chemotherapeutic agents and metal oxide (MxOy) nanoparticles has ignited a swiftly growing interest in onco-nanomedicine. Fundamentally, the idea was to maintain the concentration and residence of drug inside the malignant cells, primarily, and evade the issue of multidrug resistance, which generally reduces the drug efficacy by reducing the intracellular concentrations in malignant cells (Mirjolet et al. 2014; Castro Nava et al. 2015; Wang et al. 2019; Feng et al. 2017; Aayanifard et al. 2021; Loiseau et al. 2019, 2021).

### 4.5.3 Endometrial (Cervical Cancer)

Nanohybrids are interestingly showing considerable promising biomedical applications like bioimaging and drug delivery carriers because of their additional advantages of distinct components. Furthermore, improvement of the reproducible synthesis methods and stability (colloidal), to enable applied biomedical purposes, still faced considerable challenges. Novel approach to fabricate magneto-fluorescent nanohybrids encompassing fluorescent carbon dots and magnetic iron oxide nanoparticles through polyglycerol (PG) facilitated covalent linkage in water, superparamagnetic iron oxide-reduced graphene oxide (Fe3O4-RGO) nanohybrid and encapsulation of Hydroxyapatite nanocrystals (HAP NCs) were encapsulated (Bach et al. 2013; Gupta et al. 2018; Lee and Kim 2020; Wen et al. 2019; Girma et al. 2019).

### 4.5.4 Ovarian Cancer

Some of the researches suggest a new hesperidin and Sperm protein-loaded nanohybrid carrier-based drug delivery targeted to the progesterone receptor, which is selectively overexpressed in some cancers including ovarian malignancy,

and generally less expressed or absent in most normal cells. Phytomedicines are enthusiastically being investigated as anticancer agents substituting synthetic antineoplastic agents. Genistein (phytomedicine), an antineoplastic agent, loaded casein (milk protein)-based pH and magnetic field-triggered nanohybrid carriers conjugated with progesterone for the site specific delivery were synthesized. Hence, casein in combination with calcium ferrite, a super paramagnetic agent, showed improved surface area for drug entrapment, biocompatibility, colloidal stability, and magnetically triggered release properties (Purushothaman and KM MS. 2020; Gasparotto et al. 2017; Bindhya et al. 2021; Liu et al. 2017).

## References

Aayanifard Z, Alebrahim T, Pourmadadi M, Yazdian F, Dinani HS, Rashedi H, Omidi M (2021) Ultra pH-sensitive detection of total and free prostate-specific antigen using electrochemical aptasensor based on reduced graphene oxide/gold nanoparticles emphasis on TiO2/carbon quantum dots as a redox probe. Eng Life Sci 21(11):739–752

Aich N, Plazas-Tuttle J, Lead JR, Saleh NB (2014) A critical review of nanohybrids: synthesis, applications and environmental implications. Environ Chem 11(6):609–623

American Cancer Society (2019) What is breast cancer? [Internet]. [updated 2019 September 18] https://www.cancer.org/cancer/breast-cancer/about/what-is-breast-cancer.html. Accessed on 22 Jan, 2022

American Cancer Society (2020) How hormone therapy is used to treat cancer [Internet]. [updated 2020 July 21]. https://www.cancer.org/treatment/treatments-and-side-effects/treatment-types/hormone-therapy.html. Accessed on 22 Jan, 2022

Bach LG, Islam MR, Vo TS, Kim SK, Lim KT (2013) Poly (allyl methacrylate) functionalized hydroxyapatite nanocrystals via the combination of surface-initiated RAFT polymerization and thiol–ene protocol: a potential anticancer drug nanocarrier. J Colloid Interface Sci 394:132–140

Bindhya KP, Maheswari PU, Begum KM (2021) Milk protein inspired multifunctional magnetic carrier targeting progesterone receptors: improved anticancer potential of soybean-derived genistein against breast and ovarian cancers. Mater Chem Phys 272:125055

Brenner BM, Hostetter TH, Humes HD (1978) Glomerular permselectivity: barrier function based on discrimination of molecular size and charge. Am. J. Physiol. Ren. Physiol 234(6):F455–F460

Carmeliet P, Jain RK (2000) Angiogenesis in cancer and other diseases. Nature 407(6801):249–257

Castro Nava A, Cojoc M, Peitzsch C, Cirillo G, Kurth I, Fuessel S, Erdmann K, Kunhardt D, Vittorio O, Hampel S, Dubrovska A (2015) Development of novel radiochemotherapy approaches targeting prostate tumor progenitor cells using nanohybrids. Int J Cancer 137(10):2492–2503

Cheng D, Ji Y, Wang B, Wang Y, Tang Y, Fu Y, Xu Y, Qian X, Zhu W (2021) Dual-responsive nanohybrid based on degradable silica-coated gold nanorods for triple-combination therapy for breast cancer. Acta Biomater 128:435–446

Choi G, Rejinold NS, Piao H, Choy JH (2021) Inorganic–inorganic nanohybrids for drug delivery, imaging and photo-therapy: recent developments and future scope. Chem Sci

Cramer DW, Welch WR (1983) Determinants of ovarian cancer risk. II. Inferences regarding pathogenesis. J Natl Cancer Inst 71(4):717–721

Duncan R (2006) Polymer conjugates as anticancer nanomedicines. Nat Rev Cancer 6(9):688–701

Feng J, Li Y, Li M, Li F, Han J, Dong Y, Chen Z, Wang P, Liu H, Wei Q (2017) A novel sandwich-type electrochemical immunosensor for PSA detection based on PtCu bimetallic hybrid (2D/2D) rGO/g-C3N4. Biosens Bioelectron 91:441–448

Fujita T, Nishikawa M, Tamaki CH, Takakura YO, Hashida MI, Sezaki HI (1992) Targeted delivery of human recombinant superoxide dismutase by chemical modification with mono- and polysaccharide derivatives. J Pharmacol Exp Ther 263(3):971–978

Gasparotto G, Costa JP, Costa PI, Zaghete MA, Mazon T (2017) Electrochemical immunosensor based on ZnO nanorods-au nanoparticles nanohybrids for ovarian cancer antigen CA-125 detection. Mater Sci Eng C 76:1240–1247

Girma WM, Dehvari K, Ling YC, Chang JY (2019) Albumin-functionalized CuFeS2/photosensitizer nanohybrid for single-laser-induced folate receptor-targeted photothermal and photodynamic therapy. Mater Sci Eng C 101:179–189

Gupta J, Prakash A, Jaiswal MK, Agarrwal A, Bahadur D (2018) Superparamagnetic iron oxide-reduced graphene oxide nanohybrid-a vehicle for targeted drug delivery and hyperthermia treatment of cancer. J Magn Magn Mater 448:332–338

Harbeck N, Penault-Llorca F, Cortes J, Gnant M, Houssami N, Poortmans PK, Ruddy J, Tsang F, Cardoso F (2019) Breast cancer. Nat Rev Dis Primer 5:1–31

Ho SM (2003) Estrogen, progesterone and epithelial ovarian cancer. Reprod Biol Endocrinol 1(1): 1–8

Hossieny IbrahimIbrahim H, Ibrahim M, Temerk Y (2019) A novel megestrol acetate electrochemical sensor based on conducting functionalized acetylene black–CeO2NPs nanohybrids decorated glassy carbon microspheres. Talanta 200:324–332

Jain RK (2001) Delivery of molecular medicine to solid tumors: lessons from in vivo imaging of gene expression and function. J Control Release 74(1–3):7–25

Jia L, Li Z, Zheng D, Li Z, Zhao Z (2021) A targeted and redox/pH-responsive chitosan oligosaccharide derivatives based nanohybrids for overcoming multidrug resistance of breast cancer cells. Carbohydr Polym 251:117008

Jin C, Wang K, Oppong-Gyebi A, Hu J (2020) Application of nanotechnology in cancer diagnosis and therapy-a mini-review. Int J Med Sci 17(18):2964

Key TJ (1995) Hormones and cancer in humans. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 333(1–2):59–67

Lee CS, Kim TH (2020) Label-free assay of protein kinase a activity and inhibition in cancer cell using electrochemically-prepared AuNP/rGO nanohybrid electrode modified with C-Kemptide. Talanta 1(215):120899

Liu L, Mirandola L, Chiriva-Internati M, Chaudhuri J (2017) CdS quantum dot-chitosan-anti SP17 nanohybrid as a potential cancer biomarker. Mater Lett 199:5–8

Loiseau A, Boudon J, Mirjolet C, Morgand V, Millot N (2021) About the influence of PEG spacers on the cytotoxicity of Titanate nanotubes-docetaxel Nanohybrids against a prostate cancer cell line. Nano 11(10):2733

Loiseau A, Boudon J, Oudot A, Moreau M, Boidot R, Chassagnon R, Mohamed Saïd N, Roux S, Mirjolet C, Millot N (2019) Titanate nanotubes engineered with gold nanoparticles and docetaxel to enhance radiotherapy on Xenografted prostate Tumors. Cancers 11(12):1962

Luo T, Nie Y, Lu J, Bi Q, Cai Z, Song X, Ai H, Jin R (2021) Iron doped carbon dots based Nanohybrids as a Tetramodal imaging agent for gene delivery promotion and Photothermal-Chemodynamic cancer synergistic Theranostics. Mater Des 10:109878

Ma SF, Nishikawa M, Katsumi H, Yamashita F, Hashida M (2005) Cationic charge-dependent hepatic delivery of amidated serum albumin. J Control Release 102(3):583–594

Maack T, Johnson V, Kau ST, Figueiredo J, Sigulem D (1979) Renal filtration, transport, and metabolism of low-molecular-weight proteins: a review. Kidney Int 16(3):251–270

Maeda H (2001) SMANCS and polymer-conjugated macromolecular drugs: advantages in cancer chemotherapy. Adv Drug Deliv Rev 46(1–3):169–185

Maeda HA, Matsumura Y (1989) Tumoritropic and lymphotropic principles of macromolecular drugs. Crit Rev Ther Drug Carrier Syst 6(3):193–210

Mahato RI, Kawabata K, Takakura Y, Hashida M (2003) Erratum: in vivo disposition characteristics of plasmid DNA complexed with cationic liposomes (journal of drug targeting (1995) vol. 3 (149-157)). J Drug Target 11(7):457

Maheswari PU, Muthappa R, Bindhya KP, Begum KM (2021) Evaluation of folic acid functionalized BSA-CaFe2O4 nanohybrid carrier for the controlled delivery of natural cytotoxic drugs hesperidin and eugenol. J Drug Deliv Sci Technol 61:102105

Matsumura Y, Maeda H (1986) A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. Cancer Res 46(12 Part 1):6387–6392

Mayo Clinic (2021) Endometrial cancer [Internet]. [updated 2021 May 20]. https://www.mayoclinic.org/diseases-conditions/endometrial-cancer/symptoms-causes/syc-20352461. Accessed on 22 Jan, 2022

Mihara K, Hojo T, Fujikawa M, Takakura Y, Sezaki H, Hashida M (1993) Disposition characteristics of protein drugs in the perfused rat kidney. Pharm Res 10(6):823–827

Mirjolet C, Boudon J, Loiseau A, Chevrier S, Gautier T, Boidot R, Paris J, Millot N, Crehange G (2014) 207 the enhancement of radiotherapy efficacy with docetaxel-titanate nanotubes as a new nanohybrid for localized high risk prostate cancer. Eur J Cancer 50:67

Munkley J, Li L, Krishnan SG, Hysenaj G, Scott E, Dalgliesh C, Oo HZ, Maia TM, Cheung K, Ehrmann I, Livermore KE (2019) Androgen-regulated transcription of ESRP2 drives alternative splicing patterns in prostate cancer. elife 8:e47678

Munkley J, McClurg UL, Livermore KE, Ehrmann I, Knight B, Mccullagh P, Mcgrath J, Crundwell M, Harries LW, Leung HY, Mills IG (2017) The cancer-associated cell migration protein TSPAN1 is under control of androgens and its upregulation increases prostate cancer cell migration. Sci Rep 7(1):1–1

Nabih S, Hassn SS (2021) Nonchemical integration of au/ag-based reduced graphene nanohybrid combined with 5-fluorouracil drug to treat cancer cells. Life Sci 272:119262

Neidle S, Thurston DE (2005) Chemical approaches to the discovery and development of cancer therapies. Nat Rev Cancer 5(4):285–296

Pelicano H, Martin DS, Xu RA, Huang P (2006) Glycolysis inhibition for anticancer treatment. Oncogene 25(34):4633–4646

Purushothaman BK, KM MS. (2020) Magnetic casein-CaFe2O4 nanohybrid carrier conjugated with progesterone for enhanced cytotoxicity of citrus peel derived hesperidin drug towards breast and ovarian cancer. Int J Biol Macromol 151:293–304

Riman T, Dickman PW, Nilsson S, Correia N, Nordlinder H, Magnusson CM, Weiderpass E, Persson IR (2002) Hormone replacement therapy and the risk of invasive epithelial ovarian cancer in Swedish women. J Natl Cancer Inst 94(7):497–504

Rodriguez C, Patel AV, Calle EE, Jacob EJ, Thun MJ (2001) Estrogen replacement therapy and ovarian cancer mortality in a large prospective study of US women. JAMA 285(11):1460–1465

Senapati S, Mahanta AK, Kumar S, Maiti P (2018) Controlled drug delivery vehicles for cancer treatment and their performance. Signal Transduct Target Ther 3(1):1–9

Ulbrich K, Šubr V (2004) Polymeric anticancer drugs with pH-controlled activation. Adv Drug Deliv Rev 56(7):1023–1050

Vajhadin F, Mazloum-Ardakani M, Shahidi M, Moshtaghioun SM, Haghiralsadat F, Ebadi A, Amini A (2022) MXene-based cytosensor for the detection of HER2-positive cancer cells using CoFe2O4@ ag magnetic nanohybrids conjugated to the HB5 aptamer. Biosens Bioelectron 195:113626

Wang S, Blaha C, Santos R, Huynh T, Hayes TR, Beckford-Vera DR, Blecha JE, Hong AS, Fogarty M, Hope TA, Raleigh DR (2019) Synthesis and initial biological evaluation of boron-containing prostate-specific membrane antigen ligands for treatment of prostate cancer using boron neutron capture therapy. Mol Pharm 16(9):3831–3841

Wen Y, Xu M, Liu X, Jin X, Kang J, Xu D, Sang H, Gao P, Chen X, Zhao L (2019) Magnetofluorescent nanohybrid comprising polyglycerol grafted carbon dots and iron oxides: colloidal synthesis and applications in cellular imaging and magnetically enhanced drug delivery. Colloids Surf B: Biointerfaces 173:842–850

# Conventional to Nanoscale-Based Carrier Systems in the Management of Ovarian Cancer

5

Sabya Sachi Das, Ishan Moitra, Subhrajeet Das, Neeru Singh, Priya Ranjan Prasad Verma, and Sandeep Kumar Singh

**Abstract**

Ovarian cancer (OC) is one of the most common and dreadful cancer types which has affected the female population worldwide with an increased number of cases. Broadly, OC has been categorized into two types based on its mutational changes. These are Type 1 OC which is found to show less aggressive tumors without any mutation of the tumor protein p53 gene and Type 2 OC which exhibits tumors resulting from significant mutation of the P53 gene. The major molecular pathways and cellular targets responsible for the progression of OC include VEGF, PDGF, FGF, (PI3K)/AKT pathway, PARP, and others. The conventional therapies for the management of OC include surgery, chemotherapy, hormonal therapy, radiotherapy, monoclonal antibodies, and others. However, conventional

S. S. Das
Department of Pharmaceutical Sciences and Technology, Birla Institute of Technology, Ranchi, Jharkhand, India

School of Pharmaceutical and Population Health Informatics, DIT University, Dehradun, Uttarakhand, India

I. Moitra
Formulation R&D, IPDO, Dr. Reddy's Laboratories, Bachupally, Hyderabad, India

S. Das
Department of Chemistry, Birla Institute of Technology, Ranchi, Jharkhand, India

N. Singh
Department of Biomedical Laboratory Technology, University Polytechnic, Birla Institute of Technology, Ranchi, Jharkhand, India

P. R. P. Verma · S. K. Singh (✉)
Department of Pharmaceutical Sciences and Technology, Birla Institute of Technology, Ranchi, Jharkhand, India
e-mail: sandeep.singh@bitmesra.ac.in



M. Rahman et al. (eds.), *Hormone Related Cancer Mechanistic and Nanomedicines*,
https://doi.org/10.1007/978-981-19-5558-7_5

therapies are restricted due to a few limitations such as unintended toxicities, relapse of the conditions, high dose, less bioavailability, and prolonged therapeutic regimen. Thus, to overcome these limitations nanotherapeutic approaches can be used for the effective delivery of drugs with superior targetability and negligible or no toxicity. In this chapter, we have discussed the major molecular pathways and cellular targets causing ovarian cancer. Also, the significant role of nanotherapeutic approaches and targeting mechanisms of encapsulated bioactive/drugs against ovarian cancer cells is summarized.

**Keywords**

Ovarian cancer · Cellular responses · Molecular pathways · Nanotherapy · Targeted delivery systems · Bioavailability

## 5.1 Introduction

The need for advancements in therapeutics is often triggered by the severity of disease and the statistics that revolve around it. Ovarian cancer (OC) is one such dreadful disease that finds itself in the list of common cancers around the globe. As per the American Institute of Cancer Research, OC is the 18th most common cancer, while being the eighth most common for women (Ferlay et al. 2019). In 2021, an estimated 21,410 cases received the diagnosis and around 13,770 lives were expected to be claimed by OC in the USA. All of these data accompanied with a delayed and limited and complicated diagnosis calls for effective treatment of the disease, which shall not only lessen hospital stay, but also prevent the recurrence of the disease. Even though patients respond to treatments initially, about 50–75% of the cases see a relapse (Das and Bast Jr. 2008; Jacobs and Menon 2004; Sarojini et al. 2012). OC can be of two types; Type 1 and Type 2. Type 1 OC houses less aggressive tumors without any mutation of the Tumor Protein p53 gene; whereas Type 2 houses tumors resulting from significant mutation of the P53 gene (Kurman and IeM 2010). OC of Serous type associated with BRCA1, BRCA2, MMR, BARDI, etc. mutations is responsible for more than half the cases. Their area of metastasis is similar(Ducie et al. 2017).

As per FIGO Committee on Gynaecologic Oncology, OC can be classified under 4 stages based on the extent of metastasis. In stage 1, the tumor growth is confined to the ovaries. In stage 2, the tumor has spread to the pelvic region. When metastasis has occurred to the organs of the peritoneal cavity such as the liver and intestine, and lymph nodes, the cancer is in stage 3. If further metastasis occurs, then it can be classified as stage 4 (Prat 2014). Epithelial carcinoma constitutes 85–90% of the cases. Such type of OC includes endometrioid, mucinous, and a few other cell growths such as Brenner; but mostly constitutes epithelial cell growths. Epithelial cell growths have two subtypes; namely high-grade serous carcinoma (HGSC), which is quite common, and the uncommon low-grade serous carcinoma (LGSC) (Chen et al. 2014).

A rare type of OC includes Ovarian Malignant Germ Cell Tumors (OMGCTS), a condition arising from the malignancy of the primitive germ cells of the embryonic gonads. Their various types include the less malignant but more common, dysgerminoma, and the more malignant teratomas, yolk sac tumors, embryonal carcinomas, and polyembryomas. The tumor markers include alpha-fetoprotein, beta-human chorionic gonadotropin, and lactic dehydrogenase, all of which may or may not be present for a tumor type (Shaaban et al. 2014).

## 5.1.1 Conventional Therapies Used to Combat Ovarian Cancer

### 5.1.1.1 Surgery

In the early stages of OC, especially in stages 1 and 2, medical practitioners turn to surgeries such as Bilateral Salpingo-Oophorectomy (BSO), which has not only reduced the chance of recurrence, but also minimized the dependence on chemotherapy. BSO is a cytoreduction surgery done to remove small tumors (less than 1 cm diameter). To increase the efficacy of the treatment, the patient may be put through a cycle of cisplatin or paclitaxel administered intravenously every 21 days for 6 cycles. Carboplatin and/or cisplatin may also be used, albeit with some side effects like neuropathy (Wang and Fang 2021; McGuire et al. 1996; Elit et al. 2007).

### 5.1.1.2 Chemotherapy

Cytoreductive surgeries are usually followed by a few cycles of chemotherapy as mentioned above. However, the relapse of the disease in most cases remains a major challenge. Initially, the disease is seen to progress during platinum-based medication regime; possibly due to platinum resistance or sensitivity. In later stages of the regime, usually after 6–12 months, the condition is again seen to relapse due to resistance to platinum-based therapeutics. Anticancer agents, being low molecular weight compounds, are limited by their inability to attack specific targets; i.e., the site of cancer. Moreover, such agents are required in high doses during the treatment regime; thus, chemotoxicity also becomes a major hurdle. Their low bioavailability needs to be tackled as well (Bharadwaj et al. 2021; Pignata et al. 2011; Das et al. 2020d).

### 5.1.1.3 Intraperitoneal Chemotherapy

OC has been observed to not metastasize beyond the abdominal cavity. This is a type of treatment where the drug is administered into the abdomen region intraperitoneally (IP). A study conducted by Gynecologic Oncology Group (GOG), a randomized controlled trial with 876 patients, showed that the mean survival for intraperitoneal therapy was 61.8 months, while the same being only 51.4 months for conventional IV therapy. Thus, IP therapy was judged to be more effective when compared to IV therapy(Tewari et al. 2015; Becker et al. 2019; Barlin et al. 2012).

#### 5.1.1.4 Hormone Therapy

Luteinizing Hormone Releasing Hormone (LHRH) receptor agonists like goserelin and leuprolide are administered to lower the gonadotropin secretion leading to lower estradiol level, which is highly increased in cases of OC. Vaginal bleeding, osteoporosis, and vaginal dryness are common side effects of the treatment (Katchman et al. 2017). Tamoxifen, a nonspecific estrogen receptor antagonist, is often used in the treatment of stromal OC. Its anticancer activity is due to the ability to competitively inhibit the binding of estrogen to its receptor leading to a reduction in freely circulating estradiol. Tamoxifen reduces tumor growth factor α. Hot flushes and vaginal dryness are some of the major limitations of this treatment (Katchman et al. 2017). Apart from this, various aromatase inhibitors such as anastrozole, exemestane, letrozole, etc. exhibit potential effects in inhibiting the binding of Oestrogen to the growth hormone receptors of tumors. It acts by competitive inhibition of aromatase enzyme, which is responsible for conversion of androgens into estrogen, primarily in women after menopause. This treatment also has its fair share of side effects like osteoporosis and hot flushes among others (Katchman et al. 2017; Kok et al. 2019).

#### 5.1.1.5 Radiation Therapy

This therapy involves the use of powerful electromagnetic waves like X-rays to treat malignancy. The radiation can be administered either externally; i.e., outside the body; or internally, also known as brachytherapy. In external beam radiation therapy, a linear accelerator machine emits x-ray or photon beams in a controlled manner to attack the malignant areas, while leaving the healthy cells unharmed. External beam radiation therapy can be of various types like Intensity-modulated radiation therapy (IMRT), Proton beam therapy, Image-guided radiation therapy (IGRT), and Stereotactic Radiation Therapy (SRT). Internal radiation therapy or brachytherapy, on the other hand, employs a radioactive material to target the tumor. This material is delivered using capsules, catheters, needles, or other special kinds of applicators. The therapy is permanent if the capsule or any other such delivery system containing the radioactive therapeutic agent is implanted in the affected tissues. The therapy is temporary if the fluidized form of the radioactive therapeutic agent is delivered using needles or catheters. Apart from this, some other specific types of radiation therapy are also used to mitigate OC.

##### Intraoperative Radiation Therapy

When vital organs are close to the tumors, surgeons make use of this therapy to deliver the radiation. This therapeutic procedure is carried out during surgical operation, where any effect due to the radiation on healthy tissues can be subsided by making way for the radiation to directly reach the affected area.

##### Systemic Radiation Therapy

For this therapy, the patient has to swallow a pill containing a radioactive therapeutic agent that is supposed to target the tumor tissues. The radioactive material gets excreted from the body through feces, urine, and sweat. (Rai et al. 2014).

#### Radioimmunotherapy

This type of therapy employs monoclonal antibodies that identify specific markers surrounding tumor tissues. IgG1 antibody, Ibritumomab, is one such therapeutic agent that has found itself useful in cases of lymphomas. It is administered in a combination with a tiuxetan, a chelator that carries a radioactive isotope like yttrium or indium. This system binds to a CD20 antigen present on malignant and normal B cells at tumor sites. The therapeutic activity occurs through two mechanisms; one, through irradiation of the tumor tissue by the beta waves emitted by the radioactive isotopes; and second, through the attachment of the antibody to the antigen to bring about antibody-dependent cell-mediated cytotoxicity. (Song and Sgouros 2011).

#### 5.1.1.6 Monoclonal Antibodies

Following various targets present on tumor tissues and those exhibited during the physiological process of tumor progression, many monoclonal antibodies have paved the way for the effective treatment of OC. A trifunctional antibody molecule currently in Phase 2 of clinical trials, called Catumaxomab, works by targeting Epithelial Cell Adhesion Molecule (EpCAM) on cancer sites and CD3 on T cells. In doing so, it allows macrophages, dendritic cells, natural killer cells, and T cells to make their way to the cancer site that would result in tumor cell death (Das et al. 2020a). This treatment is aimed to be effective in patients who face a recurrence of cancer or in cases where their condition has become resistant to conventional platinum-based chemotherapy. This can be attributed to EpCAM-positive OC cells, which remain viable even after conventional chemotherapy (Tayama et al. 2017). CD25 is another lucrative which targets cancer for immunosuppressant monoclonal antibody in treating serous ovarian; a candidate for which is Daclizumab that suppresses T cell population; although the binding seems to be nonspecific (Santoiemma and Powell Jr. 2015).

## 5.2 Molecular Targets and Cellular Pathways Associated with Ovarian Cancer

Various molecular targets and pathways have been associated directly or indirectly with ovarian tumors leading to cancer. Some of the major targets have been discussed below.

### 5.2.1 Vascular Endothelial Growth Factor (VEGF), Platelet-Derived Growth Factor (PDGF), Fibroblast Growth Factor (FGF)

Several factors contribute to the progression of the tumor. Some of the prominent factors include VEGF, PDGF, and FGF. Anti-VEGF agents result in inhibition of angiogenesis at tumor sites by either blocking the receptor-mediated pathways of neutralization of VEGF. Bevacizumab is a monoclonal antibody formulation that inhibits angiogenesis by binding to VEGF and prevents it from binding to the

**Table 5.1** Clinical status of a few targeted therapeutic products in ovarian cancer therapy

| Drug | Molecular target | Mechanism of action | Phase of development |
|---|---|---|---|
| Aflibercept | VEGF A&B, PIGF | Binds to VEGF A&B and placental growth factor (PGF) and inhibits angiogenesis | Approved Drugs@FDA: FDA-Approved Drugs (n.d.) |
| Ramucirumab | VEGF receptor tyrosine kinase inhibitor | Blocks the interaction of VEGFR-2 with its ligands, VEGF-A, VEGF-C, and VEGF-D and inhibits angiogenesis | Approved FDA approves ramucirumab plus erlotinib for first-line metastatic NSCLC (n.d.) |
| Sunitinib | Multiple receptor tyrosine kinase (RTK) | Inhibition of multiple RTKs including PDGF-R and VEGF-R leading to inhibition of angiogenesis | Phase 2 Chan et al. (2018) |
| Sorafenib with bevacizumab | Multiple receptor tyrosine kinase (RTK) | Interact with multiple intracellular (CRAF, BRAF, and mutant BRAF) and cell surface kinases (KIT, FLT-3, VEGFR-2, VEGFR-3, and PDGFR-ß), thus inhibiting tumor cell proliferation | Phase 2 Drugs@FDA: FDA-Approved Drugs US-FDA (n.d.), Drugs@FDA: FDA-Approved Drugs US-FDA (n.d.) |
| Pazopanib | VEGFR-1, − 2 and − 3, PDGFR-α and -β, and c-kit | Inhibition of intracellular tyrosine kinase of VEGF and PDGF receptor leading to antiangiogenesis action | Phase 3 Kasper and Hohenberger (2011); Vergote et al. (2019); Novartis Pharmaceuticals (2013) |
| Sirolimus, Everolimus | mTOR | Inhibits cytokine by blocking PI3K/AKT signaling pathway | Approved |
| Olaparib, Rucaparib | PARP | Binds to PARP enzyme and inactivates DNA repair mechanisms | Approved |

receptor. Although some side effects like cardiotoxicity, hypertension, and dermatological conditions have been associated with the treatment, Bevacizumab has become a preferred addition to the conventional therapeutic regimen (Burger 2011; Amini et al. 2012). A few targeted therapeutic products that are either under clinical trial or approved are mentioned in Table 5.1.

### 5.2.2 (PI3K)/AKT Pathway

A signaling pathway that has a major contribution towards cancerous cell growth is the phosphatidylinositol-3-kinase (PI3K)/AKT pathway. A protein kinase in this pathway is the mTOR protein that is encoded by the mTOR gene.

Immunosuppressant cytokine inhibitor drugs like Sirolimus and Everolimus target mTOR protein and inhibit cell proliferation (Das et al. 2020b).

### 5.2.3 Poly (ADP-Ribose) Polymerase (PARP)

Another lucrative target for beating OC is the enzyme Poly (ADP-ribose) Polymerase (PARP). This enzyme is responsible for DNA repair; in both normal and cancerous cells. PARP detects single-strand breakage on DNA and binds to it and initiates repair by synthesizing poly-ADP ribose. This acts as a trigger for other DNA repairing enzymes like DNA Ligases. Programmed cell death is also regulated by this enzyme. PARP enzyme (PARP 1, PARP 2, etc.) inhibitors like Olaparib, Rucaparib, and Niraparib have been approved for the treatment of OC (AstraZeneca 2021; Barkat et al. 2021).

## 5.3 Novel Drug Delivery Systems for Treatment of Ovarian Cancer

Conventional cancer therapies come with several limitations; especially unintended toxicities, relapse of the conditions, high dose, less bioavailability, and prolonged therapeutic regimen. This not only makes the treatment inaccessible to the common public, but also increases the chance of propagation of the disease to the next generation. Thus, there is a need for innovation that makes the treatment not only economic, but also effective. Various drug delivery systems based on passive and active mechanisms, aimed to improve the Spatio-temporal response of the therapeutic system, are being continuously developed.

### 5.3.1 Passive Drug Delivery Systems

Sound knowledge of the physiology of the cancerous tissues helps in designing a drug delivery system that can take advantage of the existing conditions to make the therapeutic agent reach the desired location of action in appropriate amounts. The regions of cancer are characterized by irregular and uncontrolled growth of fenestrated blood vessels that result in the proliferation of the cancerous tissues by a supply of nutrients; while also resulting in hypoxia at that region (Danhier et al. 2010). There are also few angiogenic regulators present at the site that cause the endothelium to become "leaky". Matrix metalloproteinases are one such regulator whose inhibition (e.g., MMP inhibitor like Doxycycline) can form a treatment method. Moreover, the poor drainage of the lymphatic system makes the drug delivery systems like nanocarriers carrying the therapeutic moiety leak out of the fenestrated vasculatures and reach the target cancer tissue; while also staying in circulation for a longer time. This mechanism is termed enhanced permeation and retention effect and is widely employed for targeted therapy (Torchilin 2006).

The process by which passive targeting occurs is diffusion while escaping the reticuloendothelial system (RES). For diffusion to occur effectively, the nanocarriers need to have certain characteristics to remain circulating. Few of the desired characteristics include a particle size between 10 and 100 nm, a mostly hydrophilic composition, and a neutrally charged surface. Few materials that are widely used to develop nanoparticles (NPs) are poly-(vinyl alcohol) and poly-(ethylene glycol). Being immunologically inactive, PEG has been employed widely in varying molecular weights by altering PEG chain length (Tirelli et al. 2002).

Some passive drug delivery systems have exploited the potential of decorating the surface of nanocarriers with a copolymer block made using both hydrophilic and hydrophobic polymers. Hydrophilic polymers like PEG conjugated with hydrophobic polymers like Polyoxypropylene (PPO) form a PEG/PPO block copolymer. The PPO chains are bound to the nanocarrier by hydrophobic interaction and the PEG chains face the surrounding hydrophilic medium providing steric stability. The stability of the therapeutic system is an important consideration for sufficient circulation time in the body for an optimum therapeutic effect (Illum et al. 1987). A Phase 2 trial of combination therapy of carboplatin and pegylated liposomal doxorubicin yielded satisfactory results and the treatment was deemed feasible for gynaecologic sarcomas (Harter et al. 2016).

### 5.3.2 Active Drug Delivery System

The guiding principle behind designing site-specific targeting drug delivery systems is being able to recognize the receptors on the cancerous cells' membranes. To target these receptors, various ligands, including new generation monoclonal antibodies, can be attached to the nanocarrier. The nanocarriers after getting attached to the cells utilize uptake mechanisms like phagocytosis and endocytosis (Das et al. 2020c; Barkat et al. 2020). Nanoparticulate systems to which PEG is to be attached can be done so by attaching the PEG to polymers like poly-α-hydroxy acids (PLAs) or polylactic-co-glycolic acid (PLGA) copolymers (Milane et al. 2011). A lipidic nanocarrier like that made of distearylphosphatidylethanolamine (DSPE) can be also decorated with PEG, non-covalently; as with the case of adamantane inserted into a cyclodextrin system (Bartlett and Davis 2007; Chenjie et al. 2009). To stabilize a liposomal system, formulators often use PEG of molecular weight 2000 dalton or more. This affords a thermodynamically stable system (Garbuzenko et al. 2005).

PEG chains attached to liposomes have to be attached to a functional group like carbonyl, amide, or sulfhydryl; which can serve as active points (thus forming a bridge) where a therapeutic agent can be attached (van Vlerken et al. 2007). The attachment can take place in the presence of a linking or coupling agent like N, N'-dicyclohexylcarbodiimide or cyanuric chloride, which acts as a catalyst (Cirstoiu-Hapca et al. 2010). One of the major disadvantages of the above technique is the remains of unreacted end groups of PEG chains, those that have not been associated with any functional groups. This can lead to unsuitable interactions of the liposome

with the surrounding system; while also providing hindrances to conjugation of the therapeutic moiety or other ligands to the system (Zalipsky et al. 1997). A strategy to overcome this barrier has been to form a conjugated system consisting of Lipid-PEG-Ligand and make them attach to a preformulated nontargeting liposome. This not only positions the conjugated system outside the liposomal bilayer unlike the previous strategy where the conjugate would be trapped inside the liposome, but also allows a great deal of flexibility in designing an effective drug delivery system that has highly specific actions. Commonly used linkages include peptides, transferrin, and folate moieties (Uster et al. 1996).

These actively targeting drug delivery systems mainly target various receptors, biomarkers, and some biochemicals like proteins that are expressed during tumor proliferation for delivery of the therapeutic moiety. The Follicle Stimulating Hormone or FSH is known to activate FSH Receptor (FSHR) on the ovarian epithelium. This receptor is overexpressed in OC cells which can be targeted by the drug delivery systems (Zhang et al. 2009a). Paclitaxel-loaded NPs conjugated with a peptide derived from FSH have been developed (Zhang et al. 2009b). A tumor in its course of proliferation releases various proteins or antigens that get reflected during the evaluation of the serum of the patient. One such antigen is CA-125. This gets expressed in more than 80% of the cases of OC; which makes it an ideal target for designing therapeutic agents (Bast Jr et al. 1981; Moore and Maclaughlan 2010).

HER-2 is another receptor that is overexpressed in OC that leads to tumor proliferation and various pathways attached to it. A monoclonal antibody approved by FDA and EMA that is currently being used is Trastuzumab in the treatment of HER2-positive breast cancer, but has not proven itself effective in the treatment of epithelial OC (Wilken et al. 2010). Another biomarker expressed during cancer angiogenesis is nucleolin, which is a 31-amino acid F3 peptide. This F3 peptide is synthesized from the protein High Mobility Group (HMG) 2. F3 can bind to nucleolin protein expressed at tumor sites. Thus, it was considered as a targeting moiety; the same has been demonstrated in a study conducted by designing PEG-ylated liposomes functionalized with nucleolin targeting F3 peptide and loaded with Doxorubicin and C6-Ceramide synergistic combination and results show to be effective in generating cytotoxicity towards cancerous ovarian cells (Cruz et al. 2021).

Angiogenesis is also known to be regulated by integrins. Thus, a certain combination of amino acids sequence results in a peptide such as RGD (Arginine-Glycine-Aspartic acid) that can bind to integrin molecules (Janssen et al. 2002). The Transferrin Receptor (Tfr) is overexpressed in OC and is known to be stimulated by certain antibodies and transferrin. Thus, a drug delivery system associated with transferrin and loaded with a monoclonal antibody like R17217 and OX26 (Ulbrich et al. 2009; Béduneau et al. 2007) or Docetaxel-loaded liposomal drug delivery system modified with transferrin can target the OC sites (Yuan et al. 2014).

### 5.3.3 Materials for Fabrication of Nanoparticulate Drug Delivery Systems

While fabricating a nano delivery system, the materials used are particularly important for compatibility with the therapeutic agent, toxicity and biodegradability, and any adverse effects. Factors governing the feasibility of scaling up the product to the industrial level are also taken into consideration. The ultimate goal shall be to design an effective drug delivery system that possesses enhanced permeation and retention effect (EPR), an optimal dissolution profile, and a good Spatio-temporal response that delivers the drug at the particular cancerous site without causing any harm to the surrounding healthy cells.

#### 5.3.3.1 Biodegradable Polymers Used in the Fabrication of Nanoparticles

Biodegradable polymers are preferred materials for the fabrication of NPs. They are made up of naturally occurring polymers containing amide, ester, and ether functional groups which can be broken down by the biological mechanisms in the body without the generation of toxic residues (Sivasankarapillai et al. 2021). A few examples are mentioned in Table 5.2.

### 5.3.4 Nanocarriers for Targeted Drug Delivery in Ovarian Cancer Therapy

Various types of nanocarriers, including metallic NPs, polymeric NPs, liposomes, solid-lipid NPs, micelles, and others, have been reported for effective management of OC. These nanocarriers due to their versatile size, shape, and surface charge assist the targeted delivery of loaded drugs/bioactive to the targeted diseases to the site rather than affecting the healthy cells or tissues, thus reducing the chances of systemic toxicity.

#### 5.3.4.1 Nanoparticles

Various metallic elements like gold, silver, and metallic oxides, especially Iron oxide NPs, have been explored to fabricate NPs (Horák et al. 2017). Iron oxide NPs have been shown to exhibit significant cytotoxic effects. The in vitro study performed on OC cell lines found an increased level of ROS, resulting in damaging the mitochondrial membrane of cancer cells. This resulted in the caspase-mediated apoptosis in the OC PA-1 cells (Ramalingam et al. 2020). The success of such systems is attributed to the superparamagnetic characteristic of iron oxide that renders enhanced Spatio-temporal control over drug delivery (Zhi et al. 2020).

Chitosan is another biopolymer that due to its low toxicity and biocompatibility characteristics has found its way to the fabrication of NPs. Several amine and hydroxyl groups in their structure mean that they can be effective sites of associations with therapeutic moieties (Muddineti et al. 2017). NPs fabricated using chitosan and conjugated with proteins, drugs, and nucleic acids have exhibited

**Table 5.2** List of some major biodegradable polymers used in the fabrication of novel drug delivery systems used in the management of ovarian cancer

| Natural biodegradable polymers | | | |
|---|---|---|---|
| Polymers | Structure | Examples | Reference |
| Chitosan | Units of D-glucosamide and N-acetyl-D-glucosamide | Nanogel composed of poly (ethylene glycol)-b-poly(L-glutamic acid)-b-poly(L-phenylalanine) copolymer block incorporating cisplatin and paclitaxel; surface decorated with a folic acid ligand using PEG as a spacer. | Desale et al. (2015) |
| Proteins | Chains of amino acids of 20–30 residues | Albumin, collagen, gelatine<br>Albumin NPs incorporating Albendazole | Zhang (2008)<br>Noorani et al. (2015) |
| Polysaccharides | Polymeric carbohydrate molecule composed of monosaccharides | Cellulose, starch, glycogen | Varki et al. (2015) |
| Synthetic biodegradable polymers | | | |
| Poly (lactic-co-glycolic) acid (PLGA) | A dimer of lactic acid and glycolic acid in varying ratios results in different drug release profiles. | Lupron depot developed for prostate cancer | Astete and Sabliov (2006) |
| Polylactic acid (PLA) | A polyester structure obtained from the condensation of lactic acid monomers | Chitosan-coated Polylactic acid nanoparticle | Babu et al. (2014) |
| Polycaprolactone (PCL) | A polyester formed by polymerization of ε-caprolactone | Tumor-targeting polycaprolactone NPs with codelivery of paclitaxel and IR780 for combinational therapy of drug-resistant OC | Pan et al. (2020) |
| Polyhydroxyalkanoates (PHA) | A class of biopolyester consisting of various side chains and fatty acids | Poly(3-hydroxybutyrate-co-3-hydroxy valerate) or PHBV NPs loaded with paclitaxel show anti-OC activity | Vilos et al. (2013) |

enhanced localization at cancerous sites and taken up by cells through endocytosis among other internalization phenomena. In a bid to explore the possibility of employing biodegradable photoresponsive NPs in the treatment of OC, chitosan and PLGA-based NPs were designed and loaded with carboplatin (CP) and the near-infrared (NIR) photosensitizer indocyanine green (ICG). The study found effective cytotoxic effects against OC cell lines when the target cells were irradiated with 800 nm laser (Sánchez-Ramírez et al. 2020).

Another nanoparticulate drug delivery system fabricated from PLGA and loaded with Cannabidiol (CBD) has been shown to significantly induce apoptosis by inducing the expression PARP. The drug-loaded nanoparticle was found to have a high encapsulation efficiency of 95% and a drug release lasting about 96 h. The internalization of the NPs into the OC cell line was found to be between 2 and 4 h. Although CBD-NPs showed a tumor growth inhibition similar to CBD in solution, the IC50 value of CBD-NPs was found to be significantly lower than that of CBD solution (Fraguas-Sánchez et al. 2020).

#### 5.3.4.2 Liposomes

Liposomes are nanoparticulate drug delivery systems in the size range of 2 to 400 nm. They are composed of lipid materials that are easily biodegradable and generally nontoxic to the patient's body. Liposomes show high encapsulation efficiency for both hydrophilic and lipophilic pharmaceuticals while also showing Spatio-temporal control. The circulation time of liposomes is of prime importance when it comes to their efficacy. PEG-ylation is a well-explored technique to do the same. A study with PEG-ylated liposomal loaded with paclitaxel observed that the liposomal drug delivery system was able to contain the growth of OC cells both in vitro and in vivo by inducing their apoptosis by TNF-mediated ERK/AKT signaling (Alizadeh et al. 2020).

In a study involving cisplatin-resistant OC cells, Cisplatin-loaded liposomal particles functionalized to target transferrin receptors were designed. Their cell uptake in both resistant and nonresistant cells was found to be similar, but significantly better than that of free cisplatin. Cytotoxicity was related to the accumulation of platinum in both resistant and nonresistant cancer cells when treated with targeting liposomes. However, Tfr targeting was not found to have any significant advantage over nontargeting liposomes (İnce et al. 2020).

Often the idea of co-encapsulating two or more drugs in the liposome gains research attractions for their ability to synergistically act on the cancerous cells. Such a system was designed by encapsulating a topoisomerase inhibitor, Irinotecan, and a DNA transcription inhibitor through various mechanisms, Doxorubicin (in the ratio 1:1 in circulation), by the process of coordination complexation and pH gradient. The encapsulation efficiency was found to be more than 80%. This liposomal formulation was delivered in a xenograft model intraperitoneally growing a human ovarian tumor and found to have effective therapeutic potentiality (Qi et al. 2018).

#### 5.3.4.3 Nanomicelles

Micelles are formed as self-aggregating amphiphilic structures in an aqueous medium above a certain concentration called the critical micellar concentration. Like liposomes, Nanomicelles can also be loaded with hydrophilic and hydrophobic pharmaceuticals. Nanomicelles have been shown to exhibit higher drug loading capacity than other drug delivery systems (Krieger et al. 2010; Shaikh et al. 2013). Small size and effective penetrability and endocytosis at OC sites, accompanied by their unique characteristics like in vivo stability, biocompatibility, long plasma

circulation time, and site-specific action, have made them a quite sought-after candidate for OC therapeutics (Yu et al. 2018; Fathi et al. 2018).

In a study, Paclitaxel-loaded redox-sensitive nanomicelles were designed to target OC cells. Cytotoxicity studies revealed the therapeutic potential of the system (Pantshwa et al. 2020).

Similar effective cytotoxic effects were observed for a nanomicellar system loaded with Docetaxel and functionalized to target folate receptors on OC sites (Feng et al. 2014). A nanomicellar drug delivery system designed to incorporate two synergistically acting drugs has shown promising results. The study fabricated the nanomicelles from an amphiphilic copolymer, phenylboronic acid-coated methoxy poly (ethylene glycol)- *block*-poly-((N-2-hydroxyethyl)-aspartamide). The stability of the system was attributed to an electron donor-acceptor mechanism between the polymer particles and the encapsulated drugs doxorubicin and irinotecan. The nanomicelles were found to be biocompatible, while also affording significant cytotoxicity by the production of ROS. Apart from this, various functionalized nanocarriers have been listed in Table 5.3.

## 5.4 Conclusion and Future Perspectives

In western countries, OC has been reported as the second most common and the most fatal gynaecologic malice. Till now, there is a deficiency of approaches suggested for screening and early diagnosis of the disease, and if left untreated then at the final-stage OC is incurable in most of the clinical cases. In respect of treatment approaches, several conventional therapies, surgical technologies, and modern therapeutic regimes, entering the preclinical/clinical stages, have led to hope for the effective treatment of OC. In recent times, researchers have been able to develop potential nanocarriers for the establishment of targeted drug delivery systems to transport therapeutic regimens into ovarian carcinogenic cells with more precision. In this review, we have highlighted the outcomes and major findings of the recent articles and clinical trials associated with OC therapy and also have highlighted the significant impact of NPs-based drug delivery systems in OC therapeutics.

Studies have shown that as compared to other routes of administration, the intraperitoneal might be more beneficial for effectively targeting the folate-receptor in OC in vivo. Moreover, the amalgamation of intraperitoneal administration and targeting of the functionalized (peptides, antibodies, and aptamers)-NPs to the tumor-specific proteins might lead to decreased systemic toxicities and also increase the drug payload within the tumors. Apart from this, certain nanomaterial-mediated biosensors, due to superior sensitivity and selectivity, have exhibited a potential role in OC diagnosis. In the future, it is very important to explore the diverse aspects of NPs-based technologies and study their effects in improved tumor targeting, drug efficiency, and toxicity profiles.

**Table 5.3** Various functionalized nanocarriers for targeting chemotherapeutic agents in ovarian cancer therapy

| Functionalized Nanocarriers | Examples | Major outcomes/ mechanism | Reference |
|---|---|---|---|
| Nanoparticles (metallic/ polymeric functionalized with biodegradable polymers) | Chitosan-silicon dioxide nanoparticle ($SiO_2$-CS) loaded with epigallocatechin gallate (EGCG) | Aptamer conjugation resulted in effective internalization into OC cell lines through nucleolin recognition | Mutlu-Agardan et al. (2020) |
| | NPs functionalized with radio-iodinated folic acid | Showed improved targeting of SCOV3 cancer cell lines when compared to free thymoquinone | Zhou et al. (2021) |
| Dendrimers (regularly branched spherical system able to load drugs either attached to the branches or internalized) | Cisplatin-dendrimer conjugate | 33% reduction in ovarian tumor size; relative to the control group which was administered free cisplatin; at the highest tolerable dose of 6 mg/ kg. | Kirkpatrick et al. (2011) |
| | Cisplatin-paclitaxel co-encapsulated telodendrimer micelles | Co-encapsulation was done through the introduction of carboxylic acid and cholic acid functional groups in the layers of the dendrimer. The highest in vitro antitumor activity was observed at 1: 2 PTX/CDDP loading ratio. Corresponding results were observed in xenograft mouse model studies including demonstration of efficient targeted drug delivery and lesser side effects. | Cai et al. (2015) |
| | Folate targeted polyurea dendrimer for delivery of L-buthionine sulfoxamine (L-BSO) | Effective inhibition of glutathione (GSH) (usually responsible for chemoresistance) by the designed dendrimer while also reducing the systemic toxicity of L-BSO by targeted delivery. | Cruz et al. (2020) |

(continued)

**Table 5.3** (continued)

| Functionalized Nanocarriers | Examples | Major outcomes/ mechanism | Reference |
|---|---|---|---|
| | Polyelectrolyte nanocapsules loaded with Lapatinib and paclitaxel | In vitro studies on multidrug-resistant OC cell lines yielded promising results for inhibition of cell growth. | Vergara et al. (2012) |
| Nanocapsules (a vesicular membrane-covered system having a core to load drugs while the targeting ligands or antibodies can be attached to the outer membrane) | Paclitaxel-loaded SLNs composed of trimyristin (TM) as a solid lipid core and egg phosphatidylcholine and pegylated phospholipid as stabilizers. | OVCAR-3 human OC cell line and the MCF-7 breast cancer cell line with paclitaxel-loaded SLNs yielded cytotoxicity comparable to those of a commercially available Cremophor EL-based paclitaxel formulation | Lee et al. (2007) |
| Solid lipid nanoparticles (SLNs) (polymeric colloidal nanoparticles composed of lipids and surfactants) | Verteporfin, a photosensitizer encapsulated nanostructured lipid carrier (NLC) for targeted photodynamic therapy | In vitro and in vivo studies demonstrated effective targeting of cancer cells while also affording a long circulation time for NLC, thus being more effective than conventionally administered free verteporfin. Laser light exposure of tumors after intravenous administration of NLC-verteporfin ($8\ mg{\cdot}kg^{-1}$) significantly inhibited tumor growth without visible toxicity; unlike free verteporfin which exhibited severe phototoxic adverse reactions | Michy et al. (2019) |
| | Paclitaxel-loaded solid lipid microparticles | While affording a sustained release profile in peritoneum as observed through pharmacokinetic studies on Wistar rats, the in vitro studies on SKOV-3 OC cells demonstrated strong cytotoxic activity when compared to Taxol®. | Han et al. (2019) |

## References


Alizadeh L, Alizadeh E, Zarebkohan A, Ahmadi E, Rahmati-Yamchi M, Salehi R (2020) AS1411 aptamer-functionalized chitosan-silica nanoparticles for targeted delivery of epigallocatechin gallate to the Skov-3 ovarian cancer cell lines. J Nanopart Res 22(1):9123. https://doi.org/10.1007/s11051-019-4735-7

Amini A, Masoumi Moghaddam S, Morris DL, Pourgholami MH (2012) Utility of vascular endothelial growth factor inhibitors in the treatment of ovarian cancer: from concept to application. J Oncol 2012:540791. https://doi.org/10.1155/2012/540791

Astete CE, Sabliov CM (2006) Synthesis and characterization of PLGA nanoparticles. J Biomater Sci Polym Ed 17(3):247–289. https://doi.org/10.1163/156856206775997322

AstraZeneca (2016, December-2021, January) Olaparib tablets as a treatment for ovarian cancer subjects with different hrd tumor status. Identifier NCT02983799. https://clinicaltrials.gov/ct2/show/NCT02983799?term=olaparib &recrs=e&cond=Ovarian+Cancer&draw=2&rank=5. Accessed on 14 Jan, 2022

Babu A, Wang Q, Muralidharan R, Shanker M, Munshi A, Ramesh R (2014) Chitosan coated polylactic acid nanoparticle-mediated combinatorial delivery of cisplatin and siRNA/plasmid DNA chemosensitizes cisplatin-resistant human ovarian cancer cells. Mol Pharm [Internet] 11(8):2720–2733. https://doi.org/10.1021/mp500259e. Accessed on 28, Nov, 2021

Barkat HA, Barkat MA, Taleuzzaman M, Das SS, Rizwanullah M, Hadi HA (2021) Receptor-based combinatorial nanomedicines: a new Hope for cancer management. In: Kumar S, Rizvi MA, Verma S (eds) Handbook of research on advancements in cancer therapeutics. IGI Global, pp 339–355. https://doi.org/10.4018/978-1-7998-6530-8.ch011

Barkat HA, Das SS, Barkat MA, Beg S, Hadi HA (2020) Selective targeting of cancer signaling pathways with nanomedicines: challenges and progress. Future Oncol 16(35):2959–2979. https://doi.org/10.2217/fon-2020-0198

Barlin JN, Dao F, Bou Zgheib N, Ferguson SE, Sabbatini PJ, Hensley ML, Bell-McGuinn KM, Konner J, Tew WP, Aghajanian C, Chi DS (2012) Progression-free and overall survival of a modified outpatient regimen of primary intravenous/intraperitoneal paclitaxel and intraperitoneal cisplatin in ovarian, fallopian tube, and primary peritoneal cancer. Gynecol Oncol 125(3):621–624. https://doi.org/10.1016/j.ygyno.2012.03.027

Bartlett DW, Davis ME (2007) Physicochemical and biological characterization of targeted, nucleic acid-containing nanoparticles. Bioconjug Chem 18(2):456–468. https://doi.org/10.1021/bc0603539

Bast RC Jr, Feeney M, Lazarus H, Nadler LM, Colvin RB, Knapp RC (1981) Reactivity of a monoclonal antibody with human ovarian carcinoma. J Clin Invest 68(5):1331–1337. https://doi.org/10.1172/jci110380

Becker DA, Leath CA 3rd, Walters-Haygood CL, Smith BQ, Bevis KS (2019) Utilization of an alternative docetaxel-based intraperitoneal chemotherapy regimen in patients with ovarian, fallopian tube or primary peritoneal carcinoma: a continued need for ovarian cancer patients. Am J Clin Oncol 42(1):12–16. https://doi.org/10.1097/COC.0000000000000468

Béduneau A, Saulnier P, Hindré F, Clavreul A, Leroux JC, Benoit JP (2007) Design of targeted lipid nanocapsules by conjugation of whole antibodies and antibody Fab' fragments. Biomaterials 28(33):4978–4990. https://doi.org/10.1016/j.biomaterials.2007.05.014

Bharadwaj P, Das SS, Beg S, Rahman M (2021) Formulation and biological stability of nanomedicines in cancer treatment. In: Beg S, Choudhry H, Ahmad FJ, Rahman M, Souto EB (eds) Nanoformulation strategies for cancer treatment-micro and Nano Technologies. Elsevier, pp 277–289. https://doi.org/10.1016/C2019-0-01815-8

Burger RA (2011) Overview of anti-angiogenic agents in development for ovarian cancer. Gynecol Oncol 121(1):230–238. https://doi.org/10.1016/j.ygyno.2010.11.035

Cai L, Xu G, Shi C, Guo D, Wang X, Luo J (2015) Telodendrimer nanocarrier for co-delivery of paclitaxel and cisplatin: a synergistic combination nanotherapy for ovarian cancer treatment. Biomaterials 37:456–468. https://doi.org/10.1016/j.biomaterials.2014.10.044

Chan JK, Brady W, Monk BJ, Brown J, Shahin MS, Rose PG, Kim JH, Secord AA, Walker JL, Gershenson DM (2018) A phase II evaluation of sunitinib in the treatment of persistent or recurrent clear cell ovarian carcinoma: an NRG oncology/Gynecologic oncology group study (GOG-254). Gynecol Oncol 150(2):247–252. https://doi.org/10.1016/j.ygyno.2018.05.029

Chen M, Jin Y, Bi Y, Yin J, Wang Y, Pan L (2014) A survival analysis comparing women with ovarian low-grade serous carcinoma to those with high-grade histology. Onco Targets Ther 7: 1891–1899. https://doi.org/10.2147/OTT.S67812

Chenjie X, Yuan Z, Kohler N, Kim J, Chung MA, Sun S (2009) J Am Chem Soc 131(42): 15346–15351. https://doi.org/10.1021/ja905938a

Cirstoiu-Hapca A, Buchegger F, Lange N, Bossy L, Gurny R, Delie F (2010) Benefit of anti-HER2-coated paclitaxel-loaded immuno-nanoparticles in the treatment of disseminated ovarian cancer: therapeutic efficacy and biodistribution in mice. J Control Release 144(3):324–331. https://doi.org/10.1016/j.jconrel.2010.02.026

Cruz AF, Caleiras MB, Fonseca NA, Gonçalves N, Mendes VM, Sampaio SF, Moura V, Melo JB, Almeida RD, Manadas B, Simões S, Moreira JN (2021) The enhanced efficacy of intracellular delivery of doxorubicin/C6-ceramide combination mediated by the F3 peptide/Nucleolin system is supported by the downregulation of the PI3K/Akt pathway. Cancers 13(12):3052. https://doi.org/10.3390/cancers13123052

Cruz A, Mota P, Ramos C, Pires RF, Mendes C, Silva JP, Nunes SC, Bonifácio VDB, Serpa J (2020) Polyurea dendrimer folate-targeted Nanodelivery of l-Buthionine sulfoximine as a tool to tackle ovarian cancer Chemoresistance. Antioxidants (Basel) 9(2):133. https://doi.org/10.3390/antiox9020133

Danhier F, Feron O, Préat V (2010) To exploit the tumor microenvironment: passive and active tumor targeting of nanocarriers for anti-cancer drug delivery. J Control Release 148(2): 135–146. https://doi.org/10.1016/j.jconrel.2010.08.027

Das SS, Alkahtani S, Bharadwaj P, Ansari MT, ALKahtani MDF, Pang Z, Hasnain MS, Nayak AK, Aminabhavi TM (2020b) Molecular insights and novel approaches for targeting tumor metastasis. Int J Pharm 30(585):119556. https://doi.org/10.1016/j.ijpharm.2020.119556

Das PM, Bast RC Jr (2008) Early detection of ovarian cancer. Biomark Med 2(3):291–303. https://doi.org/10.2217/17520363.2.3.291

Das SS, Bharadwaj P, Bilal M, Barani M, Rahdar A, Taboada P, Bungau S, Kyzas GZ (2020c) Stimuli-responsive polymeric Nanocarriers for drug delivery, imaging, and Theragnosis. Polymers (Basel) 12(6):1397. https://doi.org/10.3390/polym12061397

Das SS, Hussain A, Verma PRP, Imam SS, Altamimi MA, Alshehri S, Singh SK (2020d) Recent advances in liposomal drug delivery system of quercetin for cancer targeting: a mechanistic approach. Curr Drug Deliv 17(10):845–860. https://doi.org/10.2174/1567201817666200415112657

Das SS, Verma PRP, Kar S, Singh SK (2020a) Quercetin-loaded nanomedicine as oncotherapy. In: Rahman M, Beg S, Kumar V, Ahmad FJ (eds) Nanomedicine for bioactives: healthcare applications, 1st edn. Springer, Singapore, pp 155–183. https://doi.org/10.1007/978-981-15-1664-1_5

Desale SS, Soni KS, Romanova S, Cohen SM, Bronich TK (2015) Targeted delivery of platinum-taxane combination therapy in ovarian cancer. J Control Release 220(Pt B):651–659. https://doi.org/10.1016/j.jconrel.2015.09.007

Drugs@FDA: FDA-Approved Drugs (n.d.-a) US-FDA https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm?event=overviewprocess&ApplNo=125418. Accessed on 14 Jan, 2022

Drugs@FDA: FDA-Approved Drugs (n.d.-b) US-FDA https://www.accessdata.fda.gov/drugsatfda_docs/label/2005/021923lbl.pdf. Accessed on 14 Jan, 2022

Drugs@FDA: FDA-Approved Drugs (n.d.-c) US-FDA https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm?event=BasicSearch. process. Accessed on 14 Jan, 2022

Ducie J, Dao F, Considine M, Olvera N, Shaw PA, Kurman RJ, Shih IM, Soslow RA, Cope L, Levine DA (2017) Molecular analysis of high-grade serous ovarian carcinoma with and without

associated serous tubal intra-epithelial carcinoma. Nat Commun 8(1):990. https://doi.org/10.1038/s41467-017-01217-9

Elit L, Oliver TK, Covens A, Kwon J, Fung MF, Hirte HW, Oza AM (2007) Intraperitoneal chemotherapy in the first-line treatment of women with stage III epithelial ovarian cancer: a systematic review with metaanalyses. Cancer 109(4):692–702. https://doi.org/10.1002/cncr.22466

Fathi M, Majidi S, Zangabad PS, Barar J, Erfan-Niya H, Omidi Y (2018) Chitosan-based multifunctional nanomedicines and theranostics for targeted therapy of cancer. Med Res Rev 38(6):2110–2136. https://doi.org/10.1002/med.21506

FDA approves ramucirumab plus erlotinib for first-line metastatic NSCLC (n.d.) US-FDA https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-ramucirumab-plus-erlotinib-first-line-metastatic-nsclc. Accessed on 14 Jan, 2022

Feng ST, Li J, Luo Y, Yin T, Cai H, Wang Y, Dong Z, Shuai X, Li ZP (2014) pH-sensitive nanomicelles for controlled and efficient drug delivery to human colorectal carcinoma LoVo cells. PLoS One 9(6):e100732. https://doi.org/10.1371/journal.pone.0100732

Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Piñeros M, Znaor A, Bray F (2019) Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int J Cancer 144(8):1941–1953. https://doi.org/10.1002/ijc.31937

Fraguas-Sánchez AI, Torres-Suárez AI, Cohen M, Delie F, Bastida-Ruiz D, Yart L, Martin-Sabroso C, Fernández-Carballido A (2020) PLGA nanoparticles for the intraperitoneal administration of CBD in the treatment of ovarian cancer: in vitro and in Ovo assessment. Pharmaceutics [Internet] MDPI AG 12(5):439. https://doi.org/10.3390/pharmaceutics12050439

Garbuzenko O, Barenholz Y, Priev A (2005) Effect of grafted PEG on liposome size and on compressibility and packing of lipid bilayer. Chem Phys Lipids 135(2):117–129. https://doi.org/10.1016/j.chemphyslip.2005.02.003

Han S, Dwivedi P, Mangrio FA, Dwivedi M, Khatik R, Cohn DE, Si T, Xu RX (2019) Sustained release paclitaxel-loaded core-shell-structured solid lipid microparticles for intraperitoneal chemotherapy of ovarian cancer. Artif Cells Nanomed Biotechnol 47(1):957–967. https://doi.org/10.1080/21691401.2019.1576705

Harter P, Sehouli J, Reuss A, Baumann K, Hanker L, Kimmig R, Schröder W, Burges A, Gropp-Meier M, Kurzeder C, Mahner S, Canzler U, Lück HJ, Meier W, Fehm T, du Bois A (2016) Phase II study evaluating PegLiposomal doxorubicin and carboplatin combination chemotherapy in Gynecologic sarcomas and mixed epithelial-mesenchymal Tumors a phase II protocol of the Arbeitsgemeinschaft Gynaekologische Onkologie study group (AGO-GYN 7). Int J Gynecol Cancer 26(9):1636–1641. https://doi.org/10.1097/IGC.0000000000000831

Horák D, Pustovyy VI, Babinskyi AV, Palyvoda OM, Chekhun VF, Todor IN, Kuzmenko OI (2017) Enhanced antitumor activity of surface-modified iron oxide nanoparticles and an α-tocopherol derivative in a rat model of mammary gland carcinosarcoma. Int J Nanomedicine 12:4257–4268. https://doi.org/10.2147/IJN.S137574

Illum L, Davis SS, Müller RH, Mak E, West P (1987) The organ distribution and circulation time of intravenously injected colloidal carriers sterically stabilized with a block copolymer–poloxamine 908. Life Sci 40(4):367–374. https://doi.org/10.1016/0024-3205(87)90138-x

İnce İ, Yıldırım Y, Güler G, Medine Eİ, Ballıca G, Kuşdemir BC et al (2020) Synthesis and characterization of folic acid-chitosan nanoparticles loaded with thymoquinone to target ovarian cancer cells. J Radioanal Nucl Chem 324(1):71–85. https://doi.org/10.1007/s10967-020-07058-z

Jacobs IJ, Menon U (2004) Progress and challenges in screening for early detection of ovarian cancer. Mol Cell Proteomics 3(4):355–366. https://doi.org/10.1074/mcp.R400006-MCP200

Janssen M, Oyen WJ, Massuger LF, Frielink C, Dijkgraaf I, Edwards DS, Radjopadhye M, Corstens FH, Boerman OC (2002) Comparison of a monomeric and dimeric radiolabeled RGD-peptide for tumor targeting. Cancer Biother Radiopharm 17(6):641–646. https://doi.org/10.1089/108497802320970244

Kasper B, Hohenberger P (2011) Pazopanib: a promising new agent in the treatment of soft tissue sarcomas. Future Oncol 7(12):1373–1383. https://doi.org/10.2217/fon.11.116

Katchman BA, Chowell D, Wallstrom G, Vitonis AF, LaBaer J, Cramer DW, Anderson KS (2017) Autoantibody biomarkers for the detection of serous ovarian cancer. Gynecol Oncol 146(1): 129–136. https://doi.org/10.1016/j.ygyno.2017.04.005

Kirkpatrick GJ, Plumb JA, Sutcliffe OB, Flint DJ, Wheate NJ (2011) Evaluation of anionic half generation 3.5-6.5 poly(amidoamine) dendrimers as delivery vehicles for the active component of the anticancer drug cisplatin. J Inorg Biochem 105(9):1115–1122. https://doi.org/10.1016/j.jinorgbio.2011.05.017

Kok PS, Beale P, O'Connell RL, Grant P, Bonaventura T, Scurry J, Antill Y, Goh J, Sjoquist K, De Fazio A, Mapagu C, Amant F, Friedlander M (2019) PARAGON investigators. PARAGON (ANZGOG-0903): a phase 2 study of anastrozole in asymptomatic patients with estrogen and progesterone receptor-positive recurrent ovarian cancer and CA125 progression. J Gynecol Oncol 30(5):e86. https://doi.org/10.3802/jgo.2019.30.e86

Krieger ML, Eckstein N, Schneider V, Koch M, Royer HD, Jaehde U, Bendas G (2010) Overcoming cisplatin resistance of ovarian cancer cells by targeted liposomes in vitro. Int J Pharm 389(1–2):10–17. https://doi.org/10.1016/j.ijpharm.2009.12.061

Kurman RJ, IeM S (2010) The origin and pathogenesis of epithelial ovarian cancer: a proposed unifying theory. Am J Surg Pathol 34(3):433–443. https://doi.org/10.1097/PAS.0b013e3181cf3d79

Lee MK, Lim SJ, Kim CK (2007) Preparation, characterization and in vitro cytotoxicity of paclitaxel-loaded sterically stabilized solid lipid nanoparticles. Biomaterials 28(12): 2137–2146. https://doi.org/10.1016/j.biomaterials.2007.01.014

McGuire WP, Hoskins WJ, Brady MF, Kucera PR, Partridge EE, Look KY, Clarke-Pearson DL, Davidson M (1996) Cyclophosphamide and cisplatin compared with paclitaxel and cisplatin in patients with stage III and stage IV ovarian cancer. N Engl J Med 334(1):1–6. https://doi.org/10.1056/NEJM199601043340101

Michy T, Massias T, Bernard C, Vanwonterghem L, Henry M, Guidetti M, Royal G, Coll JL, Texier I, Josserand V, Hurbin AA (2019) Verteporfin-loaded lipid nanoparticles improve ovarian cancer photodynamic therapy in vitro and in vivo. Cancers (Basel) 11(11):1760. https://doi.org/10.3390/cancers11111760

Milane L, Duan Z, Amiji M (2011) Development of EGFR-targeted polymer blend nanocarriers for combination paclitaxel/lonidamine delivery to treat multi-drug resistance in human breast and ovarian tumor cells. Mol Pharm 8(1):185–203. https://doi.org/10.1021/mp1002653

Moore RG, Maclaughlan S (2010) Current clinical use of biomarkers for epithelial ovarian cancer. Curr Opin Oncol 22(5):492–497. https://doi.org/10.1097/CCO.0b013e32833c3351

Muddineti OS, Kumari P, Ray E, Ghosh B, Biswas S (2017 Jun) Curcumin-loaded chitosan-cholesterol micelles: evaluation in monolayers and 3D cancer spheroid model. Nanomedicine (Lond) 12(12):1435–1453. https://doi.org/10.2217/nnm-2017-0036

Mutlu-Agardan NB, Sarisozen C, Torchilin VP (2020) Cytotoxicity of novel redox sensitive $PEG_{2000}$-S-S-PTX micelles against drug-resistant ovarian and breast cancer cells. Pharm Res 37(3):65. https://doi.org/10.1007/s11095-020-2759-4

Noorani L, Stenzel M, Liang R, Pourgholami MH, Morris DL (2015) Albumin nanoparticles increase the anticancer efficacy of albendazole in ovarian cancer xenograft model. J Nanobiotechnology. 13:25. https://doi.org/10.1186/s12951-015-0082-8

Novartis Pharmaceuticals (2009, March–2013, December) Efficacy and safety of pazopanib monotherapy after first line chemotherapy in ovarian, fallopian tube, or primary peritoneal cancer. Identifier NCT00866697. https://clinicaltrials.gov/ct2/show/NCT00866697?term=pazopanib&cond=Ovarian+Cancer&draw=2&rank=9. Accessed on 14 Jan, 2022

Pan Q, Tian J, Zhu H, Hong L, Mao Z, Oliveira JM, Reis RL, Li X (2020) Tumor-targeting Polycaprolactone nanoparticles with Codelivery of paclitaxel and IR780 for combinational therapy of drug-resistant ovarian cancer. ACS Biomater Sci Eng 6(4):2175–2185. https://doi.org/10.1021/acsbiomaterials.0c00163

Pantshwa JM, Kondiah PPD, Choonara YE, Marimuthu T, Pillay V (2020) Nanodrug delivery Systems for the Treatment of ovarian cancer. Cancers (Basel). 12(1):213. https://doi.org/10.3390/cancers12010213

Pignata S, Cannella L, Leopardo D, Pisano C, Bruni GS, Facchini G (2011) Chemotherapy in epithelial ovarian cancer. Cancer Lett 303(2):73–83. https://doi.org/10.1016/j.canlet.2011.01.026

Prat J (2014) FIGO committee on Gynecologic oncology. Staging classification for cancer of the ovary, fallopian tube, and peritoneum. Int J Gynaecol Obstet 124(1):1–5. https://doi.org/10.1016/j.ijgo.2013.10.001

Qi Z, Yin L, Xu Y, Wang F (2018) Pegylated liposomal-paclitaxel induces ovarian cancer cell apoptosis via TNF-induced ERK/AKT signaling pathway. Mol Med Rep 17(6):7497–7504. https://doi.org/10.3892/mmr.2018.8811

Rai B, Bansal A, Patel FD, Sharma SC (2014) Radiotherapy for ovarian cancers–redefining the role. Asian Pac J Cancer Prev 15(12):4759–4763. https://doi.org/10.7314/apjcp.2014.15.12.4759

Ramalingam V, Mohan Harshavardhan S, Kumar D, Malathi S, devi. (2020) Wet chemical mediated hematite α-Fe2O3 nanoparticles synthesis: preparation, characterization and anticancer activity against human metastatic ovarian cancer. Journal of Alloys and Compounds 834:155118. https://doi.org/10.1016/j.jallcom.2020.155118

Sánchez-Ramírez DR, Domínguez-Ríos R, Juárez J, Valdés M, Hassan N, Quintero-Ramos A, Del Toro-Arreola A, Barbosa S, Taboada P, Topete A, Daneri-Navarro A (2020) Biodegradable photoresponsive nanoparticles for chemo-, photothermal- and photodynamic therapy of ovarian cancer. Mater Sci Eng C Mater Biol Appl 116:111196. https://doi.org/10.1016/j.msec.2020.111196

Santoiemma PP, Powell DJ Jr (2015) Tumor infiltrating lymphocytes in ovarian cancer. Cancer Biol Ther 16(6):807–820. https://doi.org/10.1080/15384047.2015.1040960

Sarojini S, Tamir A, Lim H, Li S, Zhang S, Goy A, Pecora A, Suh KS (2012) Early detection biomarkers for ovarian cancer. J Oncol 2012:709049. https://doi.org/10.1155/2012/709049

Shaaban AM, Rezvani M, Elsayes KM, Baskin H Jr, Mourad A, Foster BR, Jarboe EA, Menias CO (2014) Ovarian malignant germ cell tumors: cellular classification and clinical and imaging features. Radiographics 34(3):777–801. https://doi.org/10.1148/rg.343130067

Shaikh IM, Tan KB, Chaudhury A, Liu Y, Tan BJ, Tan BM, Chiu GN (2013) Liposome co-encapsulation of synergistic combination of irinotecan and doxorubicin for the treatment of intraperitoneally grown ovarian tumor xenograft. J Control Release 172(3):852–861

Sivasankarapillai V, Das SS, Sabir F, Sundaramahalingam M, Colmenares J, Prasannakumar S, Rajan M, Rahdar A, Kyzas G (2021) Progress in natural polymer engineered biomaterials for transdermal drug delivery systems. Materials Today Chemistry 19:100382. https://doi.org/10.1016/j.mtchem.2020.100382

Song H, Sgouros G (2011) Radioimmunotherapy of solid tumors: searching for the right target. Curr Drug Deliv 8(1):26–44. https://doi.org/10.2174/156720111793663651

Tayama S, Motohara T, Narantuya D, Li C, Fujimoto K, Sakaguchi I, Tashiro H, Saya H, Nagano O, Katabuchi H (2017) The impact of EpCAM expression on response to chemotherapy and clinical outcomes in patients with epithelial ovarian cancer. Oncotarget 8(27):44312–44325. https://doi.org/10.18632/oncotarget.17871

Tewari D, Java JJ, Salani R, Armstrong DK, Markman M, Herzog T, Monk BJ, Chan JK (2015) Long-term survival advantage and prognostic factors associated with intraperitoneal chemotherapy treatment in advanced ovarian cancer: a gynecologic oncology group study. J Clin Oncol 33(13):1460–1466. https://doi.org/10.1200/JCO.2014.55.9898

Tirelli N, Lutolf MP, Napoli A, Hubbell JA (2002) Poly(ethylene glycol) block copolymers. J Biotechnol 90(1):3–15. https://doi.org/10.1016/s1389-0352(01)00057-5

Torchilin VP (2006) Multifunctional nanocarriers. Adv Drug Deliv Rev 58(14):1532–1555. https://doi.org/10.1016/j.addr.2006.09.009

Ulbrich K, Hekmatara T, Herbert E, Kreuter J (2009) Transferrin- and transferrin-receptor-antibody-modified nanoparticles enable drug delivery across the blood-brain barrier (BBB). Eur J Pharm Biopharm 71(2):251–256. https://doi.org/10.1016/j.ejpb.2008.08.021

Uster PS, Allen TM, Daniel BE, Mendez CJ, Newman MS, Zhu GZ (1996) Insertion of poly (ethylene glycol) derivatized phospholipid into pre-formed liposomes results in prolonged in vivo circulation time. FEBS Lett 386(2–3):243–246. https://doi.org/10.1016/0014-5793(96)00452-8

van Vlerken LE, Vyas TK, Amiji MM (2007) Poly(ethylene glycol)-modified nanocarriers for tumor-targeted and intracellular delivery. Pharm Res 24(8):1405–1414. https://doi.org/10.1007/s11095-007-9284-6

Varki A, Cummings RD, Esko JD, Stanley P, Hart GW, Aebi M, et al. Essentials of Glycobiology (3rd). Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2015–2017. https://www.ncbi.nlm.nih.gov/books/NBK310274/

Vergara D, Bellomo C, Zhang X, Vergaro V, Tinelli A, Lorusso V, Rinaldi R, Lvov YM, Leporatti S, Maffia M (2012) Lapatinib/paclitaxel polyelectrolyte nanocapsules for overcoming multidrug resistance in ovarian cancer. Nanomedicine 8(6):891–899. https://doi.org/10.1016/j.nano.2011.10.014

Vergote I, du Bois A, Floquet A, Rau J, Kim JW, Del Campo JM, Friedlander M, Pignata S, Fujiwara K, Colombo N, Mirza MR, Monk BJ, Tsibulak I, Calvert PM, Herzog TJ, Hanker LC, Meunier J, Lee JY, Bologna A, Carrasco-Alfonso MJ, Harter P (2019) Overall survival results of AGO-OVAR16: a phase 3 study of maintenance pazopanib versus placebo in women who have not progressed after first-line chemotherapy for advanced ovarian cancer. Gynecol Oncol 155(2):186–191. https://doi.org/10.1016/j.ygyno.2019.08.024

Vilos C, Morales FA, Solar PA, Herrera NS, Gonzalez-Nilo FD, Aguayo DA, Mendoza HL, Comer J, Bravo ML, Gonzalez PA, Kato S, Cuello MA, Alonso C, Bravo EJ, Bustamante EI, Owen GI, Velasquez LA (2013) Paclitaxel-PHBV nanoparticles and their toxicity to endometrial and primary ovarian cancer cells. Biomaterials 34(16):4098–4108. https://doi.org/10.1016/j.biomaterials.2013.02.034

Wang P, Fang L (2021) Salpingo-oophorectomy versus cystectomy in patients with borderline ovarian tumors: a systemic review and meta-analysis on postoperative recurrence and fertility. World J Surg Oncol 19(1):132. https://doi.org/10.1186/s12957-021-02241-2

Wilken JA, Webster KT, Maihle NJ (2010) Trastuzumab sensitizes ovarian cancer cells to EGFR-targeted therapeutics. J Ovarian Res 3:7. https://doi.org/10.1186/1757-2215-3-7

Yu F, Jiang F, Tang X, Wang B (2018) N-octyl-N-arginine-chitosan micelles for gambogic acid intravenous delivery: characterization, cell uptake, pharmacokinetics, and biodistribution. Drug Dev Ind Pharm 44(4):615–623. https://doi.org/10.1080/03639045.2017.1405973

Yuan MQ, Zhu F, Lou JY, Yuan WM, Fu L, Liu S, Zhang ZZ, Liu CY, He Q (2014) The antitumoral efficacy of a docetaxel-loaded liposomal drug delivery system modified with transferrin for ovarian cancer. Drug Res (Stuttg) 64(4):195–202. https://doi.org/10.1055/s-0033-1355335

Zalipsky S, Mullah N, Harding JA, Gittelman J, Guo L, DeFrees SA (1997) Poly(ethylene glycol)-grafted liposomes with oligopeptide or oligosaccharide ligands appended to the termini of the polymer chains. Bioconjug Chem 8(2):111–118. https://doi.org/10.1021/bc9600832

Zhang Y (2008) Progress and challenges in protein structure prediction. Curr Opin Struct Biol 18(3):342–348. https://doi.org/10.1016/j.sbi.2008.02.004

Zhang XY, Chen J, Zheng YF, Gao XL, Kang Y, Liu JC, Cheng MJ, Sun H, Xu CJ (2009b) Follicle-stimulating hormone peptide can facilitate paclitaxel nanoparticles to target ovarian carcinoma in vivo. Cancer Res 69(16):6506–6514. https://doi.org/10.1158/0008-5472.CAN-08-4721

Zhang Z, Jia L, Feng Y, Zheng W (2009a) Overexpression of follicle-stimulating hormone receptor facilitates the development of ovarian epithelial cancer. Cancer Lett 278(1):56–64. https://doi.org/10.1016/j.canlet.2008.12.024

Zhi D, Yang T, Yang J, Fu S, Zhang S (2020) Targeting strategies for superparamagnetic iron oxide nanoparticles in cancer therapy. Acta Biomater 102:13–34. https://doi.org/10.1016/j.actbio.2019.11.027

Zhou M, Luo Y, Zeng W, Yang X, Chen T, Zhang L, He X, Yi X, Li Y, Yi X (2021) A co-delivery system based on a dimeric prodrug and star-shaped polymeric prodrug micelles for drug delivery. Front Chem 9:765021. https://doi.org/10.3389/fchem.2021.765021

# Pancreatic Cancer: Nanoparticle Targeted Therapy Via Epidermal Growth Factor Receptor

6

Vivek Dwivedi, Shahnawaz Sameem, Mahfoozur Rahman, Prateek Pathak, and Amita Verma

**Abstract**

Pancreatic cancer is the malignant neoplasm of pancreas and has the worst mortality rate with a 5-year survival rate of only 7%. Pancreatic cancer is tough to detect, hard to diagnose, and early to metastasize. Hence, by the time symptoms are diagnosed it may be at higher phases and could have been spread to other organs. Pancreatic cancer is a malignant neoplasm that arises from the cells of pancreas, a glandular organ made of both endocrine and exocrine cells behind the stomach. The role and significance of EGFR in the development and progression of human malignancies highlight the fact that EGFR blockade offers to be a promising therapeutic intervention strategy for cancer. The ligand or the targeting moiety which could be exploited for the development of an efficient targeted nanotherapeutics has to be chosen only after clear understanding about the various strategies which specifically target and block EGFR signaling cascade representing various strategies which have been exploited for targeting EGFR overexpressed on cancer cells as a therapeutic intervention approach for cancer treatment.

V. Dwivedi · S. Sameem · A. Verma (✉)
Bioorganic and Medicinal Chemistry Research Laboratory, Department of Pharmaceutical Sciences, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, India
e-mail: amita.verma@shiats.edu.in

M. Rahman
Department of Pharmaceutical Sciences, Faculty of Health Sciences, SHUATS, Allahabad, Uttar Pradesh, India

P. Pathak
Laboratory of Computational Modeling of Drugs, Higher Medical and Biological School, South Ural State University, Chelyabinsk, Russia



M. Rahman et al. (eds.), *Hormone Related Cancer Mechanistic and Nanomedicines*,
https://doi.org/10.1007/978-981-19-5558-7_6

**Keywords**

Pancreatic cancer · Nanoparticle · EGFR

## 6.1 Introduction

Pancreatic cancer is the malignant neoplasm of pancreas and has the worst mortality rate with a 5-year survival rate of only 7% (Siegel et al. 2015). Pancreatic cancer is tough to detect, hard to diagnose, and early to metastasize. Hence, by the time symptoms are diagnosed, it may be at higher phases and could have been spread to other organs. Nearly 52% of all patients are diagnosed when the cancer has already metastasized, leaving little hope for survival (Tingstedt et al. 2011). In general, the treatment options for pancreatic cancer include surgery, radiation therapy, and chemotherapy. However, chemotherapy is the only inevitable treatment modality when the cancer has advanced and has spread beyond the pancreas (Wood et al. 2022).

The chemotherapy drugs approved by FDA for treating pancreatic cancer include gemcitabine, 5-fluorouracil (5-FU), irinotecan, oxaliplatin, cisplatin, paclitaxel, etc. (Klatte et al. 2022). Although chemotherapy is useful for advanced cancers as it reaches all areas of the body through the bloodstream, it is always associated with several far-reaching side effects (Klatte et al. 2022). Some of the common side effects of chemo-drugs used against pancreatic cancer include fatigue, nausea, mouth sores, diarrhea, hair loss, hand/foot syndrome, loss of appetite, neuropathy, increased chance of infection, etc. (Klatte et al. 2022; Huang et al. 2022). The most commonly used chemo-drug, gemcitabine, causes specific side effects such as myelosuppression, pulmonary toxicity, hepatic toxicity, capillary leak syndrome, etc. (Hamidi-Sofiani et al. 2022). The adverse effects of chemotherapy depend largely on the type and dose of drug administered and length of the treatment (Hamidi-Sofiani et al. 2022). Therefore, any treatment modality having minimum side effects or that can reduce the therapeutic dose of chemo-drugs hold great promise for pancreatic cancer therapy.

New strategies are being explored to improve the treatment of pancreatic cancer, such as new chemotherapeutic agent, combination therapy, and potential drug delivery system. Among the potential new chemotherapeutic agent for pancreatic cancer therapy, drugs such as metformin, aspirin, chloroquine, etc. are gaining attention because of its long-established safety profile in clinics in addition to its low cost and experimentally proven anticancer effect (Li et al. 2009; Currie et al. 2009; Sadeghi et al. 2012; Wang et al. 2014; Cerullo et al. 2016; Tan et al. 2011; Bonifazi et al. 2010; Cavalla 2015). While considering the therapeutic perspective, in addition to the anticancer potential of a chemo-drug, its effective delivery is also a key issue to attain ideal efficacy. Recent advancement from preclinical studies using drug delivery systems holds great promise for improving pancreatic cancer therapy. Encapsulation of drugs into suitable nanocarrier can significantly enhance its anti-cancer effect by modifying the drug solubility, extending drug exposure time,

selectively delivering the drug to target, improving therapeutic outcome, reducing toxic effects, and also by decreasing drug resistance (Yu et al. 2010). For pancreatic cancer treatment, the most commonly investigated nanocarriers for drug delivery include nanoparticles, liposomes, and carbon nanotubes (Yu et al. 2010). Development of nanocarriers for delivering common chemotherapy drugs such as irinotecan and paclitaxel has led to improved survival benefits through combination approach in pancreatic cancer patients (Passero et al. 2016).

In the clinical scenario, combination therapy using two or more chemo-drugs is often prescribed in order to achieve the best outcome. FOLFIRINOX, a combination of 5-FU/leucovorin, irinotecan, and oxaliplatin, is generally used for the treatment of metastatic pancreatic cancer (Reni and Orsi 2022). Currently, drug-loaded nanoparticles have been utilized for combination therapy to elicit better anticancer effect, thereby reducing therapeutic dose and adverse side effects. Clinical trials conducted using drug-loaded nanocarriers in combination with other chemo-drugs showed a beneficial effect against pancreatic cancer. For instance, Abraxane® (albumin-bound paclitaxel nanoparticles) in combination with gemcitabine exhibited superior effects in clinical trials and has been used as a promising second-line treatment option for metastatic pancreatic cancer (Hosein et al. 2013). Onivyde®, a nano liposomal formulation of irinotecan, was recently approved by FDA as a combination regimen with 5-FU/leucovorin for gemcitabine-resistant metastatic pancreatic cancer (Zhang 2016). Even though the combination therapy improves overall survival, the patient might experience added side effects due to the overburden of toxic drugs involved in the combination regimen.

Metformin is a well-accepted and FDA-approved drug for treating Type II diabetes, an increasingly common metabolic disorder which has also been listed as a risk factor and a potential consequence of pancreatic cancer (Wang et al. 2003). The prevalence of diabetes in 50–80% of patients diagnosed with pancreatic cancer further emphasizes the clinical potential of metformin as a dual-therapy for pancreatic cancer and associated diabetes (Permert et al. 1993; Wakasugi et al. 2001; Pannala et al. 2008). The potential of metformin is widely investigated for repurposing it as an anticancer agent against pancreatic cancer. Studies from pharmacy and disease database show decreased cancer incidence in individuals taking metformin, emphasizing the cancer preventive effect of metformin (Lee et al. 2012; Zhang et al. 2011; Wright and Stanford 2009; Bodmer et al. 2011; Donadon et al. 2010; Donadon et al. 2009). Studies analyzing the influence of metformin in pancreatic cancer cells revealed that the drug effectively decreases insulin/insulin growth factor (IGF) signaling, disrupts mitochondrial respiration, and inhibits the mammalian target of rapamycin (mTOR) pathway. The antineoplastic effects of metformin also include its ability to downregulate specific protein transcription factors and associated genes, alter miRNAs, reduce proliferation of cancer stem cell, and decrease DNA damage and inflammation (Karnevi et al. 2013; Nair et al. 2013; Rozengurt et al. 2010; Gou et al. 2013; Lonardo et al. 2013; Mohammed et al. 2013).

However, one of the major disadvantages of metformin is its fast renal clearance with an elimination half-life of 6.2 h from plasma (RIOMET® (metformin

hydrochloride oral solution) n.d.; Sexton et al. 2022). Hence, it is required to have longer sustainable therapeutic time to elicit a better therapeutic response. Nanoparticles (NPs)-mediated drug-delivery system can be an effective and radical new approach for delivering metformin for its application against pancreatic cancer. Nano-encapsulation of metformin can provide extended circulation time and controlled release of metformin, thereby reducing drug clearance. In addition, the better in vivo retention of metformin nanoparticles might also improve the antineoplastic effect of metformin towards pancreatic cancer cells, minimizing the therapeutic dose required. The advantages of combination therapy and the better outcome from the combinatorial chemotherapeutic regimen urged clinical trials to assess the combinatorial effect of metformin with chemo-drug towards pancreatic cancer (Kordes et al. 2015; Braghiroli et al. 2015; Reni et al. 2016). There have been no prior studies of nano-encapsulated metformin used as a combination therapy for pancreatic cancer. Hence, the current clinical scenario demands combinatorial effect assessment of metformin nanoparticles with other standard chemo-drugs to evaluate its potential as an adjuvant to chemotherapy. Hence, we propose that nanoparticles drug delivery system encapsulated with metformin, a clinically safe antidiabetic drug with potent anticancer effect, can be a promising supplement to the existing chemotherapy for better management of pancreatic cancer with minimum side effects. In addition, the metformin-encapsulated nanoparticles with better in vivo retention than the conventional free metformin can also have its application as a prospective antidiabetic therapy.

## 6.2 Pancreatic Cancer

Pancreatic cancer is a malignant neoplasm that arises from the cells of pancreas, a glandular organ made of both endocrine and exocrine cells behind the stomach. The pancreatic cancer cells which grow, divide, and spread uncontrollably form a malignant tumor. Although cancer can occur from either exocrine or endocrine cells of pancreas, the majority of pancreatic cancer starts in the exocrine cells contributing almost 95% of cases (pancreatic adenocarcinomas) (Sexton et al. 2022). Pancreatic cancer is referred as a "silent cancer" as its early symptoms remain unrecognized and it quickly spreads to other organs or tissues before it is being diagnosed (Gugenheim et al. 2022). Only 10–15% of pancreatic cancers are diagnosed at an early stage when it is limited only to the pancreas (Heinemann et al. 2022). In 2016, pancreatic cancer became the third leading cause of cancer-related mortality in U.S surpassing breast cancer (Goldberg 2022). According to the GLOBOCAN 2012 estimates, pancreatic cancer is responsible for more than 3,31,000 annual deaths worldwide (Kalthoff 2022). Despite years of research, the survival advances for pancreatic cancer have been slow in contrast to most cancers where there is a steady increase in survival. Currently, the 5-year relative survival for pancreatic cancer is 8% in U.S, as estimated by the American Cancer Society (Siegel et al. 2017). According to the latest estimates in India, the annual pancreatic cancer

load was approximately 17,000 and has predicted to increase in future (Gopinath 2015).

### 6.2.1 Treatment Options for Pancreatic Cancer

Based on the type and stage of the disease, treatment options for pancreatic cancer include surgery, ablation or embolization treatment, radiation therapy, and chemotherapy (Kalthoff 2022). Among them, the only potential curative therapy for pancreatic cancer is surgery. However, only 20% patients are eligible for surgery because most patients are diagnosed with pancreatic cancers at an advanced stage. Even if the cancer is resected, the median survival among these patients is only 13–18 months with a 5-year relative survival of 10–20% (Kalthoff 2022; Wilkowski et al. 2008). For locally advanced pancreatic cancer, where the cancer has spread to nearby tissues or organs, chemotherapy alone or in combination with radiation therapy is the treatment choice. In metastatic stage, when the cancer has spread to organs such as liver, lungs, and peritoneal cavity, chemotherapy is the only option to improve survival (Kalthoff 2022). Although ablation or embolization treatment alone is very unlikely to cure the cancer, they are sometimes used to treat pancreatic cancer especially when it has metastasized to the liver. Sometimes, more than one type of treatment may be combined to increase the effectiveness of pancreatic cancer therapy (Schmidt et al. 2022). Pancreatic cancer patients are also recommended to go for clinical trials when the standard treatments are not effective in tumors (Immunotherapy for Pancreatic Cancer n.d.; Kalthoff 2022) (Fig. 6.1).

### 6.2.2 Chemotherapy

Chemotherapy uses drugs to prevent cancer cells from growing and dividing thereby killing the cancer cells (Kalthoff 2022). In chemotherapy, anticancer drugs are administered either as IV injection or as oral formulation. Although chemotherapy can be used at any stage of pancreatic cancer, it is inevitable at advanced stage when the tumor is unresectable or if resection is not an option due to some other reason. Further, as the chemo-drugs can reach all parts of the body through bloodstream, it is the only potentially useful treatment when the cancer has spread beyond the pancreas (Kalthoff 2022). Chemo-drugs commonly used to treat pancreatic cancer include gemcitabine, 5-FU, irinotecan, oxaliplatin, abraxane, capecitabine, cisplatin, paclitaxel, docetaxel, and onivyde. Depending on patients' general health, combination of two or more chemo-drugs is usually given for better effect (Schmidt et al. 2022). For instance, FOLFIRINOX (combination of 5-FU, leucovorin, oxaliplatin, and irinotecan) is given when the patient is in good health. However, when patients are not healthy enough, single drugs like gemcitabine, 5-FU, or capecitabine are generally given (Huang et al. 2022). Further, chemotherapy can be given alone or alongside other treatments like radiotherapy and/or surgery to enhance the effect of treatment (Gugenheim et al. 2022).

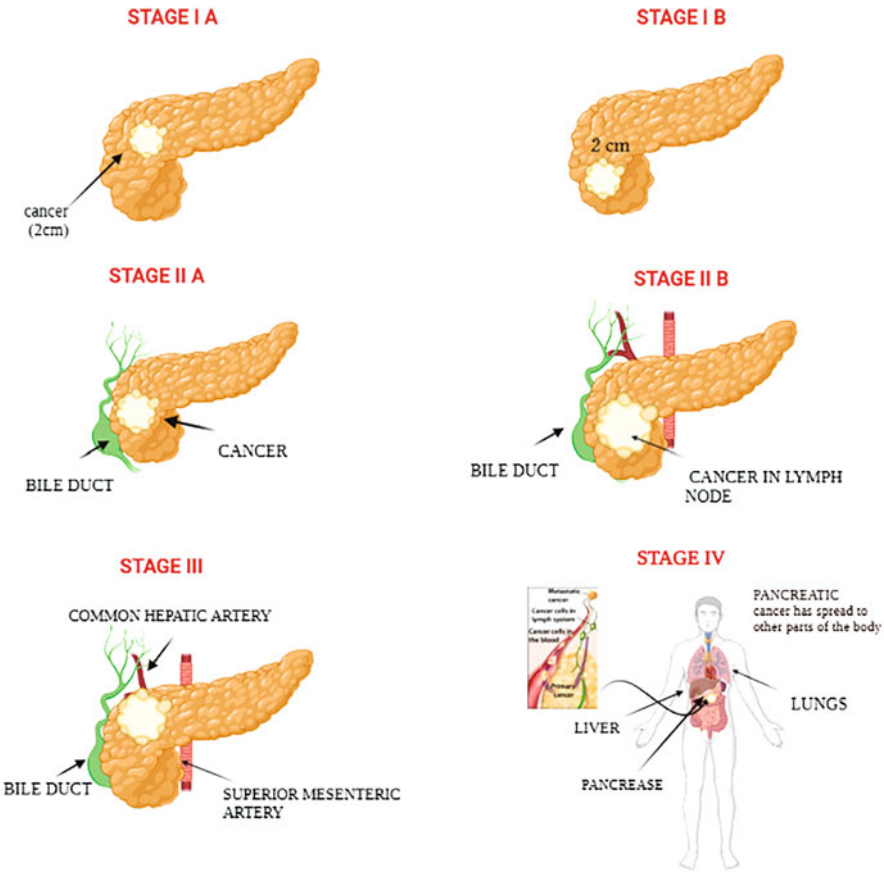



**Fig. 6.1** Different stages of pancreatic cancer

### 6.2.2.1 Limitations of Current Chemotherapy for Pancreatic Cancer

As chemo-drug kills fast growing cancer cell, it can also damage rapidly dividing normal cells such as blood cells, bone marrow cells, cells in the reproductive and digestive tracts, etc. (Heinemann et al. 2022). Hence, chemotherapy can cause side effects which will also differ from person to person (Klatte et al. 2022). Common side effects of chemotherapy include nausea, poor appetite, hair loss, mouth sores, constipation or diarrhea, increased risk of infection, bleeding or bruising, fatigue, etc. (Hamidi-Sofiani et al. 2022). In addition, some drugs can also induce other specific side effects; for example, gemcitabine causes flu-like symptoms, neuropathy, hand/foot syndrome, pulmonary problems, etc. and 5-FU causes photophobia, skin reactions, cardiac problems, etc. (Huang et al. 2022; Reni and Orsi 2022). Side effects of chemotherapy mainly depend on the type and dose of drugs used, as well as the duration of the treatment (Hamidi-Sofiani et al. 2022). Hence, any treatment modality which can replace the toxic chemo-drug with a biocompatible and potent

alternative or which can reduce the dose or treatment duration required for current chemo-drug can substantially minimize the side effects of chemotherapy.

Another major limitation of chemotherapy is that the current chemo-drugs only modestly improve overall survival. In locally advanced pancreatic cancer, the median survival with chemotherapy has been reported as only 6–11 months (Thomasset and Lobo 2010). Gemcitabine, the standard drug for pancreatic cancer since its FDA approval in 1996, has a survival benefit of only 6 months (Klatte et al. 2022; Burris et al. 1997). The combination of abraxane with gemcitabine which was approved as first-line treatment for metastatic pancreatic cancer in 2013 has added only an overall survival advantage of only 2.6 months compared to that with gemcitabine as single agent (Reni and Orsi 2022; Von Hoff et al. 2013; Goldstein et al. 2015). FOLFIRINOX, another drug combination commonly used for metastatic pancreatic cancer, has reported a significant clinical survival benefit of 11.1 months, but only at the expense of more severe side effects, making the combination suitable only for patients with good performance status (Conroy et al. 2011). Even with the most recently approved drug combination of onivyde with 5-FU and leucovorin, the median overall survival was improved to only 6.2 months compared to 4.2 months with 5-FU and leucovorin alone in gemcitabine-resistant metastatic pancreatic cancer patients (Wang-Gillam et al. 2016b). New therapeutic approaches are being investigated such as more effective therapeutic agent, combination therapy, and improved drug delivery systems to prolong survival time and to increase quality of life in pancreatic cancer patients.

Most of the chemo-drugs are metabolically unstable and are rapidly cleared from the blood circulation which reduces its therapeutic efficacy. Efforts to compensate these limitations usually result in administration of high dose of these cytotoxic drugs which in turn increases side effects. For example, gemcitabine is administered at a dose of 1 g/m2 causing severe side effects such as neutropenia, breathlessness, nausea, and kidney failure (Huang et al. 2022). To circumvent this, novel strategies such as nanocarrier-based approaches are being explored to improve drug delivery and to extend retention time (Amrutkar and Gladhaug 2017; Moysan et al. 2013). The latest advancement in overall survival with the drug-loaded nanocarrier such as onivyde emphasizes the benefit of nanoparticles-mediated drug delivery in pancreatic cancer therapy (Zhang 2016).

The chemo-drugs are expensive, especially the newer ones, as they are required for long-term and more than one chemo-drug may be needed. According to the U.S. Centers for Disease Control and Prevention, 20% of cancer patients below the age of 65 years delayed or avoided chemo treatment solely due to the high cost involved (Uddin n.d.; Huang et al. 2022; The Cost of Cancer Treatment with Chemothreapy is Going Up n.d.; Sexton et al. 2022). Hence, a relatively cost-effective new drug with similar or better antitumor efficacy as that of current chemo-drugs can alleviate the economic burden in patients.

## 6.3 Alternative to Conventional Chemotherapy for Pancreatic Cancer Management

### 6.3.1 Potential Drugs Other than Standard of Care

Increasing prevalence of pancreatic cancer in the world, as well as the limited effects and substantial toxicity of conventional drugs, has created a strong need for novel therapeutic agents. Several innovative drugs are being explored for pancreatic cancer treatment including FDA-approved drugs which were initially developed for other diseases but have been incidentally shown as having anticancer effect towards pancreatic cancer (Schmidt et al. 2022). Generally, these drugs are used off-label as an alternative in the absence of an approved therapy or may be used instead of the standard drug to improve efficacy or to minimize side effects (Levêque 2008; Levêque 2016). The off-label use is only recommended if there is clear evidence from published research, ongoing research, or from molecular tumor testing that the drug can work for the disease (Heinemann et al. 2022). Based on clinical studies suggesting beneficial use, the anticancer drugs which are Compendia-listed for off-label use against pancreatic cancer are doxorubicin HCl, epirubicin, flutamide, octreotide, tamoxifen, etc. (Kristin n.d.; Goldberg 2022). In addition to the off-label use of cancer drugs, there is also another category of drugs used for other disease but are extensively studied due to their potential off-label use for pancreatic cancer. This includes metformin (antidiabetic drug), aspirin (anti-inflammatory drug), chloroquine (antimalarial drug), etc. (Cavalla 2015; Gugenheim et al. 2022).

### 6.3.2 Different Types of Nanocarriers Used against Pancreatic Cancer

The poor response of pancreatic cancer to the conventional systemic chemotherapy can be also attributed to the inefficient delivery of chemo-drugs to the tumor. Nanotherapeutics is a fast-developing area in cancer research with an intention to resolve limitations of conventional chemotherapy (Sebastian 2017). New strategies using nanocarriers-based systems, such as nanoparticles, liposomes, polymersomes, etc., have been utilized to deliver drugs to cancer cells through enhanced permeability and retention (EPR) effects. In EPR effect, the increased permeability of blood vessels and dysfunctional lymphatic drainage can increase permeation and retention of nanocarriers in the tumor which permit drug release near the tumor cells (Peer et al. 2007). The encapsulation of chemo-drug into nanocarrier can protect the drug from degradation and rapid clearance from the blood circulation before it reaches the target site (Vieira and Gamarra 2016). Hence, the nanocarrier can improve the pharmacokinetics and tissue distribution of chemo-drugs which in turn enhance drug delivery to target, thereby increasing the therapeutic index of the drug (Hare et al. 2017). In addition, with suitable surface modification of nanocarriers, it can be directed to specific cancer cells via active targeting, increasing the likelihood of destroying cancer cells without causing much damage to normal tissue (Sebastian

2017; Peer et al. 2007). Nanocarriers can impart controlled release of drugs and serve to reduce undesirable off-target toxicity of chemo-drugs. Nanocarrier has also been reported to be capable of multidrug delivery to combat drug resistance and to improve cancer treatment (Sebastian 2017). The first nanotherapeutic approved by FDA was Doxil, a PEGylated liposomal formulation encapsulated with doxorubicin for breast cancer (Barenholz 2012). Advances in nanotechnology have also developed chemo-drug-loaded nanocarrier system that has added clinical benefit in pancreatic cancer therapy. Abraxane was approved for pancreatic cancer in advanced stages as it has improved overall survival in combination with gemcitabine, by increasing intra-tumoral concentration of paclitaxel and gemcitabine. Further, the toxicity of abraxane such as peripheral neuropathy and myelosuppression was reported as manageable compared to that with the conventional cremaphor formulation of paclitaxel (Von Hoff et al. 2013; Von Hoff et al. 2011; Ma and Hidalgo 2013). Nano liposomal irinotecan (Onivyde) was approved by FDA in combination with leucovorin and 5-FU for gemcitabine-refractory metastatic pancreatic cancer based on significant improvement observed in overall survival due to prolonged circulation and a higher concentration of irinotecan in the tumor (Passero et al. 2016; Wang-Gillam et al. 2016a).

#### 6.3.2.1 Polymer

Polymers include PCL, PLA, PEG, PLGA, chitosan, etc. Cationic SLNs were loaded with carmustine and functionalized with an anti-EGFR antibody which was found to inhibit the growth of U87MG glioma cells in vitro by enhancing the transport and reducing the administered dosage. Anti-EGFR antibody-conjugated liposomes and PLGA nanoparticles were reported to be effective for EGFR-specific delivery of gemcitabine in A549-based lung cancer model and orthotopic pancreatic carcinoma model.

#### 6.3.2.2 Carbon Nanotubes

Carbon nanotubes have been functionalized with cetuximab antibody for EGFR targeted delivery of different imaging and therapeutic moieties. The majority of these targeted nanosystems exhibited EGFR-specific internalization for effective imaging and more than 80% therapeutic potential in vitro. Cetuximab was also reported to enable targeted drug delivery following noncovalent functionalization of carbon nanovectors.

#### 6.3.2.3 Iron

Conjugated quantum dots or iron oxide nanoparticles were found to internalize efficiently in an orthotopic pancreatic model proving to be used as a molecularly targeted in vivo tumor imaging agent. EGFR-specific iron-oxide Nps, silica-coated polystyrene-loaded Nps, and quantum dots have been developed as targeted contrast agents.

#### 6.3.2.4 Gold Nano

Gold nanoparticles were utilized for EGFR targeted photo thermal therapy and RF (radiofrequency) ablation reported to induce complete cancer cell death in vitro. Antibody conjugation enhanced the receptor-specific uptake of these Nps and the accumulated gold Nps generated heat upon light or RF exposure and resulted in enhanced cancer cell death conjugated gold nanoparticles within EGFR+ve A431 cells. 2′-fluoro RNA aptamers were used as a cell internalization SELEX approach by gold nanorods (Apt EGFR-PGNRs) and were reported to exhibit excellent tumor targeting ability and feasibility of effective photothermal ablation pancreatic cancer therapy. Despite these promising targetability, aptamer-based targeting is challenged by their stability and degradation properties in physiological conditions.

## 6.4 Combination Chemotherapy

For pancreatic cancer, combination chemotherapy has emerged as a new standard of care. In combination chemotherapy, more than one chemo-drug are used simultaneously to treat cancer. Use of drug combinations has several advantages in cancer therapy such as it can reduce the risk of tumor resistance, can act on multiple molecular targets, and can make the heterogeneous tumor more susceptible to therapy, which collectively can increase the probability of eliminating the cancer. In addition, a successful combination of drugs can reduce the dose required which in turn can minimize the side effects of chemotherapy (What Is Combination Chemotherapy? n.d.; Kartal et al. 2022). Criteria for developing drug combination for cancer therapy involve that the different drugs should combat the malignancy through a different mechanism of action, reducing the chance of further mutation. Toxicity of drugs selected for combination must also be considered to avoid any additive toxic effect to any organ system (de la Pinta 2022). FOLFIRINOX is the first introduced combination chemotherapy regimen showing improved overall survival for treatment of metastatic pancreatic cancer. However, the combination is recommended as a first-line treatment option only for patients having good performance status because of its severe side effects (McDonald 2022). Abraxane plus gemcitabine is another combination approved by FDA for the treatment of metastatic pancreatic cancer because of its better safety profile, although overall survival was not good as FOLFIRINOX (Baines et al. 2016). Finally, a combination regimen of onivyde with leucovorin and 5-FU is the first and only treatment option for gemcitabine-refractory advanced or metastatic pancreatic cancer approved by FDA (Onivyde (Irinotecan Liposome Injection) n.d.; Cabasag et al. 2022).

### 6.4.1 Cancer Nanotherapeutics

In order to improve the availability of onco drugs at the targets, researchers developed strategies for enhancing the accumulation of therapeutic payloads at the target site. Tumor accumulation of therapeutic macromolecules was reported by

Matsumura and Maeda for the distribution and retention of poly (styrene-co-maleic acid)-Neocarzinostatin (SMANCS) in the tumor interstitium. The presence of fenestrations in the tumor blood vessels and poor lymphatic drainage to the tumor were inferred as the main factor to this macromolecular accumulation and this phenomenon was known by the name Enhanced Permeation and Retention (EPR). Based on this, people started looking into the efficiency of nanometer scale (10–100 nm) delivery systems or nanotherapeutics with high surface to volume ratio in accumulating the tumors by utilizing EPR effects. The possibility to design nanoparticles to encapsulate poorly soluble anticancer drugs and tune their systemic circulation and tissue distribution profiles was the focus of cancer researchers to formulate nanotherapeutics for the wide range of potential cytotoxic. Nanotherapeutics or nanomedicines can improve drug solubility by providing hydrophilic and hydrophobic environments, protect the payload from premature degradation by functioning as a sustained release systems thereby reducing dosing frequencies, control the rate of drug release which avoids unexpected tissue damage due to accidental extravasation, enhance the transport across biological barriers, improve pharmacokinetic and pharmacodynamic profiles, and reduce toxicities. To be more precise, the nanoengineered formulations yielded longer circulation half-lives and superior bioavailability and also evaded efflux proteins (e.g.,: Pgp), thereby overcoming drug resistance. The modular design capabilities with improved biological and therapeutic effects, thereby improving patient outcomes, would make this nanometric drug delivery systems an efficient class of cancer therapeutics. Various nanoparticle systems under current focus to be developed as cancer therapeutics include dendrimers, polymeric nanoparticles, protein nanoparticles, viral nanoparticles, metallic nanoparticles, and carbon nanotubes. The size, particle shape, and surface characteristics of a nanoparticle govern the lifespan of a nanoparticle within the circulation and can be modulated by its interactions with the above-mentioned physical characteristics and the environment in which the nanoparticles are existing. The significance of EPR in delivering therapeutic moieties in nanoparticulate form has been studied for past 20 to 30 years and well-established and first-generation nanomedicines (e.g.,: Abraxane, Doxil) rely on this mechanism for controlling the pharmacokinetics and biodistribution of a compound by modulating its physicochemical properties.

### 6.4.2 Targeted Pancreatic Cancer Nanomedicines

Targeted Pancreatic Cancer Nanomedicines targeted cancer therapies include drug molecules which specifically interfere with the molecular pathways involved in tumor growth and progression. In general, these targeted drugs are broadly classified into a) monoclonal antibodies (mAb) and b) small molecule inhibitors. Therapeutic mAbs target specific cell surface antigens such as transmembrane receptors or extracellular growth factors, whereas small molecule inhibitors penetrate the cell membrane and act on intracellular targets. Table 6.1 provides a list of the

**Table 6.1** FDA-approved targeted pancreatic cancer therapies

| Targeting agent | Target | FDA-approved indications |
|---|---|---|
| Everolimus (Afinitor) | mTOR | Pancreatic, gastrointestinal cancer |
| Erlotinib (Tarceva) | EGFR (HER1/ERBB1) | Pancreatic cancer |

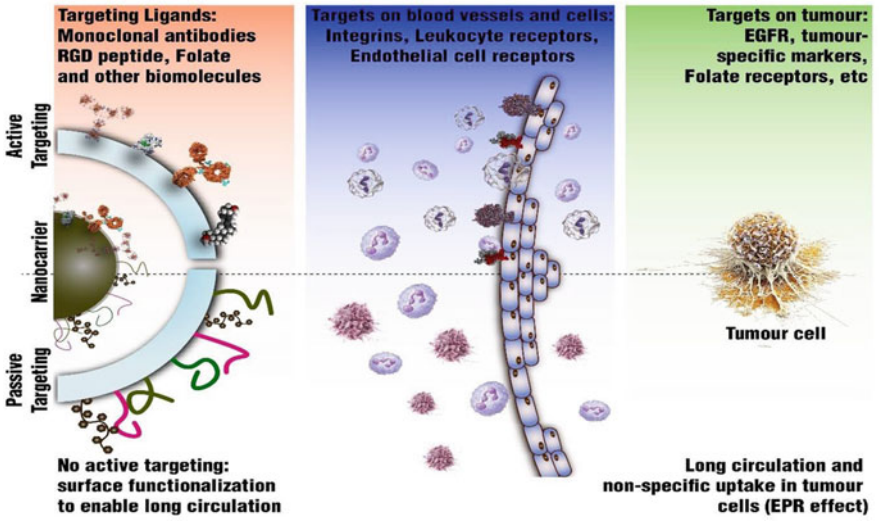



**Fig. 6.2** Schematic representation showing passive and active targeting of nanoparticles attached with various ligands specific for cancer cell surface markers which could improve tumor targeting and in turn therapeutic efficacy

FDA-approved targeted pancreatic cancer therapies for solid malignancies and their molecular targets.

Active targeting depends upon the recognition of the ligand by the target substrates wherein ligands could be antibodies, proteins, peptides, nucleic acids, or sugars, whereas targets could be cell surface proteins, sugars, or lipids. Active targeting enhances the therapeutic outcome by increasing cellular internalization, thereby improving the availability of the therapeutic agent at the target site. In short, the long-circulating nanoparticles allow EPR-mediated effective transport to the tumor and simultaneously are taken up by the tumor cells by targeted endocytosis mechanism by which the nanoparticles are internalized and release the therapeutic moiety, thereby enhancing therapeutic effect (Fig. 6.2).

## 6.5 Significance of EGFR as a Target

The significance of epidermal growth factor receptor (EGFR) in the development and prognosis of epithelial malignancies is being exploited by the researchers for developing therapeutic interventions. The over proliferating cancer cells are known to overexpress growth factor receptor molecules like EGFR which serve as an endogenous tool or docking sites for developing targeted nanotherapeutics. Receptor-ligand interactions belong to the most important aspect of active targeting of nanocarriers and could be further potentiated by EPR. EGFR plays a crucial role in controlling cell survival and apoptosis, angiogenesis, cell motility, and metastasis. Many established studies reported EGFR to be overexpressed in many of the human solid tumors, including glioma, head and neck, non-small cell lung, breast, gastric, colon, pancreatic, renal, ovarian, and bladder carcinomas and they are associated with poor prognosis. These receptors are single chain transmembrane glycoproteins (170 kDa) with an extracellular ligand binding ectodomain, a transmembrane domain, a short juxta membrane section, a tyrosine kinase domain, and a tyrosine containing C-terminal tail. Specific ligands bind to the ectodomain promoting homo/hetero dimerization, which further activates intracellular tyrosine kinase domain and eventually the C-terminus tail. EGFR stimulation activates downstream components of Ras/MAPK, PLCg1/PKC, PI (3) kinase/Akt, and STAT signaling pathways. EGFR family comprises of four structurally related members: EGFR (ErbB1, HER1), ErbB2 (HER2, neu in rodents), ErbB3 (HER3), and ErbB4 (HER4). EGF, TGF-β, heparin-binding EGF, amphiregulin, and betacellulin fall in the list of endogenous ligands for EGFR. These receptors undergo internalization followed by nanoparticles carrying therapeutic cargo by targeting EGFR using EGFR-specific ligand (Fig. 6.3).

**Strategies for Targeting EGFR**

The discussion about the role and significance of EGFR in the development and progression of human malignancies highlights the fact that EGFR blockade offers to be a promising therapeutic intervention strategy for cancer. The main strategies reported to target EGFR includes the following: a) anti-EGFR monoclonal antibodies which bind to extracellular domain of the receptor, thereby blocking the natural ligand binding and EGFR pathway activation, b) Small molecule inhibitors which specifically block the tyrosine kinase intracellular pathway, c) immunoconjugates where the anti-EGFR antibodies could be conjugated to toxin, d) ligand conjugates where the toxins could be conjugated to EGFR-specific ligands, and finally e) antisense therapy from the genetic level by targeting EGFR using DNA or RNA oligonucleotides. The ligand or the targeting moiety which could be exploited for the development of an efficient targeted nanotherapeutics has to be chosen only after clear understanding about the various strategies which specifically target and block EGFR signaling cascade. Further, various strategies which have been exploited for targeting EGFR overexpressed on cancer cells as a therapeutic intervention approach for cancer treatment.

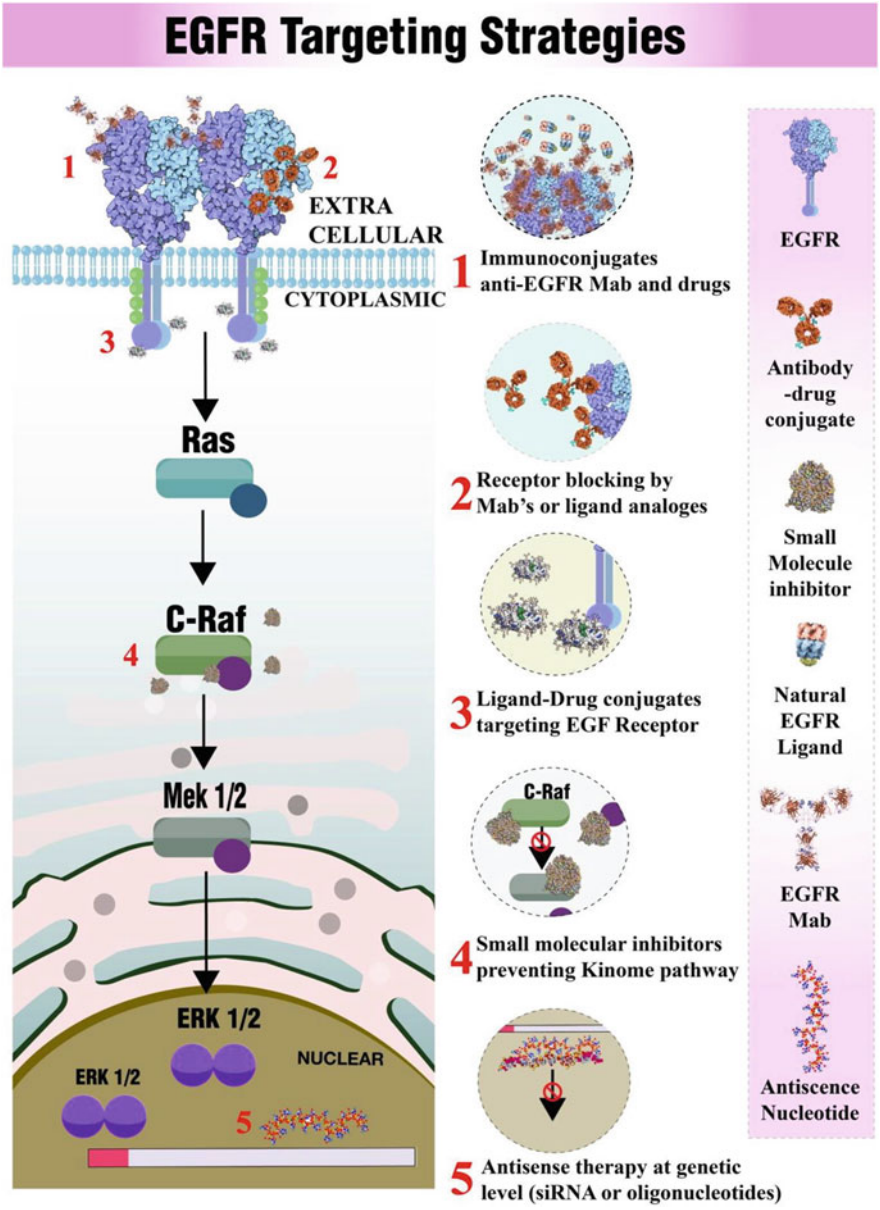



**Fig. 6.3** EGFR targeting strategies which have been exploited as therapeutic intervention approach for cancer treatment

## References

Amrutkar M, Gladhaug IP (2017) Pancreatic cancer Chemoresistance to gemcitabine. Cancers (Basel) 9

Baines AT, Martin PM, Rorie CJ (2016) Current and emerging targeting strategies for treatment of pancreatic cancer. Prog Mol Biol Transl Sci 144:277–320

Barenholz Y (2012) Doxil®--the first FDA-approved Nano-drug: lessons learned. J Control Release 160:117–134

Bodmer M, Becker C, Meier C, Jick SS, Meier CR (2011) Use of metformin and the risk of ovarian cancer: a case-control analysis. Gynecol Oncol 123:200–204

Bonifazi M, Gallus S, Bosetti C, Polesel J, Serraino D, Talamini R, Negri E, La Vecchia C (2010) Aspirin use and pancreatic cancer risk. Eur J Cancer Prev 19:352–354

Braghiroli MI, de Celis Ferrari ACR, Pfiffer TE, Alex AK, Nebuloni D, Carneiro AS, Caparelli F, Senna L, Lobo J, Hoff PM et al (2015) Phase II trial of metformin and paclitaxel for patients with gemcitabine–refractory advanced adenocarcinoma of the pancreas. Ecancermedicalscience 9:563

Burris HA, Moore MJ, Andersen J, Green MR, Rothenberg ML, Modiano MR, Cripps MC, Portenoy RK, Storniolo AM, Tarassoff P et al (1997) Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. J Clin Oncol 15:2403–2413

Cabasag CJ, Ferlay J, Laversanne M, Vignat J, Weber A, Soerjomataram I, Bray F (2022) Pancreatic cancer: an increasing global public health concern. Gut 71(8):1686–1687

Cavalla D (2015) Do no harm. In: Off-label prescribing–justifying unapproved medicine. John Wiley & Sons, Ltd, pp 71–103

Cerullo M, Gani F, Chen SY, Canner J, Pawlik TM (2016) Metformin use is associated with improved survival in patients undergoing resection for pancreatic cancer. J Gastrointest Surg 20: 1572–1580

Conroy T, Desseigne F, Ychou M, Bouché O, Guimbaud R, Bécouarn Y, Adenis A, Raoul J-L, Gourgou-Bourgade S, de la Fouchardière C et al (2011) FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. N Engl J Med 364:1817–1825

Currie CJ, Poole CD, Gale EAM (2009) The influence of glucose-lowering therapies on cancer risk in type 2 diabetes. Diabetologia 52:1766–1777

de la Pinta C (2022) Radiomics in pancreatic cancer for oncologist: present and future. Hepatobiliary Pancreat Dis Int 21(4):356–361

Donadon V, Balbi M, Ghersetti M, Grazioli S, Perciaccante A, Della Valentina G, Gardenal R, Dal Mas M, Casarin P, Zanette G et al (2009) Antidiabetic therapy and increased risk of hepatocellular carcinoma in chronic liver disease. World J Gastroenterol 15:2506–2511

Donadon V, Balbi M, Mas MD, Casarin P, Zanette G (2010) Metformin and reduced risk of hepatocellular carcinoma in diabetic patients with chronic liver disease. Liver Int 30:750–758

Goldberg R (2022) Toward precision pancreatic cancer care. J Natl Compr Cancer Netw 20(5):547–548

Goldstein D, El-Maraghi RH, Hammel P, Heinemann V, Kunzmann V, Sastre J, Scheithauer W, Siena S, Tabernero J, Teixeira L et al (2015) Nab–paclitaxel plus gemcitabine for metastatic pancreatic cancer: long-term survival from a phase III trial. J Natl Cancer Inst 107

Gopinath KS (2015) Indian J Surg Oncol 6:1–2

Gou S, Cui P, Li X, Shi P, Liu T, Wang C (2013) Low concentrations of metformin selectively inhibit $CD133^+$ cell proliferation in pancreatic cancer and have anticancer action. PLoS One 8: e63969

Gugenheim J, Crovetto A, Petrucciani N (2022) Neoadjuvant therapy for pancreatic cancer. Updat Surg 74(1):35–42

Hamidi-Sofiani V, Rakhshi R, Moradi N, Zeynali P, Nakhaie M, Behboudi E (2022) Oncolytic viruses and pancreatic cancer. Cancer Treat Res Commun 31:100563

Hare JI, Lammers T, Ashford MB, Puri S, Storm G, Barry ST (2017) Challenges and strategies in anti-cancer Nanomedicine development: an industry perspective. Adv Drug Deliv Rev 108: 25–38

Heinemann V, Boeck S, Westphalen CB (2022) Treatment of metastatic pancreatic cancer. Inn Med (Heidelb) 63(8):851–862

Hosein PJ, de Lima Lopes G, Pastorini VH, Gomez C, Macintyre J, Zayas G, Reis I, Montero AJ, Merchan JR, Rocha Lima CM (2013) A phase II trial of nab-paclitaxel as second-line therapy in patients with advanced pancreatic cancer. Am J Clin Oncol 36:151–156

Huang X, Zhang G, Liang T (2022) Subtyping for pancreatic cancer precision therapy. Trends Pharmacol Sci 43(6):482–494

Immunotherapy for Pancreatic Cancer (n.d.). https://www.cancerresearch.org/we-are-cri/home/cancer-types/pancreatic-cancer. Accessed Feb 5, 2018

Kalthoff H (2022) How open is the therapeutic horizon for pancreatic cancer patients? Hepatobiliary Pancreat Dis Int. 21(1):1–3

Karnevi E, Said K, Andersson R, Rosendahl AH (2013) Metformin-mediated growth inhibition involves suppression of the IGF-I receptor Signalling pathway in human pancreatic cancer cells. BMC Cancer 13:235

Kartal K, Guan Z, Tang R, Griffin M, Wang Y, Braun D, Klein AP, Hughes KS (2022) Familial pancreatic cancer: who should be considered for genetic testing? Ir J Med Sci 191(2):641–650

Klatte DCF, Wallace MB, Löhr M, Bruno MJ, van Leerdam ME (2022) Hereditary pancreatic cancer. Best Pract Res Clin Gastroenterol 58–59:101783

Kordes S, Pollak MN, Zwinderman AH, Mathôt RA, Weterman MJ, Beeker A, Punt CJ, Richel DJ, Wilmink JW (2015) Metformin in patients with advanced pancreatic cancer: a double-blind, randomised, placebo–controlled phase 2 trial. Lancet Oncol 16:839–847

Kristin A (n.d.) Esposito. medications used for the treatment of pancreatic cancer associated with ICD-10 codes. http://oncpracticemanagement.com/issue-archive/2016/january-2016-volume-6-number-1/medications-used-treatment-pancreatic-cancer-associated-icd-10-codes/. Accessed Feb 5, 2018

Lee JH, Kim TI, Jeon SM, Hong SP, Cheon JH, Kim WH (2012) The effects of metformin on the survival of colorectal cancer patients with diabetes mellitus. Int J Cancer 131:752–759

Levêque D (2008) Off-label use of anticancer drugs. Lancet Oncol 9:1102–1107

Levêque D (2016) Off-label use of targeted therapies in oncology. World J Clin Oncol 7:253–257

Li D, Yeung S-CJ, Hassan MM, Konopleva M, Abbruzzese JL (2009) Antidiabetic therapies affect risk of pancreatic cancer. Gastroenterology 137:482–488

Lonardo E, Cioffi M, Sancho P, Sanchez-Ripoll Y, Trabulo SM, Dorado J, Balic A, Hidalgo M, Heeschen C (2013) Metformin targets the metabolic Achilles heel of human pancreatic cancer stem cells. PLoS One 8:e76518

Ma WW, Hidalgo M (2013) The winning formulation: the development of paclitaxel in pancreatic cancer. Clin Cancer Res 19:5572–5579

McDonald OG (2022) The biology of pancreatic cancer morphology. Pathology 54(2):236–247

Mohammed A, Janakiram NB, Brewer M, Ritchie RL, Marya A, Lightfoot S, Steele VE, Rao CV (2013) Antidiabetic drug metformin prevents progression of pancreatic cancer by targeting in part cancer stem cells and mTOR signaling. Transl Oncol 6:649–659

Moysan E, Bastiat G, Benoit J-P (2013) Gemcitabine versus modified gemcitabine: a review of several promising chemical modifications. Mol Pharm 10:430–444

Nair V, Pathi S, Jutooru I, Sreevalsan S, Basha R, Abdelrahim M, Samudio I, Safe S (2013) Metformin inhibits pancreatic cancer cell and tumor growth and downregulates Sp transcription factors. Carcinogenesis 34:2870–2879

Onivyde (Irinotecan Liposome Injection) (n.d.) A new treatment option for patients with metastatic pancreatic cancer. http://jhoponline.com/top-issues/2016-issues/august-2016-vol-9-no-3/16849. Accessed Feb 5, 2018

Pannala R, Leirness JB, Bamlet WR, Basu A, Petersen GM, Chari ST (2008) Prevalence and clinical profile of pancreatic cancer-associated diabetes mellitus. Gastroenterology 134:981–987

Passero FC, Grapsa D, Syrigos KN, Saif MW (2016) The safety and efficacy of Onivyde (irinotecan liposome injection) for the treatment of metastatic pancreatic cancer following gemcitabine-based therapy. Expert Rev Anticancer Ther 16:697–703

Peer D, Karp JM, Hong S, Farokhzad OC, Margalit R, Langer R (2007) Nanocarriers as an emerging platform for cancer therapy. Nat Nanotechnol 2:751–760

Permert J, Ihse I, Jorfeldt L, von Schenck H, Arnqvist HJ, Larsson J (1993) Pancreatic cancer is associated with impaired glucose metabolism. Eur J Surg 159:101–107

Reni M, Orsi G (2022) Pancreatic cancer: still a cold case? Lancet Oncol 23(8):964–965

Reni M, Dugnani E, Cereda S, Belli C, Balzano G, Nicoletti R, Liberati D, Pasquale V, Scavini M, Maggiora P et al (2016) (Ir)relevance of metformin treatment in patients with metastatic pancreatic cancer: an open-label, randomized phase II trial. Clin Cancer Res 22:1076–1085

RIOMET® (metformin hydrochloride oral solution) (n.d.). https://www.accessdata.fda.gov/drugsatfda_docs/label/2005/021591s003lbl.pdf. Accessed Feb 5, 2018

Rozengurt E, Sinnett-Smith J, Kisfalvi K (2010) Crosstalk between insulin/insulin-like growth Factor-1 receptors and G protein-coupled receptor signaling systems: a novel target for the antidiabetic drug metformin in pancreatic cancer. Clin Cancer Res 16:2505–2511

Sadeghi N, Abbruzzese JL, Yeung S-CJ, Hassan M, Li D (2012) Metformin use is associated with better survival of diabetic patients with pancreatic cancer. Clin Cancer Res 18:2905–2912

Schmidt T, Belyaev O, Uhl W, Bruns CJ (2022) Surgical treatment of pancreatic cancer-What is new? Chirurg 93(5):446–452

Sebastian R (2017) Nanomedicine–the future of cancer treatment: a review. J Cancer Prev Curr Res 8

Sexton RE, Uddin MH, Bannoura S, Khan HY, Mzannar Y, Li Y, Aboukameel A, Al-Hallak MN, Al-Share B, Mohamed A, Nagasaka M, El-Rayes B (2022) Azmi AS connecting the human microbiome and pancreatic cancer. Cancer Metastasis Rev 41(2):317–331

Siegel RL, Miller KD, Jemal A (2015) Cancer statistics, 2015. CA Cancer J Clin 65:5–29

Siegel RL, Miller KD, Jemal A (2017) Cancer statistics, 2017. CA Cancer J Clin 67:7–30

Tan X-L, Reid Lombardo KM, Bamlet WR, Oberg AL, Robinson DP, Anderson KE, Petersen GM (2011) Aspirin, nonsteroidal anti-inflammatory drugs, acetaminophen, and pancreatic cancer risk: a clinic-based case-control study. Cancer Prev Res 4:1835–1841

The Cost of Cancer Treatment with Chemothreapy is Going Up (n.d.). http://cancercelltreatment.com/2015/05/13/the-cost-of-cancer-treatment-with-chemothreapy-is-going-up/. Accessed Feb 5, 2018

Thomasset SC, Lobo DN (2010) Pancreatic Cancer Surg 28:198–204

Tingstedt B, Weitkämper C, Andersson R (2011) Early onset pancreatic cancer: a controlled trial. Ann Gastroenterol 24:206–212

Uddin R (n.d.) The disadvantages of chemotherapy. https://www.livestrong.com/article/71774-disadvantages-chemotherapy/. Accessed Feb 5, 2018

Vieira DB, Gamarra LF (2016) Advances in the use of Nanocarriers for cancer diagnosis and treatment. Einstein (Sao Paulo) 14:99–103

Von Hoff DD, Ervin T, Arena FP, Chiorean EG, Infante J, Moore M, Seay T, Tjulandin SA, Ma WW, Saleh MN et al (2013) Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. N Engl J Med 369:1691–1703

Von Hoff DD, Ramanathan RK, Borad MJ, Laheru DA, Smith LS, Wood TE, Korn RL, Desai N, Trieu V, Iglesias JL et al (2011) Gemcitabine plus nab-paclitaxel is an active regimen in patients with advanced pancreatic cancer: a phase I/II trial. J Clin Oncol 29:4548–4554

Wakasugi H, Funakoshi A, Iguchi H (2001) Clinical observations of pancreatic diabetes caused by pancreatic carcinoma, and survival period. Int J Clin Oncol 6:50–54

Wang F, Herrington M, Larsson J, Permert J (2003) The relationship between diabetes and pancreatic cancer. Mol Cancer 2:4

Wang Z, Lai S-T, Xie L, Zhao J-D, Ma N-Y, Zhu J, Ren Z-G, Jiang G-L (2014) Metformin is associated with reduced risk of pancreatic cancer in patients with type 2 diabetes mellitus: a systematic review and meta-analysis. Diabetes Res Clin Pract 106:19–26

Wang-Gillam A, Li C-P, Bodoky G, Dean A, Shan Y-S, Jameson G, Macarulla T, Lee K-H, Cunningham D, Blanc JF et al (2016a) Nanoliposomal Irinotecan with fluorouracil and Folinic acid in metastatic pancreatic cancer after previous gemcitabine-based therapy (NAPOLI-1): a global, randomised, open-label, phase 3 trial. Lancet 387:545–557

Wang-Gillam A, Li C-P, Bodoky G, Dean A, Shan Y-S, Jameson GS, Macarulla T, Lee K-H, Cunningham D, Blanc J-F et al (2016b) Updated overall survival analysis of NAPOLI-1: phase III study of Nanoliposomal Irinotecan (nal-IRI, MM-398), with or without 5-fluorouracil and Leucovorin (5-FU/LV), versus 5-FU/LV in metastatic pancreatic cancer (mPAC) previously treated with Gemcitabin. J Clin Oncol 34:417

What Is Combination Chemotherapy? (n.d.). https://www.verywell.com/what-is-combination-chemotherapy-2248995. Accessed Feb 5, 2018

Wilkowski R, Wolf M, Heinemann V (2008) Primary advanced Unresectable pancreatic cancer. Recent Results Cancer Res 177:79–93

Wood LD, Canto MI, Jaffee EM, Simeone DM (2022) Pancreatic cancer: pathogenesis, screening, diagnosis, and treatment. Gastroenterology 163(2):386–402

Wright JL, Stanford JL (2009) Metformin use and prostate cancer in Caucasian men: results from a population-based case-control study. Cancer Causes Control 20:1617–1622

Yu X, Zhang Y, Chen C, Yao Q, Li M (2010) Targeted drug delivery in pancreatic cancer. Biochim Biophys Acta 1805:97–104

Zhang H (2016) Onivyde for the therapy of multiple solid tumors. Onco Targets Ther 9:3001–3007

Zhang Z-J, Zheng Z-J, Kan H, Song Y, Cui W, Zhao G, Kip KE (2011) Reduced risk of colorectal cancer with metformin therapy in patients with type 2 diabetes: a meta-analysis. Diabetes Care 34:2323–2328

# Nanocarriers-Based Targeted Therapies for Pancreatic Cancer and Challenges Ahead

7

Ankit Sahoo, Kainat Alam, Shipra Daniel, Sarwar Beg, Vikas Kumar, Abdul Hafeez, Mahfoozur Rahman, and Waleed H. Almalki

**Abstract**

Pancreatic cancer is one of the deadliest types of cancer and is considered the seventh leading cause of cancer. Pancreatic cancer incidence and mortality have been steadily increasing. Over the last decade, advancements in diagnosis, pancreatectomy surgery, radiotherapy technique, and systemic therapies have made advances, but relatively small improvements in patient outcomes. Furthermore, in pancreatic cancer, most of the chemotherapy drugs respond poorly or intrinsic resistance to chemotherapeutics, and lack effective target therapies that are the key factors contributing to a dismal prognosis. Recently, significant attempts have been made to provide targeted-based nanocarriers to treat pancreatic cancer. This

A. Sahoo · V. Kumar · M. Rahman (✉)
Department of Pharmaceutical Sciences, Faculty of Health Sciences, SHUATS, Allahabad, Uttar Pradesh, India

K. Alam
KKECS College of Nursing, Bangalore, India

S. Daniel
Christian College of Nursing, Faculty of Health Sciences, Sam Higginbottom University of Agriculture, Technology & Sciences, Allahabad, Uttar Pradesh, India

S. Beg
Department of Pharmaceutics, School of Pharmaceutical Education and Research, New Delhi, India

A. Hafeez
Glocal School of Pharmacy, Glocal University, Saharanpur, Uttar Pradesh, India

W. H. Almalki
Department of Pharmacology and Toxicology, College of Pharmacy, Umm Al-Qura University, Mecca, Saudi Arabia



M. Rahman et al. (eds.), *Hormone Related Cancer Mechanistic and Nanomedicines*,
https://doi.org/10.1007/978-981-19-5558-7_7

chapter tries to give information about the new possibilities of targeting pancreatic cancer via nanocarriers and challenges ahead.

**Keywords**

Pancreatic cancer · Targeted therapy · Signalling pathway · Receptors · Resistance · Challenges

## 7.1 Introduction

The pancreas is a vital organ in the digestion and absorption as well as the use and storage of energy. The pancreas consists of two structurally different but functionally linked glandular systems, the exocrine and endocrine pancreas, which developed from the primitive gut. The exocrine pancreas secretion is regulated by neural and hormonal signals, mostly in the form of gastrointestinal peptide hormones. Pancreatic cancer (PC) is the seventh leading cause of cancer with 496,000 cases and 466,000 deaths due to its poor prognosis in both genders (Sung et al. 2021). There are many factors contributing to the poor prognosis of pancreatic cancer, such as late diagnosis, high inherent resistance to conventional chemotherapy, a lack of biomarkers, and lack of effective therapeutic alternatives. From an epidemiological viewpoint, high-risk factors for pancreatic cancer include age, genetic predisposition, diabetes mellitus, family or personal history of pancreatitis, and especially the lifestyle of a person such as chain smoking, drinking alcohol, tobacco, and obesity, which increase the chance of development of PC by up to 50–60 percent (Dariya et al. 2019; Brand et al. 2007), whereas 5–10% is due to genetic mutations such as Kristen rat sarcoma (Kras). In addition to genetic mutation, PC is associated with epigenetic aberration abnormality of oncogenes. Silencing of tumour suppressor genes such as p16, TP53, and cyclin-dependent kinase inhibitor 2A (CDKN2A) is the risk hallmark of PC (Dariya et al. 2019). PC will soon be the second most malignant cancer in the world, with an overall survival rate of 26% for 5 years in advanced stages of the disease, and 22% for early-stage detection with surgical resection of the tumour.

Currently, treatment options are limited and chemotherapy is one of the choices for the treatment of cancer. Chemotherapy often fails to completely treat and cure cancer due to the high dose of drug required, poor accessibility of antineoplastic agents to tumour, and significant toxic effects due to their nonselective nature. As a result, nanocarrier-based targeted therapies have the potential to significantly enhance cancer treatment by delivering a therapeutically effective concentration of drug at the tumour site. Nanocarriers-based targeted therapies have advantages over conventional therapy such as (1) reducing cytotoxic compound effects on healthy cells, (2) fighting drug-resistant cancerous cells, and (3) reduction in dose-limiting adverse effects (Attia et al. 2019).

### 7.1.1 Pathophysiology of Pancreatic Cancer and their Types

Pancreatic cancer develops from epithelial cells that are responsible for the formation of digestive pancreatic enzymes and line the pancreatic duct, known as pancreatic exocrine or pancreatic ductal adenocarcinoma (PDAC). There are many characteristic precursor lesions in PDAC, including intraductal papillary mucinous neoplasm, mucinous cystic neoplasm, and pancreatic intra-epithelial neoplasia (most common). Others include the type of exocrine that includes acinar cell carcinoma, intraductal papillary-mucinous neoplasm, and mucinous cystadenocarcinoma, which are less prevalent but common (Chiaravalli et al. 2017). Pancreatic cancer may also grow from the pancreas's endocrine cells called islets of Langerhans, which produce hormones such as glucagon and insulin that are released into the blood circulation and regulate the blood glucose level in the body. This particular cancer is referred to as pancreatic endocrine cancer. It is necessary to identify the type of tumour that has developed in order to treat it effectively because they act and respond differently to treatment. Exocrine tumours are the most common type of pancreatic cancer, accounting for about 93% of cases and nearly 7% of neuroendocrine tumours (pancreatic NETs or PNETs), also known as islet cell tumour. Pancreatic NET grows slower than the endocrine tumour. PNET is generally non-functional (i.e. it does not produce hormones), but in some cases it may be functional (produce hormones), which make it important due to a major glucose homeostasis imbalance. Pancreatic cancer was described in revolutionary terms by Alvin et al. (Makohon-Moore and Iacobuzio-Donahue 2016) They classify it into three stages: the initiation of normal cells driven by gene mutation caused by environmental exposure and clone expansion, in which mutant cells continue to divide; the formation of a clonal population; and lastly, the introduction into a foreign microenvironment, in which tumour cells break through the basement membrane and invade the surrounding stroma. Multiple pancreatic cancer begins with pancreatic intraepithelial neoplasia (PanIN-IA, IB, II, and III), which is caused by a gene mutation, and progresses to invasive neoplastic lesions in the pancreas (Dariya et al. 2019). PanIN are the histological precursors of PC, characterized by enlarged nuclei, polarity loss, crowding of the nucleus, and pseudo-stratification hyper-chromatin. The process begins with dysplastic epithelial cells such as PanIN-IA and B, progresses into dysplasia cells including PanIN-II and III, and ultimately transforms into invasive carcinoma characterized by mutations in oncogenes like Kras and tumour suppressor genes like TP53, CDKN2A, and SMAD4 (Aslan et al. 2018). In one manner, pancreatic cancer is caused by epigenetic modulation and digestive enzyme inhibition that begin in childhood and eventually become chronic in adults (Table 7.1).

Cancer cells have a high degree of plasticity (i.e. the capacity to change to adapt to the intense tumour environment), which mostly involves the transformation from epithelial to mesenchymal form and metabolic alterations. These alterations provide cancer cells unique phenotype characteristics, including strong invasiveness and resistance to apoptosis. Changes in environmental factors have been suggested as potential causes of cell plasticity. However, genetic alteration (p53 and NFATc1

**Table 7.1** A comparison of key features of different types of pancreatic cancer

| Type of pancreatic cancer | Features | |
|---|---|---|
| Exocrine | | References |
| Pancreatic adenocarcinoma | Highly aggressive<br>Presence of typical precursor lesions viz. pancreatic intraepithelial<br>Prevalence: > 95% of reported cases<br>Neoplasia (most common), mucinous cystic Neoplasm, and intraductal papillary mucinous neoplasm | Desai et al. (2019) |
| Acinar cell carcinoma | Vary rare type<br>Possibility of excessive pancreatic lipase secretion | Desai et al. (2019) |
| Intraductal papillary-mucinous neoplasm | Potential precursor of PDAC<br>Main duct carcinoma is more severe<br>Pancreatic duct carcinoma (grows from main pancreatic duct or branches of the duct) | Desai et al. (2019) |
| Mucinous cystadenocarcinoma | Rare type<br>More commonly observed in tail of pancreas<br>Type of cystic tumour<br>Predominant in women | Desai et al. (2019) |
| Pancreatic neuroendocrine tumours (pancreatic NETs or PNETs) islet cell tumours | | |
| Prevalence: < 5% of reported cases<br>Less aggressive than PDAC<br>Can be functional (produce hormones) or non-functional (produce no hormones)<br>Majority are non-functional tumours | | Desai et al. (2019) |

signalling activity) is the most common cause. The classic desmoplastic/stromal reaction is an important histopathological feature of pancreatic cancer, and it is caused by the interaction of various factors including pancreatic stellate cells (PSCs), fibroblasts, inflammatory cells, and derived factors including fibronectin, fibronectin, extracellular matrix (ECM) proteins, and different growth factors (Liu et al. 2016; Apte et al. 2013). Pancreatic cancer cells upregulate various growth factors that cause tumourigenesis and contribute to stromal reaction. These factors include hepatocyte growth factors, transforming growth factors-β, insulin-like growth factor 1, fibroblast growth factor, epidermal growth factor, vascular endothelial growth factor (VEGF), platelet-derived growth factor, and connective tissue growth factors.

## 7.2 Targeted Therapy

As previously stated, PDAC is genetically heterogeneous, and traditional treatments that target distinct biological processes fail to differentiate between malignant and normal cells, resulting in severe side effects. Hence, small-molecule inhibitors and monoclonal antibodies targeting cancer cell surface receptors, growth factors, or

other proteins that contribute to disease development are needed for targeted treatments utilizing small molecules.

### 7.2.1 Targeting Surface Receptors

Multiple surface receptors have been linked to pancreatic cancer progression. Only a few inhibitors have been developed, including the EGFR inhibitor erlotinib, but they do not substantially enhance patient survival. However, despite the development of new inhibitors for various receptors, more precise therapeutic targets remain a need.

#### 7.2.1.1 Epidermal Growth Factor Receptor (EGFR), VEGF and IGF Receptor Targeted Delivery

Epidermal growth factor receptor (EGFR or HER1) (Philip et al. 2010), VEGF (Martin et al. 2012), and IGF receptors have been targeted by monoclonal antibodies (Mab) and have been evaluated in both preclinical and clinical trials. Using EGFR inhibitors in a phase III clinical study did not improve outcomes in patients with advanced pancreatic cancer compared to use of gemcitabine when used alone in a patient.

#### 7.2.1.2 Targeting Transferrin Receptors (TFRC)

The membrane-bounded protein TFRC is overexpressed in 90 percent of pancreatic cancer cells, and it is thought to be a marker of malignant cells (Ryschich et al. 2004). TFRC plays a critical role in the development and progression of pancreatic cancer. Along with gemcitabine, a tumour-specific liposome-based nanocomplex conjugated with a single-chain antibody fragment (TfRsFv) targeted the transferrin receptor in vivo mice model with PDAC (Camp et al. 2013). This complex system was demonstrated to be effectively located inside the tumour tissue through the transferrin receptor, to significantly reduce tumour growth and to extend the median survival of a metastatic pancreatic cancer mouse model.

#### 7.2.1.3 Folate Receptor (FR)

FR, a glycosylphosphatidylinositol-anchored receptor, is another potential target for pancreatic cancer treatment, since it is expressed at moderate to high level in more than 80% of patients with PDAC tumours (Cai et al. 2017). FR is mostly expressed in tumour cells with limited expression in normal cells (Zwicke et al. 2012) and may serve as a good receptor for nanoparticle-based targeted drug delivery.

### 7.2.2 Targeting Signalling Pathway in PDAC

The abnormal activation or dysregulation of several signalling pathways significantly contributes to pancreatic cancer heterogeneity. Numerous attempts have been made to create effective inhibitors, including biological and small molecules. Most of these signalling inhibitors are still being investigated and have not yet been

approved for clinical use. Targeted treatments are more precise than conventional drugs in the clinic because they block a key signalling pathway that is crucial for cell proliferation, survival, metastasis, and progression.

#### 7.2.2.1 KRAS Signalling

About 90% of PDAC patients have a KRAS mutation, making it an excellent therapeutic target since KRAS triggering mutations of this oncogene are the primary cause of the disease and its development (Collins et al. 2012). The KRAS proto-oncogene is responsible for the production of the GTPase protein. The KRAS mutation G12D leads to constitutive phosphorylation and activation of this pathway (Eser et al. 2014). Many distinct signalling pathways are activated when KRAS mutations occur. These signalling pathways include RAF, MEK, ERK, and the P13K/AKT pathways (Knickelbein and Zhang 2015). These pathways play key roles in cell division, survival, and drug resistance. KRAS activity affects the microenvironment of PDAC by generating sonic Hedgehog (Ji et al. 2007), interleukin-6 (Lesina et al. 2011), and prostaglandin E (Charo et al. 2013) and therefore regulates stroma maintenance (Pylayeva-Gupta et al. 2012). KRAS signalling has also contributed to the promotion of immunosuppression (Pylayeva-Gupta et al. 2012).

Although KRAS, one of the most potent of all human oncogenes, is activated in over 90% of PDAC, there are currently no KRAS-targeted treatments in the clinic. Due to the relatively smooth surface of the 3D structure, it has been difficult to inhibit KRAS directly, making it an untreatable target (Van Cutsem et al. 2004; Zeitouni et al. 2016). Several groups have been studied and the efficacy of targeting KRAS indirectly, as well as its downstream mediators PI3K and MEK pathway. However, it is difficult to suppress PI3K due to the presence of multiple isoforms of PI3K protein and not all isoforms of PI3K interact with KRAS (Vanhaesebroeck et al. 2010). To overcome the high levels of drug resistance and improve patient survival, new therapeutic strategies for PDAC at an advanced stage are required. Inhibiting KRAS activity by targeting it offers many opportunities for new drug development. Although compounds that access an inducible pocket generated in the KRAS structure have been discovered, more optimization is needed before these compounds can be developed into a clinically useful drug.

#### 7.2.2.2 TGF-β Signalling

TGF is involved in a variety of biological processes, including homeostasis and cellular processes such as cell proliferation, differentiation, and apoptosis (Elliott and Blobe 2005). TGF- suppresses cell proliferation and has tumour suppressive activity in the early stages of tumour development, but as tumourigenesis progresses, it takes on an oncogenic role in PDAC (Shen et al. 2017). TGF-signalling is typically activated by SMAD proteins that are classified as receptor-regulated, mediators, or inhibitors. TGF-ligand binding to the type II receptor phosphorylates SMAD2 and SMAD3, which then form a complex with the tumour suppressor protein SMAD4 (Singh et al. 2015). This signalling may be disrupted by inhibiting SMADs (SMAD6 and SMAD7) from interacting with the receptor. This pathway is usually

upregulated in patients with PDAC. In about 40% of pancreatic tumour cases, SMAD4 mutations are observed, resulting in decreased SMAD4 expression and inactivation (Malkoski and Wang 2012). The SMAD4 mutation is recognized and seen in high-grade PanIN lesions (Malkoski and Wang 2012). Most pancreatic carcinoma cell lines and patients with advanced pancreatic cancer have mutations in TGF-β1 and TGF-βR-1-2, which contribute to their poor prognosis. It has been shown that immune activation of TGF-β gene knockdown mice can result in tumour cell apoptosis and prolonged survival (Javle et al. 2014). TGF-siRNA induced apoptosis by activating RGI-I signalling, and TGF-siRNA decreased serum levels and anticancer efficacy in an orthotopic PDAC mouse model (Ellermeier et al. 2013). The SnoN (Ski-related novel protein N) protein is a molecule that negatively regulates the TGF- pathway, and its silencing leads to a decrease in cancer cell proliferation and an increase in apoptosis in vitro, indicating that TGF- signalling is an important molecular target in PDAC tumours.

#### 7.2.2.3 Hedgehog Signalling

In PDAC tumours, overexpression of Sonic Hedgehog ligands (SHH) leads to cancer initiation and metastasis, as well as a substantial desmoplastic response (Olive et al. 2009). It binds to the PTCH1 receptor, which is involved in the regulation of Smoothened protein (SMO) and downstream pathways. The sHH pathway is abnormally activated in pancreatic cancer when Hedgehog SMO is overexpressed (Honselmann et al. 2015). Currently, many ongoing studies investigate the role of inhibition of SMO receptors in PDAC. The combination of saridegib and gemcitabine, which inhibits the SMO receptor, reduced desmoplasia and collagen deposition, while increasing the intratumoural gemcitabine concentration and improved overall mice survival (Olive et al. 2009). However, in clinical studies combining hedgehog signalling inhibitors with gemcitabine failed to provide positive outcomes. As a part of one pilot study and phase II clinical trial, small-molecule SMO antagonist vismodegib showed disappointing results in patients with metastatic pancreatic cancer (Kim et al. 2014) (NCT01064622). Targeting any Hedgehog pathway molecule seems to be a viable approach since it can impact both the tumour and its surrounding stroma, as well as their interaction.

#### 7.2.2.4 Notch and Wnt Signalling

Overexpression of Notch genes, Notch receptors, and ligands were recognized even in early PanIN lesions (Guo et al. 2016; Mazur et al. 2010). Notch signalling pathway activation upregulates the invasive phenotype of pancreatic cancer by interfering with oncogene pathways and decreasing EGFR and NF-kB signalling (Mccleary-Wheeler et al. 2012). Pancreatic cancer cell lines BxPC-3, HPAC, and PANC-1 exhibited a high level of Notch 1 expression, and siRNA-mediated suppression of Notch 1 substantially reduced cell proliferation and induced apoptosis (Wang et al. 2006). However, the abnormal activation of the Wnt signalling pathway was also discovered in PDAC (Mccleary-Wheeler et al. 2012; Zeng et al. 2006). Wnt receptor activation is caused by ligand binding, which in turn activates β-catenin. Normally, β-catenin is inactive; however, active-catenin levels are elevated in

pancreatic cancer. Extracellular proteins, such as Hsulf-1,2, may serve as positive regulators of the Wnt signalling pathway and are often overexpressed in tumour cells but not in normal cells (Nawroth et al. 2007), indicating that the Wnt signalling pathway is constitutively active and therefore a therapeutic target. Patients with stage IV pancreatic cancer are being treated with biological therapeutic agent OMP-54F28, a type of decoy receptor protein that binds to Wnt ligands. This treatment is being utilized in conjunction with paclitaxel and gemcitabine as part of a phase I clinical study. The study's findings have not yet been made public (NCT02050178).

### 7.2.3 Tumour-Specific Nanotherapeutics for Targeting PDAC

In pancreatic cancer, use of nanoparticles has recently emerged as a therapeutic option for pancreatic cancer. The shape, size, and charge of nanoparticles affect their ability to enter into the cell. Tumour-specific nano-delivery systems are important for improving the effectiveness of anticancer therapies in PDAC because they minimize undesired and dose-limiting damage to normal cells while targeting tumours specifically and selectively. Drugs conjugated or encapsulated into nanoparticles have improved stability and half-life, allowing for more controlled release. Using modified nanoparticles, it is possible to improve the pharmacokinetics and biodistribution profiles of drugs substantially. Size influences cargo drug biodistribution and allows them to enter tumours via the increased permeability and retention effect of nanoparticles (EPR). They can easily penetrate the cell membrane, interact with various biological molecules, and accumulate inside tumours. Charged particles generate electrostatic attraction or repulsion with other charged particles, further impeding their diffusion. Their stability can be increased by functionalizing their surfaces with molecules that prolong their circulation throughout the body. These nanoparticle nanocarriers may be used to enhance the intracellular delivery of drugs to cancer cells while also knocking down abnormal gene or protein expression in cancer cells when combined with chemotherapy drugs or therapeutic RNA molecules (siRNA) for gene therapy. Nanoparticle-mediated targeted delivery may substantially reduce drug dosage and toxicity while improving drug bioavailability and gene therapy for improved prognosis in the case of hardly treated pancreatic cancer.

#### 7.2.3.1 Chemoprotective Drug Delivery Via NPs

Low dosages of gemcitabine are administered using nanoparticles, which enhance distribution in cancer cells and enhance efficacy. In PDAC tumour models, Rejiba et al. demonstrated increased efficacy of Gem (4-(N)-tris-nor-squalenoyl-gemcitabine (SQ-Gem) nanoparticle formulation (Couvreur et al. 2008). It inhibited cell proliferation and induced apoptosis in resistant Panc1 cells when administered at a concentration of 5 microM, resulting in 40% of apoptotic cells. In contrast, treatment with free gemcitabine killed only 10% of the cells and had no effect on tumour growth or survival in mice. The gemcitabine–squalene nanoassemblies

produced similar results in vitro and in vivo, with a 70% reduction in tumour volume (Maksimenko et al. 2015).

(a) *Gelatin-based drug delivery*—Gelatin has been proven as an effective gemcitabine carrier owing to its safety, biocompatibility, and biodegradability (Maksimenko et al. 2015). To enhance absorption of NP by pancreatic cancer cells, the EGFR peptide was attached to gelatin through a PEG linker (MW 2000 Da, size: 150–250 nm) for targeted drug delivery. PEGylation is well recognized to improve and prolong the systemic circulation. Gemcitabine release into Panc-1 cells from Gem-Gel-PEG- EGFR nanoparticles occurs following a disulfide bond cleavage. Intravenous injection of an EGFR-targeted Gem-Gel-PEG nanoparticle (one per week for four weeks) substantially decreased tumour volume (approximately 70%). However, nanoparticles were also found in the liver and spleen, and no adverse effects were found. Two chemotherapeutic drugs may be incorporated into nanoparticles to enable dual or multidrug delivery. Self-assembled nanoscale coordination polymers (NCPs)-based nanoparticles containing two agents (oxaliplatin and gemcitabine) showed a significant anticancer impact by inducing apoptosis by 75% in AsPc-1 and 80% in BxPc-3 cells in vitro and 80% in vivo (Poon et al. 2015). AsPc-1 xenograft models treated with this formulation showed an 11-fold reduction in tumour size compared to the controls, indicating that it prevented tumour development.

(b) *Inorganic nanoparticles*—Some of these NPs, including iron oxide, carbon nanotubes (CNT), quantum dots (QDs), and gold nanoparticles (AuNP), have been studied as drugs or gene carriers to enhance drug treatment effectiveness and extend the lifespan of pancreatic cancer models in preclinical and clinical trials (Hwang et al. 2012). The administration of (intravenous) IONPs coupled with IGF-1 and loaded with Dox (size: 20.4 nm) into an orthotopic pancreatic PDX model (Zhou et al. 2015) showed enhanced nanoparticle selectivity and accumulation within the tumour region, resulting in substantial suppression of cell proliferation and tumour development (untreated vs. IGF1-IONP- Dox). Using a drug that selectively targets IGF-1R led to increased amounts of Dox (5 mg/kg dose) in the tumour, which helped reduce tumour mass.

#### 7.2.3.2 Nanoparticle-Based Delivery of siRNAs

In the RNA interference (RNAi) pathway, there are three primary methods to silence genes: small interfering RNAs, microRNA, and short hairpin strands of RNA (shRNA). Non-coding RNAs, such as siRNA, also known as small interfering RNA or silencing RNA, are made from double-stranded RNA molecules and have a length of 20–25 base pairs. These molecules act as part of the RNAi mechanism. Once siRNA has been delivered into cells, the enzyme Dicer cleaves it into small fragments that direct the loading of siRNA molecules into a protein complex known as the RNA-inducing silencing complex later on (RISC). RISC proteins act in the unwinding of siRNA and cleavage of siRNA sense strand, leaving anti-sense strand free to complementary bind to mRNA and induce post-transcriptional gene silencing

(Guo et al. 2013). RNAi has been used in many preclinical and clinical investigations to suppress tumour-associated oncogenes, growth factors, and angiogenesis-promoting receptors that are overexpressed and contribute to tumour development; compared to other oligonucleotides, siRNA therapies offer many benefits. They can be easily chemically synthesized and efficiently suppress gene expression. Since it doesn't directly attach to DNA, there are no concerns of new mutations being generated during gene therapy. While siRNA has many benefits, it also has certain drawbacks when it comes to cancer treatment. It is highly unstable in body fluids and serum due to nuclease-induced degradation. For successful delivery of siRNA to cancer cells, many delivery systems including liposomes, polymers, and inorganic nanoparticles have been developed and conjugated with cancer-specific targeting molecules.

**Polymeric-Based Nanoparticles for siRNA Delivery into PDAC**

NPs based on polymers have been utilized in vitro and in vivo as carriers for siRNA delivery that specifically targets the KRAS gene or other target genes (Xu and Wang 2015). Stability, safety, and effectiveness have been shown in a PDAC mouse model for the local intratumoural delivery system LODER (Local Drug EluteR) (Khvalevsky et al. 2013). When used in a mouse model of PDAC tumours, LODER effectively delivered KRAS siRNA, reduced tumour development, and extended overall survival.

Cationic poly (lactic acid) (CPLA) biodegradable nanocapsules (CPLA-NC with zeta potentials of +45 MV and diameters of 32 nm) were evaluated for their ability to silence the KRAS oncogene in PDAC models (Lin et al. 2013). Through electrostatic interactions, negatively charged siRNA was attached to the surface. In PDAC models, CPLA-NC containing anti-KRAS siRNA could reduce the expression of the KRAS gene by nearly 50%. This complex did not have any nanoparticle-based cytotoxicity, indicating that it is safe for use in in vivo experiments.

PLGA/poloxamer (polyethyleneimine-poly (lactide-coglycolide) nanoparticle for siRNA delivery into PDAC. About 67% of PDAC patients had elevated levels of hypoxia-inducible factor 2 (HIF-2), also known as endothelial PAS domain protein 1 (EPAS1). Overexpression is associated with a poor prognosis, an advanced stage, and lymph node metastases, making it a possible therapeutic target in PDAC. In vivo targeting of EPAS1 with siRNA encapsulated in a PLGA/poloxamer (polyethyleneimine-poly (lactide-coglycolide)) nanoparticle resulted in improved intracellular uptake (Pan et al. 2015). PLGA has been used for many years for siRNA delivery. However, due to the low electrostatic interaction between PLGA and siRNA, a cationic polyetheyleneimine (PEI) polymer is coated on the surface of PLGA to overcome this limitation. Treatment of a nude mouse model with EPAS1siRNA nanoparticles resulted in a substantial decrease in tumour volume, according to in vivo tests in PDAC models.

To address the issue of opsonization and improve the efficacy of gene silencing, nanoparticles were PEGylated with POEGMA (38 nm). High transfection efficiency and uptake of fluorescently labelled siRNA star-POEGMA nanoparticles into

MiaPaca-2 cells were observed. There was a significant, more than 80%, reduction in βIII- tubulin gene expression following systemic delivery (4 mg/kg) of star nanoparticles. The stability of nanoparticles with POEGMA was achieved and star polymeric nanoparticles carrying siRNA against βIII-tubulin showed a therapeutic effect in an in vivo orthotopic pancreatic mouse model (Teo et al. 2016).

The overall study suggested that using siRNA as a therapeutic agent for pancreatic cancer with the ability to image tumour response in vitro and in vivo offers a feasible approach, with numerous benefits over conventional treatments.

#### 7.2.3.3 Photothermal Therapy by Inorganic Nanomaterials

Some nanomaterials can transform light energy into heat energy, which makes them potent therapies for targeting cancerous tissues. Using this approach to treat cancer has many benefits, including less invasion, fewer side effects, controllability, and specificity to particular tumour areas. Inorganic nanoparticles such as gold, carbon nanotubes, and copper sulfide nanoparticles were shown to successfully convert photo energy into thermal energy (Bao et al. 2016).

- Gold nanorods
  Recently, gold nanorods have gained considerable interest owing to their plasmonic photothermal treatment properties. After being irradiated with a short laser pulse, gold nanorods produce vapour nanobubbles called plasmonic nanobubbles. Patino et al. (Patino et al. 2015) functionalized the surface of gold nanorods using thiol-PEG-biotin to remove the cetyltrimethylammonium bromide (CTAB) layer on the nanorod's surface, which is known to be hazardous to cells. Additionally, they coupled gold nanorods with EPPT (MUC-1-specific peptide) and MPAP (myristoylated polyarginine peptide) peptides to enable targeted delivery by MUC-1 markers and enhance cellular uptake of gold nanorods. This resulted in extremely selective apoptosis after laser irradiation with no harm in surrounding cells. High loading of nanomaterials is usually needed in photothermal treatment approaches to produce adequate heating, and in this instance, the uptake rate of gold nanorods was enhanced by dual conjugation (EPPT and MPAP). Yin and colleagues (Yin et al. 2015) investigated the triple impact of KRAS gene silencing, doxorubicin, and photothermal treatment in pancreatic cancer therapy. They utilized a multilayer arrangement to cover the surface of gold nanorods with a negatively charged PSS polymer for doxorubicin capture and a positively charged PAH polymer for siRNA capture. Doxorubicin and KRAS siRNA were released into tumour cells under the control light (665 nm), which inhibited tumour development for at least 25 days.
- Carbon nanotubes
  PEG-functionalized multi-walled carbon nanotubes showed photothermal effects on PANC-1 cells at varying nanoparticle concentrations (5, 10, 50 g/ml) (Mocan et al. 2014). At dosages of more than 10 μg, laser irradiation significantly increased the number of apoptotic cells. Exposure to 50 μg/ml resulted in a substantial increase in reactive oxygen species (ROS), with 57% of pancreatic cancer cells expressing ROS.

## 7.3 Clinical Trials

In clinical trials, the entry of nanocarriers-based formulations opens a new pathway for the treatment of pancreatic cancer. A significant number of nanocarrier-based formulations are now in various phases of clinical trials. This formulation includes polymeric nanoparticles, liposomes, amphiphilic polymers nanoparticles, small interfering ribonucleic acid (siRNA) nanoparticles, dendrimers, carbon nanotubes, gold nanoparticles, quantum dots, inorganic nanoparticles, and magnetic nanoparticles (Table 7.2).

## 7.4 Challenges for Nanocarriers-Based Targeted Therapies

### 7.4.1 Physiological Barriers

Pancreatic stellate cells (PSCs) are a major barrier to any antineoplastic nanomedicine or conventional drug delivery to pancreatic tumour cells. PSCs cells stimulate pancreatic cancer and undergo functional and morphological alterations. As a consequence, the ECM is overproduced and deposited, leading to fibrosis of pancreatic stroma. Stromal fibrosis promotes tumour development by creating a favourable environment, and it also plays a key role in distant metastasis. Additionally, stromal fibrosis restricts delivery of drugs to the tumour site, resulting in less sensitivity to drugs and, sometimes, resistance.

PDAC develops in the exocrine area of the pancreas and is graded according to the histology of intraepithelial neoplasms (PanIN-1–3). Each PanIN stage has its own histological features, and the accumulation of mutations at each stage correlates with the progression of the disease (Cowan and Maitra 2014).

The desmoplastic nature of the stroma generates solid stress and/or increased interstitial fluid pressure inside the tumour, resulting in vessel compression and insufficient perfusion and hypo-vascularity, leaving about 80% of the tumour's vessels non-functional. The tumour microenvironment in PDAC is typically hypoxic due to poor perfusion and hypo-vascularity (Chauhan et al. 2013).

PDAC has a high stromal-to-neoplastic tissue ratio and a thick desmoplastic stroma composed of cellular (endothelial, nerve, and immune cells, as well as fibroblasts) and acellular (fibrin, collagen, fibronectin, and hyaluronan) components (Cowan and Maitra 2014; Rucki and Zheng 2014). Neoplastic cells usually make up less than 20% of the tumour mass.

Inflammatory cytokines, such as IL-1 and IL-6, and growth factors, such as tumour necrosis factor (TNF) and transforming growth factor1 (TGF-1), may activate pancreatic stellate cells in the tumour stroma, causing them to secrete copious quantities of extracellular matrix components that serve as a barrier to drug extravasation into the tumour interstitium (Phillips et al. 2003).

Stellate cells secrete matrix metalloproteinases (MMP-1 and MMP-9) that destroy basement membrane proteins, causing the initiation of fibrosis and cancer cell invasion (Li et al. 2010).

**Table 7.2** Current status of nanocarriers-based targeted therapies in clinical trials

| Intervention | Targets | Phase | Result | NCT identifier |
|---|---|---|---|---|
| ATI-1123: Liposomal docetaxel | Tubulin | I | Tumour size reduced to 29% from baseline in 1 of 6 patient with PDAC | NCT01041235 |
| BIND-014: Polymeric docetaxel nanoparticle | Tubulin | I | _ | NCT01300533 |
| Doxil: PEGylated liposomal doxorubicin (in combination with topotecan) | DNA | I | _ | NCT00252889 |
| TKM-080301: Lipid nanoparticles containing PLKI siRNA | PLK1 | I | _ | NCT01437007 |
| siG12D-LODER: Biopolymeric cylindrical implant | $KRAS^{G12D}$ | I<br>II | Decreased CA19–9 levels in 70% of patients; median overall survival (OS) was 15.1 months. Phase II trial ongoing | NCT01188785<br>NCT01676259 |
| Atu027: Liposomal PKN3 siRNA | Silences PKN3 (a PKC-signalling pathway molecules) | I & II | Ongoing | NCT01808638 |
| SGT-53: Liposomal p53 plasmid DNA (with nab-paclitaxel + gemcitabine) | P53 | II | Ongoing | NCT02340117 |
| NC-6004 (Nanoplatin): Micellar polymeric nanoparticles encapsulating cisplatin (with gemcitabine) | DNA | II & III | _ | NCT00910741<br>NCT02043288 |
| MM-398 (onivyde): Liposomal irinotecan (with 5-FU/folinic acid) | Topoisomerase 1 inhibitor | III | Improve PFS, ORR, and OS versus 5-FU/ folinic acid alone The FDA has given its clearance for usage in combination as a second-line treatment. | NCT01494506 |

### 7.4.2 Challenges in Clinical

#### 7.4.2.1 Controllable and Reproducible Synthesis

It is necessary to determine the optimum physicochemical parameters for the effective development of therapeutic nanoparticles (NPs). There has been significant progress in understanding the individual factors that contribute to successful immune evasion, cell targeting and internalization, extravasation and diffusion, and controlled drug release (Alexis et al. 2008; Perrault et al. 2009). It is still difficult to systematic parallel screening the wide range of NP attributes, due to the challenges of rapid, precise, and reproducible synthesis of NP libraries with unique characteristics. For the high-speed self-assembly of NPs with smaller size distribution, adjustable physical and chemical properties, and better batch-to-batch repeatability, microfluidic methods have lately gained interest compared to conventional bulk approaches that typically produce NPs with significant polydispersity (Valencia et al. 2010; Chen et al. 2012). In the same way, particle replication in non-wetting template (PRINT) technology has allowed the synthesis of monodispersed nanoparticles with exquisite control over chemical composition, drug loading, surface characteristics, and shape and size (Rolland et al. 2005; Xu et al. 2013).

#### 7.4.2.2 Evaluation and Screening

As new biomaterials or nanostructured NPs rapidly develop, in vitro assessment is becoming more essential for identifying biocompatible candidates before moving to animal testing. Additionally, in vitro tests may help us better understand the interaction between the nanoparticle and the cell. However, since traditional in vitro models based on cell culture in multi-well plates lack the complexity of real biological tissue and the ability to regulate fluid flow, they may be unable to represent the complicated interaction of nanoparticles with physiological barriers. Recent attempts to create biomimetic 'organ/tumour on a chip' technology may overcome some constraints associated with existing in vitro models (Toh et al. 2009; Huh et al. 2010; Albanese et al. 2013). Tumour-like spheroids incorporated into a microfluidic channel may provide information on the impact of cell binding, interstitial flow, and diffusion (Albanese et al. 2013). Nanoparticle behaviour in these chip systems may be comparable to those of animals, which may provide a glimpse into the future possibilities of these biomimetic microdevices. To evaluate NP performance in vivo (for example, biodistribution, PK, efficacy, and safety), animal models are required. One well-recognized barrier is the difference seen between efficacy achieved in preclinical studies and the results of clinical trials. This is a significant area due to the paucity of tumour models that adequately replicate human malignancies, despite the fact that certain studies have shown PK scaling across various species (including humans) for different nanotherapeutics (Zuckerman et al. 2014; Schultheis et al. 2014). Many animal models are now available, including orthotopic xenografts, cell line-based subcutaneous, genetically engineered mouse models (GEMMS), and patient-derived xenografts (PDX). However, not a single model can completely replicate all aspects of human malignancy. On the other hand, EPR is usually more consistent in animals than in human cancer patients.

Additionally, since tumour metastases are a significant cause of cancer death, a model of human tumour metastasis will be crucial in evaluating EPR and nanoparticle penetration and targeting in metastasis tumour in comparison to primary tumour.

The translation of nanotherapeutics may be significantly aided by the creation of animal models that accurately replicate the heterogeneity and anatomical histology of human tumours, such as humanized mouse (Shi et al. 2017)models (Rongvaux et al. 2014), high-fidelity PDXs (Lin et al. 2014), and GEMMs with aggressive metastasis.

### 7.4.3 Manufacturing on a Large Scale

Another challenge to clinical development stems from the escalating complexity in chemistry, manufacturing and controls (CMC) and good manufacturing practice (GMP) requirements as NP technology transitions from preclinical to clinical development, subsequent commercialization and beyond, as long as the product is on the market. Both aim to ensure that a product consistently meets a specified quality standard, although their methods and regulations diverge yet overlap. Additional GMP and CMC difficulties may arise when more complex nanomedicines are scaled up. This could involve changes to current unit operations or the development of novel manufacturing processes.

Complex technology and numerous stages in the NP formulation process make large-scale and repeatable synthesis more challenging (Shi et al. 2017). A change in formulation parameters or technique is almost always required when moving a molecule from the laboratory to clinical trials, thus thinking about scaling up early is critical to NP design and engineering.

### 7.4.4 Funding

Financial issues are yet another hindrance in the development of nanocarrier-based systems, as it is difficult to demonstrate their efficacy and safety to gain regulatory approval using traditional medicine's guidelines (Rebelo and Reis 2018). The majority of currently approved nano pharmaceuticals are based on already approved conventional drugs, and their contribution is still negligible. Only a small number of nanotherapeutics are currently in the development stage and will get regulatory approval.

## 7.5 Conclusion

Several attempts have been made recently to deliver chemotherapeutic drugs such as gemcitabine and gene inhibitors such as siRNA via nanoparticles. Various nanoparticles, including polymeric, inorganic and lipid-based NPs, have been created to suppress pancreatic cancer development, and metastasis in addition to efforts

to enhance gemcitabine administration, a front-line treatment in PDAC. There is also optimism that siRNA therapies will be utilized to inhibit pancreatic cancer development by overcoming drug resistance, decreasing off-target toxicity, and improving chemotherapeutic agent antitumour effectiveness. PDAC may be treated well by targeting KRAS, EGFR, and genes. Developing nanoparticles that contain both siRNA molecules and chemotherapeutic drugs and then delivering them to cancer-specific receptors to enhance active cancer cell delivery has emerged as a viable treatment for PDAC. Most nanoparticles have desirable characteristics in vitro, such as toxicity and stability, but in vivo safety and toxicity profiles may vary. As a result, after in vivo administrations, comprehensive safety and toxicology investigations should be performed. Both cancer cells and the tumour microenvironment are anticipated to be targeted by novel formulations as well as communication networks in the stroma that support cancer cells. Targeting signalling pathways active in both the stroma and tumour compartments is effective. In conclusion, nanoparticles are probably more widely used in the era of personalized drugs to create single- or multi-gene targeting therapeutic approaches as well as chemotherapeutic or small molecule inhibitors.

## References

Albanese A, Lam AK, Sykes EA, Rocheleau JV, Chan WCW (2013) Tumour-on-a-chip provides an optical window into nanoparticle tissue transport. Nat Commun 4:2718. https://doi.org/10.1038/ncomms3718

Alexis F, Pridgen E, Molnar LK, Farokhzad OC (2008) Factors affecting the clearance and biodistribution of polymeric nanoparticles. Mol Pharm 5(4):505–515. https://doi.org/10.1021/mp800051m

Apte MV, Wilson JS, Lugea A, Pandol SJ (2013) A starring role for stellate cells in the pancreatic cancer microenvironment. Gastroenterology 144(6):1210–1219. https://doi.org/10.1053/j.gastro.2012.11.037

Aslan M, Shahbazi R, Ulubayram K, Ozpolat B (2018) Targeted therapies for pancreatic cancer and hurdles ahead. Anticancer Res 38:6591–6606

Attia MF, Anton N, Wallyn J, Omran Z, Vandamme TF (2019) An overview of active and passive targeting strategies to improve the nanocarriers efficiency to tumour sites. J Pharm Pharmacol 71(8):1185–1198. https://doi.org/10.1111/jphp.13098

Bao Z, Liu X, Liu Y, Liu H, Zhao K (2016) Near-infrared light-responsive inorganic nanomaterials for photothermal therapy. Asian J Pharmaceutical Sci 11:349–364. https://doi.org/10.1016/j.ajps.2015.11.123

Brand RE et al (2007) Advances in counselling and surveillance of patients at risk for pancreatic cancer. Gut 56(10):1460–1469. https://doi.org/10.1136/gut.2006.108456

Cai L et al (2017) Expression status of folate receptor alpha is a predictor of survival in pancreatic ductal adenocarcinoma. Oncotarget 8(23):37646–37656. https://doi.org/10.18632/oncotarget.16841

Camp ER et al (2013) Transferrin receptor targeting nanomedicine delivering wild-type p53 gene sensitizes pancreatic cancer to gemcitabine therapy. Cancer Gene Ther 20(4):222–228. https://doi.org/10.1038/cgt.2013.9

Charo C et al (2013) Prostaglandin E2 regulates pancreatic stellate cell activity via the EP4 receptor. Pancreas 42(3):467–474. https://doi.org/10.1097/MPA.0b013e318264d0f8

Chauhan VP et al (2013) Angiotensin inhibition enhances drug delivery and potentiates chemotherapy by decompressing tumour blood vessels. Nat Commun 4:2516. https://doi.org/10.1038/ncomms3516

Chen D et al (2012) Rapid discovery of potent siRNA-containing lipid nanoparticles enabled by controlled microfluidic formulation. J Am Chem Soc 134(16):6948–6951. https://doi.org/10.1021/ja301621z

Chiaravalli M, Reni M, O'Reilly EM (2017) Pancreatic ductal adenocarcinoma: state-of-the-art 2017 and new therapeutic strategies. Cancer Treat Rev 60:32–43. https://doi.org/10.1016/j.ctrv.2017.08.007

Collins MA et al (2012) Oncogenic Kras is required for both the initiation and maintenance of pancreatic cancer in mice. J Clin Invest 122(2):639–653. https://doi.org/10.1172/JCI59227

Couvreur P et al (2008) Discovery of new hexagonal supramolecular nanostructures formed by squalenoylation of an anticancer nucleoside analogue. Small 4(2):247–253. https://doi.org/10.1002/smll.200700731

Cowan RW, Maitra A (2014) Genetic progression of pancreatic cancer. Cancer J 20(1):80–84. https://doi.org/10.1097/PPO.0000000000000011

Dariya B, Alam A, Nagaraju GP (2019) Biology, pathophysiology, and epidemiology of pancreatic cancer. Theranostic approach for pancreatic cancer. Elsevier Inc. https://doi.org/10.1016/B978-0-12-819457-7.00001-3

Desai P, Ann D, Wang J, Prabhu S (2019) Pancreatic cancer: recent advances in nanoformulation-based therapies. Crit Rev Ther Drug Carrier Syst 36:59–91

Ellermeier J et al (2013) Therapeutic efficacy of bifunctional siRNA combining TGF-β1 silencing with RIG-I activation in pancreatic cancer. Cancer Res 73(6):1709–1720. https://doi.org/10.1158/0008-5472.CAN-11-3850

Elliott RL, Blobe GC (2005) Role of transforming growth factor beta in human cancer. J Clin Oncol 23(9):2078–2093. https://doi.org/10.1200/JCO.2005.02.047

Eser S, Schnieke A, Schneider G, Saur D (2014) Oncogenic KRAS signalling in pancreatic cancer. Br J Cancer 111(5):817–822. https://doi.org/10.1038/bjc.2014.215

Guo W, Chen W, Yu W, Huang W, Deng W (2013) Small interfering RNA-based molecular therapy of cancers. Chin J Cancer 32(9):488–493. https://doi.org/10.5732/cjc.012.10280

Guo J, Xie K, Zheng S (2016) Molecular biomarkers of pancreatic intraepithelial neoplasia and their implications in early diagnosis and therapeutic intervention of pancreatic cancer. Int J Biol Sci 12(3):292–301. https://doi.org/10.7150/ijbs.14995

Honselmann KC et al (2015) Regulation mechanisms of the hedgehog pathway in pancreatic cancer: a review. J Pancreas 16(1):25–32. https://doi.org/10.6092/1590-8577/2894

Huh D et al (2010) Reconstituting organ-level lung functions on a chip. Science (80-) 328(5986):1662–1668. https://doi.org/10.1126/science.1188302

Hwang RF et al (2012) Inhibition of the hedgehog pathway targets the tumor-associated stroma in pancreatic cancer. Mol Cancer Res 10(9):1147–1157. https://doi.org/10.1158/1541-7786.MCR-12-0022

Javle M et al (2014) Biomarkers of TGF-β signaling pathway and prognosis of pancreatic cancer. PLoS One 9(1):e85942. https://doi.org/10.1371/journal.pone.0085942

Ji Z, Mei FC, Xie J, Cheng X (2007) Oncogenic KRAS activates hedgehog signaling pathway in pancreatic cancer cells. J Biol Chem 282(19):14048–14055. https://doi.org/10.1074/jbc.M611089200

Khvalevsky EZ et al (2013) Mutant KRAS is a druggable target for pancreatic cancer. Proc Natl Acad Sci U S A 110(51):20723–20728. https://doi.org/10.1073/pnas.1314307110

Kim EJ et al (2014) Pilot clinical trial of hedgehog pathway inhibitor GDC-0449 (vismodegib) in combination with gemcitabine in patients with metastatic pancreatic adenocarcinoma. Clin Cancer Res 20(23):5937–5945. https://doi.org/10.1158/1078-0432.CCR-14-1269

Knickelbein K, Zhang L (2015) Mutant KRAS as a critical determinant of the therapeutic response of colorectal cancer. Genes and Diseases 2(1):4–12. https://doi.org/10.1016/j.gendis.2014.10.002

Lesina M et al (2011) Stat3/Socs3 activation by IL-6 Transsignaling promotes progression of pancreatic intraepithelial neoplasia and development of pancreatic cancer. Cancer Cell 19(4): 456–469. https://doi.org/10.1016/j.ccr.2011.03.009

Li J, Wientjes MG, Au JLS (2010) Pancreatic cancer: pathobiology, treatment options, and drug delivery. AAPS J 12(2):223–232. https://doi.org/10.1208/s12248-010-9181-5

Lin G et al (2013) Biodegradable nanocapsules as siRNA carriers for mutant k-ras gene silencing of human pancreatic carcinoma cells. Small 9(16):2757–2763. https://doi.org/10.1002/smll.201201716

Lin D et al (2014) High fidelity patient-derived xenografts for accelerating prostate cancer discovery and drug development. Cancer Res 74(4):1272–1283. https://doi.org/10.1158/0008-5472.CAN-13-2921-T

Liu MP et al (2016) Sanguisorba officinalis L synergistically enhanced 5-fluorouracil cytotoxicity in colorectal cancer cells by promoting a reactive oxygen species-mediated, mitochondria-caspase-dependent apoptotic pathway. Sci Rep 6:34245. https://doi.org/10.1038/srep34245

Makohon-Moore A, Iacobuzio-Donahue CA (2016) Pancreatic cancer biology and genetics from an evolutionary perspective. Nat Rev Cancer 16(9):553–565. https://doi.org/10.1038/nrc.2016.66

Maksimenko A, Caron J, Mougin J, Desmaële D, Couvreur P (2015) Gemcitabine-based therapy for pancreatic cancer using the squalenoyl nucleoside monophosphate nanoassemblies. Int J Pharm 482(1–2):38–46. https://doi.org/10.1016/j.ijpharm.2014.11.009

Malkoski SP, Wang XJ (2012) Two sides of the story? Smad4 loss in pancreatic cancer versus head-and-neck cancer. FEBS Lett 586(14):1984–1992. https://doi.org/10.1016/j.febslet.2012.01.054

Martin LK et al (2012) VEGF remains an interesting target in advanced pancreas cancer (APCA): results of a multi-institutional phase II study of bevacizumab, gemcitabine, and infusional 5-fluorouracil in patients with APCA. Ann Oncol 23(11):2812–2820. https://doi.org/10.1093/annonc/mds134

Mazur PK et al (2010) Notch2 is required for progression of pancreatic intraepithelial neoplasia and development of pancreatic ductal adenocarcinoma. Proc Natl Acad Sci U S A 107(30): 13438–13443. https://doi.org/10.1073/pnas.1002423107

Mccleary-Wheeler AL, Mcwilliams R, Fernandez-Zapico ME (2012) Aberrant signaling pathways in pancreatic cancer: a two compartment view. Mol Carcinog 51(1):25–39. https://doi.org/10.1002/mc.20827

Mocan T et al (2014) Photothermal treatment of human pancreatic cancer using PEGylated multi-walled carbon nanotubes induces apoptosis by triggering mitochondrial membrane depolarization mechanism. J Cancer 5(8):679–688. https://doi.org/10.7150/jca.9481

Nawroth R et al (2007) Extracellular sulfatases, elements of the Wnt signaling pathway, positively regulate growth and tumorigenicity of human pancreatic cancer cells. PLoS One 2(4):e392. https://doi.org/10.1371/journal.pone.0000392

Olive KP et al (2009) Inhibition of hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. Science (80-) 324(5933):1457–1461. https://doi.org/10.1126/science.1171362

Pan X et al (2015) PLGA/poloxamer nanoparticles loaded with EPAS1 siRNA for the treatment of pancreatic cancer in vitro and in vivo. Int J Mol Med 35(4):995–1002. https://doi.org/10.3892/ijmm.2015.2096

Patino T et al (2015) Multifunctional gold nanorods for selective plasmonic photothermal therapy in pancreatic cancer cells using ultra-short pulse near-infrared laser irradiation. Nanoscale 7:5328–5337. https://doi.org/10.1039/c5nr00114e

Perrault SD, Walkey C, Jennings T, Fischer HC, Chan WCW (2009) Mediating tumor targeting efficiency of nanoparticles through design. Nano Lett 9(5):1909–1915. https://doi.org/10.1021/nl900031y

Philip PA et al (2010) Phase III study comparing gemcitabine plus cetuximab versus gemcitabine in patients with advanced pancreatic adenocarcinoma: southwest oncology group-directed intergroup trial S0205. J Clin Oncol 28(22):3605–3610. https://doi.org/10.1200/JCO.2009.25.7550

Phillips PA et al (2003) Cell migration: a novel aspect of pancreatic stellate cell biology. Gut 52(5): 677–682. https://doi.org/10.1136/gut.52.5.677

Poon C, He C, Liu D, Lu K, Lin W (2015) Self-assembled nanoscale coordination polymers carrying oxaliplatin and gemcitabine for synergistic combination therapy of pancreatic cancer. J Control Release 201:90–99. https://doi.org/10.1016/j.jconrel.2015.01.026

Pylayeva-Gupta Y, Lee KE, Hajdu CH, Miller G, Bar-Sagi D (2012) Oncogenic Kras-induced GM-CSF production promotes the development of pancreatic neoplasia. Cancer Cell 21(6): 836–847. https://doi.org/10.1016/j.ccr.2012.04.024

Rebelo A, Reis C (2018) Emerging therapeutic nanotechnologies in pancreatic cancer: advances, risks and challenges. Ther Deliv 9:691–694

Rolland JP et al (2005) Direct fabrication and harvesting of monodisperse, shape-specific nanobiomaterials. J Am Chem Soc 127(28):10096–10100. https://doi.org/10.1021/ja051977c

Rongvaux A et al (2014) Development and function of human innate immune cells in a humanized mouse model. Nat Biotechnol 32(4):364–372. https://doi.org/10.1038/nbt.2858

Rucki AA, Zheng L (2014) Pancreatic cancer stroma: understanding biology leads to new therapeutic strategies. World J Gastroenterol 20(9):2237–2246. https://doi.org/10.3748/wjg.v20.i9.2237

Ryschich E et al (2004) Transferrin receptor is a marker of malignant phenotype in human pancreatic cancer and in neuroendocrine carcinoma of the pancreas. Eur J Cancer 40(9): 1418–1422. https://doi.org/10.1016/j.ejca.2004.01.036

Schultheis B et al (2014) First-in-human phase I study of the liposomal RNA interference therapeutic Atu027 in patients with advanced solid tumors. J Clin Oncol 32(36):4141–4148. https://doi.org/10.1200/JCO.2013.55.0376

Shen W et al (2017) TGF-β in pancreatic cancer initiation and progression: two sides of the same coin. Cell Biosci 7:39. https://doi.org/10.1186/s13578-017-0168-0

Shi J, Kantoff PW, Wooster R, Farokhzad OC (2017) Cancer nanomedicine: Progress, challenges and opportunities. Nat Rev Cancer 17:20–37

Singh D, Upadhyay G, Srivastava RK, Shankar S (2015) Recent advances in pancreatic cancer: biology, treatment, and prevention. Biochimica et Biophysica Acta–Reviews on Cancer 1856(1):13–27. https://doi.org/10.1016/j.bbcan.2015.04.003

Sung H et al (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

Teo J et al (2016) A rationally optimized nanoparticle system for the delivery of RNA interference therapeutics into pancreatic tumors in vivo. Biomacromolecules 17(7):2337–2351. https://doi.org/10.1021/acs.biomac.6b00185

Toh YC et al (2009) A microfluidic 3D hepatocyte chip for drug toxicity testing. Lab Chip 9(14): 2026–2035. https://doi.org/10.1039/b900912d

Valencia PM et al (2010) Single-step assembly of homogenous lipid-polymeric and lipid-quantum dot nanoparticles enabled by microfluidic rapid mixing. ACS Nano 4(3):1671–1679. https://doi.org/10.1021/nn901433u

Van Cutsem E et al (2004) Phase III trial of gemcitabine plus tipifarnib compared with gemcitabine plus placebo in advanced pancreatic cancer. J Clin Oncol 22(8):1430–1438. https://doi.org/10.1200/JCO.2004.10.112

Vanhaesebroeck B, Guillermet-Guibert J, Graupera M, Bilanges B (2010) The emerging mechanisms of isoform-specific PI3K signalling. Nat Rev Mol Cell Biol 11(5):329–341. https://doi.org/10.1038/nrm2882

Wang Z et al (2006) Down-regulation of Notch-1 contributes to cell growth inhibition and apoptosis in pancreatic cancer cells. Mol Cancer Ther 5(3):483–493. https://doi.org/10.1158/1535-7163.MCT-05-0299

Xu C, Wang J (2015) Delivery systems for siRNA drug development in cancer therapy. Asian J Pharmaceutical Sci 10:1–12. https://doi.org/10.1016/j.ajps.2014.08.011

Xu J et al (2013) Future of the particle replication in nonwetting templates (PRINT) technology. Angew Chemie–Int Ed 52(26):6580–6589. https://doi.org/10.1002/anie.201209145

Yin F et al (2015) A light-driven therapy of pancreatic adenocarcinoma using gold nanorods-based nanocarriers for co-delivery of doxorubicin and siRNA. Theranostics 5(8):818–833. https://doi.org/10.7150/thno.11335

Zeitouni D, Pylayeva-Gupta Y, Der CJ, Bryant KL (2016) KRAS mutant pancreatic cancer: no lone path to an effective treatment. Cancers 8(4):45. https://doi.org/10.3390/cancers8040045

Zeng G et al (2006) Aberrant Wnt/β-catenin signaling in pancreatic adenocarcinoma. Neoplasia 8(4):279–289. https://doi.org/10.1593/neo.05607

Zhou H et al (2015) IGF1 receptor targeted Theranostic nanoparticles for targeted and image-guided therapy of pancreatic cancer. ACS Nano 9(8):7976–7991. https://doi.org/10.1021/acsnano.5b01288

Zuckerman JE et al (2014) Correlating animal and human phase Ia/Ib clinical data with CALAA-01, a targeted, polymer-based nanoparticle containing siRNA. Proc Natl Acad Sci U S A 111(31):11449–11454. https://doi.org/10.1073/pnas.1411393111

Zwicke GL, Ali Mansoori G, Jeffery CJ (2012) Utilizing the folate receptor for active targeting of cancer nanotherapeutics. Nano Rev 2012:3. https://doi.org/10.3402/nano.v3i0.18496

# Pancreatic Cancer Treatment by Using Theragnostic Nanoparticles

8

Sabya Sachi Das, Amit Kumar Dubey, Neeru Singh, Priya Ranjan Prasad Verma, Shubhankar Kumar Singh, and Sandeep Kumar Singh

**Abstract**

Pancreatic cancer (PC) is becoming a major cause of cancer death and is difficult to treat due to poor detection and late-stage diagnosis. However, various approaches have been implemented for the detection of PC at an early stage. Apart from this several chemotherapeutic drugs are suggested for patients diagnosed with PC but their applications are restricted due to systemic toxicity

Sabya Sachi Das and Amit Kumar Dubey contributed equally with all other contributors.

S. S. Das
Department of Pharmaceutical Sciences and Technology, Birla Institute of Technology, Ranchi, Jharkhand, India

School of Pharmaceutical and Population Health Informatics, DIT University, Dehradun, Uttarakhand, India

A. K. Dubey
Department of Biotechnology, National Institute of Pharmaceutical Education and Research (NIPER), Hajipur, Bihar, India

Division of Microbiology, Parasite Immunology Lab, Indian Council of Medical Research (ICMR)-Rajendra Memorial Research Institute of Medical Sciences (RMRIMS), Patna, Bihar, India

N. Singh
Department of Biomedical Laboratory Technology, University Polytechnic, Birla Institute of Technology, Ranchi, Jharkhand, India

P. R. P. Verma · S. K. Singh (✉)
Department of Pharmaceutical Sciences and Technology, Birla Institute of Technology, Ranchi, Jharkhand, India
e-mail: sandeep.singh@bitmesra.ac.in

S. K. Singh
Department of Biotechnology, National Institute of Pharmaceutical Education and Research (NIPER), Hajipur, Bihar, India



M. Rahman et al. (eds.), *Hormone Related Cancer Mechanistic and Nanomedicines*,
https://doi.org/10.1007/978-981-19-5558-7_8

due to long-term usage. Nanotechnology-based therapeutic approaches have gained significant importance in both early-stage detection and targeted drug delivery in PC therapy. The nanoparticles assist in the sustained and targeted release of a drug regimen to cancer/tumor-specific sites rather than affecting healthy cells, leading to negligible or no toxicity. In this chapter, we have highlighted the significant role of various PC-specific receptors, the mechanism of nanocarrier accumulation in carcinogenic cells, biomarkers in PC diagnosis, and the role of nanocarriers in theranostic applications in the PC microenvironment.

**Keywords**

Pancreatic cancer · Molecular mechanism · Targeted nanocarrier · Drug delivery · Biomarkers · Theranostics

## 8.1 Introduction

Pancreatic cancer is claimed to be the seventh type of demise in earlier reports. Then, gradually, it becomes the fourth prime reason for tumor-related mortality in Europe and/or the USA (Kunnumakkara et al. 2007; Shamseddine et al. 2014). In most of (90%) pancreatic cancer clinical examinations, it is normally called pancreatic ductal carcinoma (Ansari et al. 2015). The delayed diagnosis and/or asymptomatic at the beginning stages is the major hurdle. Until diagnosis is done, pancreatic cancer reached late stages. This stage is unfortunate as the cancer multiplies rapidly and is resistible to treatment (Li et al. 2016). The substandard prognosis is further held responsible for delayed diagnosis. The clinical examination in 80% of cases which are presented for histopathology already outstretched to metastasis or final stages of cancer (Klein 2012). Hereafter, the only possibility that remain in treatment is surgery (of tissue or part of an organ), cut out for further spreading the tumor (Macgregor-Das and Iacobuzio-Donahue 2012). This is the only present functional approach to counteract the mortality before the tumor worsens. The previous data reveal that pancreatic adenocarcinoma has a very little survival rate (6%) in the last five years, as reported by the American cancer society (ACS). Pancreatic exocrine cancer is so much fatal that only 4.1/one lakh patients survived (Siegel et al. 2014). This tumor genesis is a mass or lump of tissues, a sclerotic mass of penetrating ductal carcinoma. The corners of those tumors persist long spread carcinoma mass that lays away from the primary tumor sites. This results in insignificant knowledge of pancreatic corners/edges. Adeno carcinomas are infiltrative abnormal growth and sometimes invade vascular and perineural areas. This affects other areas of the human body and cancer metastasis with severity (Maitra and Hruban 2008; Yachida and Iacobuzio-Donahue 2009). As discussed above, tissue resection is the last option in cancer morbidity but due to delayed diagnosis and critical situations, only a few

number (9–15%) of cases are available for surgery (Patra et al. 2008). The postsurgical morbidity complications also have been seen in pancreatic cancer cases. This happened because of delayed gastric emptying and pancreatic anastomotic leaks. About 40% of complicated cases were reported after pancreatic resection surgery (Vincent et al. 2011). The study found that chemo-radiations on patients did not better the situation and survival rate. Therefore, chemotherapy is the only treatment option with moderate results (Neoptolemos et al. 2004). These treatment modalities also have limitations because radiotherapy is generally postponed until chemotherapy goes on. About 70% systemic and exceeding 20% local recurrence occur after postsurgery of tumor and clinicians cannot perform radiotherapy due to treatment modalities. To counteract the effects of chemo-medications, the adjuvant "chemo" is given to cancer survivors (Redmond et al. 2010).

Basic treatments, such as Gemcitabine (GEM), are given to patients with advanced stages of cancer. The combination therapy such as GEM and Capecitabine is also prescribed in some other cases (Cunningham et al. 2009; Burris et al. 1997). Indeed, toxicological parameters and/or drug bioavailability are the main factors in redefining the regimen (Bramhall et al. 2002; Yallapu et al. 2013). Moreover, it was observed that inaccurate prognosis is due to drug resistance of chemo-agents in pancreatic tumor cases (Erkan et al. 2012). It was also found, in adenocarcinoma, that little blood circulation was delivered at tumor sites when seen in preclinical and clinical studies. Therefore, chemo-resistance occurs because of improper blood supply at adequate area. The drug delivery methods then become inefficient for treating cancer. One of the major discoveries elaborated that chemotherapeutic resistance can be overcome by targeting the stromal hedgehog pathway. This pathway targeted drug agent ameliorate the delivery system and profound achievement in medication fulfilled in pancreatic cancer (Olive et al. 2009). In consequence, the present decade of research work focuses on in vivo toxicological assessment of chemotherapeutic agents to elucidate and settle down resistance problems. Repurposing the existing drugs and novel drug delivery methods or nanocarriers are the solving strategies and innovation in pharm-nanobiotechnology leads to the best hits in chemotherapy. The nanoparticle-based vehicles (NPs encapsulated drugs) to target sites are the modus operandi in modern therapeutics. This approach is used these days for the exact delivery at tumor sites/bio-distribution with minimum toxicity on healthy tissues (Harshita et al. 2020). Bypassing the GIT by nanosystem drug conjugates and tracking-targeting tumor sites, this will resolve the therapeutics degradations and upgrade the adsorption, dissolution, metabolism, excretion ratios (ADME) and pharmacokinetics. These are liquid crystalline nanoparticles (LCNPs), Fe2O3/Fe3O4 NPs with curcumin as a drug moiety, versatile delivery vehicle super nanoporous silica NPs (2–50 nm) with lipid coats, solid-lipid NPs (lipid nanocarriers), etc. Recently, poly (lactic-co-glycolic acid) nanoparticles and chitosan-derived polymers encapsulation approaches are at promising level in anticancer therapy for pancreatic tumor (Freag et al. 2016).

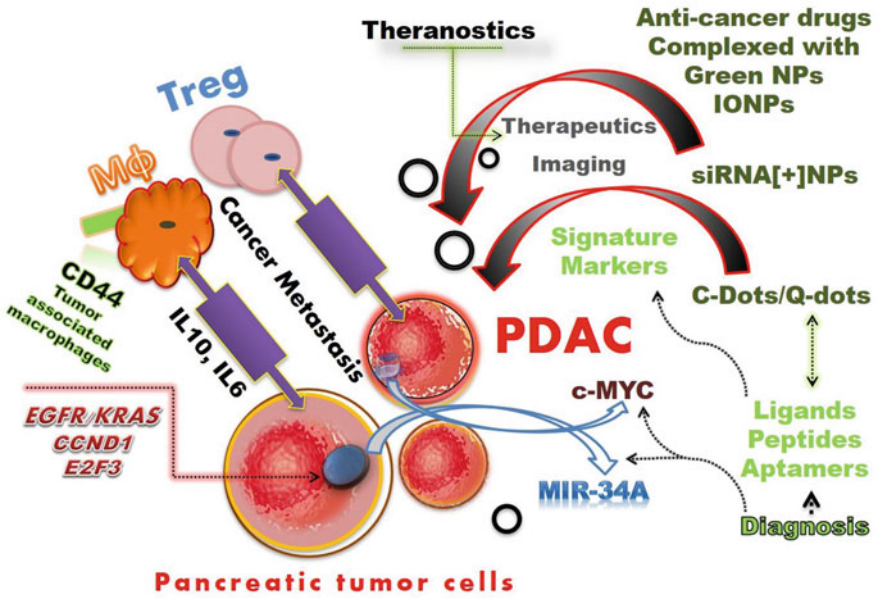



**Fig. 8.1** Schematic illustration of various molecular targets and theragnostic approaches associated with pancreatic cancer therapy

### 8.1.1 Targeting Surface Receptors

The gene expression of transmembrane receptors (cell surface-anchored) is linked to disease proliferation or metastasis. The signal transduction via several surface receptors is the prime mechanism in pancreatic cancer progression. Some antagonists are approved in anticancer therapy such as epidermal growth factor receptor (EGFR) signaling pathway inhibitors, e.g., Erlotinib; this inhibition did not improvise the patient's health appreciably. Noble inhibitors are being developed against various receptors, but more specific therapeutic targets are still needed. Moreover, the probable mechanism associated with pancreatic cancer molecular targeting and the effects of diverse theragnostic approaches have been illustrated in Fig. 8.1 and have been detailed below.

#### 8.1.1.1 Transferrin Receptors (TFRC)

Heme uptake is required for tumor cell growth onset and progression. Transferrin receptor protein 1 (TfR1)/CD71, its dysregulation, is observed in many pancreatic cancer cases. It was diagnosed that nearly 93% pancreatic tumor tissues over-expressed TFRC-markers, mostly in malignant cells. This is a very important specific biomarker significantly used in diagnostics (El-Zahaby et al. 2019; Jeong et al. 2016). A study is performed on pancreatic ductal adenocarcinoma (PDAC) in BALB/c model for preclinical data. Here, liposome-derived nanoparticles were used that conjugates with a single monomeric variable antibody domain/fragment and for

TFRC targeting. Gemcitabine was also used in combination with liposome NPs: anti-transferrin receptor single-chain antibody fragment (TfRscFv) targeted for in vivo efficacy evaluation to target tumor cells. This model was found to be highly successful and the proliferation of cancer was also decreased in in vivo animal model (Ryschich et al. 2004).

#### 8.1.1.2 Folate Receptor (FR)

Specialized biomarkers for pancreatic tumor detection. These receptors are membrane-bound surface proteins; ligate with folate molecules, glycosylphosphatidylinositol-anchored receptors. Its overexpression is found in 80% of pancreatic ductal adenocarcinoma cases, mostly in lethal tumor situations. The FRs are mostly over-expressed in PDAC cases and minimally expressed in healthy cells. Therefore, these specific receptors are an excellent target for nanoparticle-based therapeutics (Camp et al. 2013).

#### 8.1.1.3 Epidermal Growth Factor Receptor (EGFR/HER1), Vascular Endothelial Growth Factor (VEGF), and Insulin-like Growth Factor (IGF) Receptor-Targeted Delivery

These molecular receptors are also diagnosed and/or assessed in both animal models and cancer patients. In addition, these three receptors are targeted by monoclonal Abs for histopathological nonclinical/clinical considerations. A study reported in clinical oncology, phase-III clinical trials, was performed on advanced pancreatic carcinoma. Here, gemcitabine+cetuximab combination vs gemcitabine was used for human trial phase-III. The results were not satisfactory in targeting EGFR with combination therapy (Cai et al. 2017; Philip et al. 2010).

## 8.2 Pancreatic Microenvironment and Impact of Nanocarriers

To monitor patient compliances in cancer diseases, clinicians should know well about the disordered physiological processes/pathophysiology. Secondarily, nanotechnology-based bioimaging, theragnostics, nano biotechnology understanding, and nano-grafted designing are the essential advanced techniques that should be used in pancreatic cancer targeting, increasing the residential time and decreasing the dosing frequency within the tumor (Martin et al. 2012).

The two primarily involved factors in the pancreatic cancer microenvironment are stromal invasion (invasive growth of cancer cells into fibrotic septa or within the expanded portal tract) and hypo-vascularity (Fatty/hypoxic changes in vasculatures arteries and/or veins). The stroma persists in numerous cells but the specific fibrous stromal cells are known as pancreatic resident cells/stellate cells (PSCs). The fibrotic modification decreases the vasculature, the root cause behind ill pathophysiological state. These two factors lead to complications in disease management of PDACs cases. Both manifestations hype the critical situation in cancer case handling (Das et al. 2020d; Li et al. 2010). Hence, nanotechnologist is considering pancreatic stroma as a prominent target, i.e., advancement in nanocarrier targeting pancreatic

tumor. Another established molecular switch turn is over mass production of mucin in pancreatic tumor. In healthy tissues, there is minimal production of mucin but over-expression of mucin boosts cancer to migrate to different locations. Therefore, over-expression of mucin helps in the metastasis of pancreatic cancer (Bachem et al. 2005). In recent times, most of the research focuses on nanoformulations to investigate xenograft cancer models (tumor xenograft) or immune-compromised models in preclinical studies. Here, tumor cells' sensitivity and functionality are tested; tumor cells are implanted in the same organs of animals where cancer originates. Afterward, the mechanism has to be understood, but still much unique information is lacking in these pilot studies. The efficacy and toxicity parameters of nanoformulations have to be well-understood with more and more preclinical testing. To see into the depth of tumor microenvironment changes after drug nanocarrier: conjugates targeting, researchers have to test with specific animal models, e.g., EL1-luc/TAg transgenic mice. These mice are ideally suited for pancreatic cancer-related studies as specific bioluminescence signal are produced whenever the tumor progress. One can determine penetration, bioavailability, systemic toxicity, etc., of these targeted nano-grafted formulations in cases of adenocarcinoma (Kaur et al. 2013). Challenges in nanocarriers formulation related to the diseased microenvironment in both diagnosis and treatment of pancreatic cancer will be more discussed in this article in detail under relevant sections.

### 8.2.1 Mechanisms of NPs Accumulation in Tumors

There are two ways of tumor targeting by novel drug delivery system, active and passive. In active targeting, direct binding of decorated NPs as ligand-receptor (Antibody, peptides, etc.); selective accumulation and uptake of designed NPs in effected site. Another way of neoplastic tissue targeting is passive which relies on the enhanced permeability and retention (EPR) effect. High molecular weight prodrugs encapsulated in NPs (size varies) are targeted to the cancer sites. Due to different NPs sizes, two primary characteristics are involved in this EPR-based approaches of NPs targeting cancer, that is, rifty vasculature and faulty lymphatic drainage. These two properties are significant for tumor tissues (Cabral et al. 2013). The vasculature permeabilization and payload retention pave the way for NPs accumulation, and, thus, enhance neoplasm concentration. In passive targeting, membrane-bound proteins carry the drug cargo complex and this is basically known as diffusion-mediated transport. So, different NPs' topological/geometrical parameters are responsible for cellular compartment internalization. While in active targeting, receptor-facilitated transport occurs. After diagnosis, few biomarkers are detected which are upregulated from cancer cells, through them drug-loaded NPs cargos complexes are uptaken and assembled at tumor sites or tumor microenvironment (Bazak et al. 2014). Some receptors that are specifically over-expressed or only unique to cancer cells enable and facilitate the uptake of NPs and therapeutic cargo accumulation of chemotherapeutic agents in the tumor. Targeting moieties, such as high-affinity ligands (i.e., antibodies, Ab-fragments, peptides, aptamers) that bind to

the specific receptors on tumors, are usually used to functionalize the surface of the delivery system to increase NP/drug uptake.

### 8.2.2 Nanocarriers-Loaded Drug Delivery Systems for PC Therapy

The mostly used chemotherapeutic since the last 40–50 years is 5-Fluorouracil (5-FU); antineoplastic agent given usually through i.v routes. It is one of the best anticancer drugs, also as the first line of drug recommended in different cancer types, specifically highly potent and highly competent to work against breast, colon, pancreas, skin, throat, mouth cancer, etc. (Danhier et al. 2010; Neoptolemos et al. 2010). This drug is a sparingly soluble drug when given orally. Its short half-life and insufficient bioavailability lead to faster desorption and metabolism. Moreover, being the reason behind sparingly soluble and incomplete oral absorption, this drug is metabolized by dihydropyrimidine dehydrogenase (Chandran et al. 2017). If we focus on pharmacological properties, then it causes gastrointestinal irritation during absorption, interference in hematological consequences, changes in dermatology (skin), heart-related problems, other serious side effects, etc. (Diasio and Lu 1994). To decrease the dose-related side effects, nanometer size carrier with gemcitabine (GEM) is designed as de novo drug delivery against cancer. These GEM: AuNPs (Gold NPs) reduce systemic toxicity and enhance clinical aftereffect. In this study, cetuximab-C225 (EGFR antibody) is applied as a targeting agent. This novel preparation was a multistep process and had a higher inhibitory effect on pancreatic exocrine cancer cells. This approach was not only good at inhibiting cellular proliferation of cancer cells but also less toxicity profile to healthy tissues in patients. The preparation involves firstly conjugate of gold NPs (GNP) to C225 Abs, secondarily fabrication of gold rod (GEM+C225). Then, the nano-designed product is brought up to ultracentrifuge to get an excellent dispersion outcome (Chandran et al. 2017). A few years back, Trabulo S. and coworkers developed multifunctional Fe2O3/Fe3O4 nanoparticles addition with GEM. These special magnetic properties bearing iron oxide NPs (MNP) comprised of anti-CD47 Abs and drug conjugates in a vial. This research has proven the expected results and the combined therapy achieved suppression/downregulation in pancreatic adenocarcinoma outgrowth in vivo (Patra et al. 2008).

Moving further, a study was performed by Lee et al. for pancreatic tumors magnetic resonance bioimaging (contrast modified-MRI) and GEM-targeted therapy implanted via theranostic methodology. Here, sustained release of gemcitabine as an antitumor is targeted and delivered through activated peptide solution in vivo. This formulation is synthesized as iron oxide NPs-GEM-activated peptides and this is facilitated by clathrin-mediated endocytosis intra-cellular (Trabulo et al. 2017). Dai JT *research* team proposed that gemcitabine encapsulated into cell-derived nanovesicles (mesoporous silica NPs vesicles) targets pancreatic neoplasm (Lee et al. 2013). Nigam P. and his colleagues researched graphene nano-dots which are conjoint with blood serum albumin-nanoparticles (BSA-NPs); loaded with gemcitabine. The results showed negligible toxicity and 3D bioimaging of human

pancreatic cancer cell line (Panc-1) tumor cells. The cell viability found was about 90% in this case (Dai et al. 2017). Wang L et al. showed GEM-loaded magnetic albumin nanospheres and iron oxide NPs are combined for surplus chemotherapy with cetuximab. This enables the MRI performance increment overall. This is an approach of double targeting via thermo-chemotherapy. This model showed maximum destruction of pancreatic tumor cells (Nigam et al. 2014). Moving further, a phase-II testing was performed by Halford S et al., studying the tolerance and efficacy (expected degree potency) of CAELYX; a concentric phospholipid bilayer vesicles conjugates with doxorubicin. This liposomal doxorubicin is also famous by the trade name "Doxil." In preclinical evaluation, prominent tumor cell growth inhibition with tolerable toxicity was observed (Wang et al. 2015). A different kind of study was performed by Murphy E.A. and colleagues on doxorubicin NPs (DOX:NPs) conjugated formulations. Here, he synthesized anticancer targeted peptides with DOX and they were delivered to the body to understand the αvβ3-integrin receptor. These receptors are well expressed and used to target tumor vasculature which is a marking point in pancreatic cancer. The systemic delivery of peptides-DOX-nanoparticles enhances targeting competency onto cancer cells. The results shown were 15-fold increased antitumor activity (Halford et al. 2001).

Thapa et al. designed vibrating droplet synergistic chemotherapy in addition to photo-therapy for delivering the drug DOX. He invented AuNPs hold DOX chemo agent and thermoagent-graphene oxide (TGO) for cancer treatment. Therewith chitosan polymers were used for robust surface-coating onto nanoparticles to stave off the quick clearance by the RES system. The RES system associates with mononuclear phagocyte system, widely known as reticuloendothelial system. The methodology was very simple, i.e., dissociation factor, purification without heat-generated reaction. Here, laminar jet break-up technique/microencapsulation with a vibration nozzle is used firstly then afterward drying optimization (diffusion drying process) to prepare doxorubicin droplets in addition to gold-graphene nanoparticles with a layer of chitosan Zwittor ion. The characterization proved of uniform size of NPs and transmission electron microscopy data vindicate the AuNPs deposition over oxide of graphene. The results of novel AuNPs:OxG:Chitosan onto Panc-1 and Mia Paca-2 cell lines diminished the toxicity and uptake/retention at cellular level (Murphy et al. 2008). Lo et al. prepared paclitaxel (Ptx) self-emulsifying oil in water nanoformulation mixed with electrically charged surfactants proven its bio-availability and minimal tissues toxicity (Thapa et al. 2018). Hu C-MJ in 2010 developed a de novo drug-targeting method encapsulated the drug paclitaxel. The structure lays ligands; anti-human carcinoembryonic antigen (CEA) monoclonal antibody (mAb), and hybrid NPs. Hybrid NPs are synthesized by functionalized liposomal formulation loaded with 0.1 mg of paclitaxel. As cancer proteomics said that CEA is expressed at a higher rate in tumor tissue, active bound structure/functionalized NPs are targeted against pancreatic cancer cells. A therapeutic window showed that this nanoconstruct attained twofold efficacies with Ptx on pancreatic neoplasm (Lo et al. 2010). Research by Zhang et al. furnished polyethylene glycol (PEG)-farnesylthiosalicylate (FTS) conjugated nanomicelles. He used PTX drug to deliver with nanomicellar system. Therefore, FTS is a prominent antagonist

to small GTPases RAS proteins (excessive tumor proliferator); hence, it was used while nanomicelles enhance the paclitaxel solubility and controlled releases. They draw a line via in vivo experiment that nanomiceller targeted PTX is better than marketed TAXOL (Hu et al. 2010). Kim et al. formed a natural nanoconstruct by compound turmeric combined with albumin nanoparticles in which PTX is encapsulated. This natural nanoconstruct found antitumor activity when they were targeted via GP60-mediated pathway (Tyrosine kinase-dependent) (Zhang et al. 2013). In another study, Bisht S and coworkers designed curcumin-grafted polymeric NPs against pancreatic tumor. The construct is framed by *N*-isopropylacrylamide, vinylpyrrolidone, and acrylic acid. The amides, tetrahydropyrrole, and poly (ethylene glycol) units showing hydrophilic behavior and nanomicelles were used for hydrophobicity in the central core. This hydrophobic-hydrophilic moiety NPs construct gave better outcomes rather than free turmeric as a drug. The systemic circulatory curcumin drug observed a greater extent, i.e., tissue/plasma bioavailability achieved by NPs delivery (Kim et al. 2016). A study published in molecular cancer therapeutics by Yallapu et al. developed a novel turmeric encapsulated magnetic NPs for anti-pancreatic tumor targeting. He used Muc-1/EMA as a molecular target (glycoprotein with extensive O-linked glycosylation or cell surface associated protein or epithelial membrane antigen) to check "Cur"-loaded nanoparticle's efficacy. He used a gold standard mice model (CDX model), also known as mouse xenograft tumor model. The most interesting fact examined is that about 80% of mouse model showed downward Muc-1 expression. The survival rate was also prolonged after delivery by Cur-loaded magnetic NPs in preclinical studies (Bisht et al. 2007). In recent years, to understand the tumor microenvironment, micro-RNA (miRNA/miR)-based researches are going on. Presently, miRNA-based molecular therapies strategies highlighted two major points which are applicable. One is mimicking cancer suppressor micro-RNA and secondarily, miR inhibition tactics by miR-inhibitors (oncogenic miRNA blockers) and miRNA gene silencing/knockdown, etc. (Yallapu et al. 2013; Ling et al. 2013). At preclinical stages, there are various studies on miR targeted, reported such as remedial delivery of miRNA-29b by positively charged lipoplexes against cancer and anticancer miRNA delivery targeted to eGFR by exosomes after systemic administration (Cheng et al. 2014; Wu et al. 2013). While some of them have registered on clinical platform/cancer registry (Ohno et al. 2013; Li and Sarkar 2016). A drug called Miravirsen had registered or first mentioned for clinical trials. This is a small RNA short locked nucleic acid drug tried for clinical medicine and this had gone up to II phase in human trials. A dose-related study of miravirsen in the treatment of chronic Hepatitis-C subjects; the molecular target was miR-122 in liver cells infected with HCV-RNA virus (Pai et al. 2013). One more micro-RNA mimic molecular drug (MRX34) combined with lipid NPs tried in phase-I human trials for calculation of safety and efficacy. Their aim was to develop a clinical practiced drug for solid tumors, e.g., Herpetological cancer, but can also be recommended for hematological cancers (Janssen et al. 2013). Therewith, a report on MRX34 + NPs complexes was published. Here, miR-34a impregnated nano-preparations delivered and targeted, examined on Panc-1 xenograft model for an anticancer modality

improvement. These testings showed a rise in micro-RNA-34a level but parallel downregulation shown in messenger RNA of B cell lymphoma-2 (BCL-2), cellular-Myc (c-Myc) factor/MYC proto-oncogenes, cyclin D-1 (CCND1), and E2F Transcription Factor 3 (E2F3), respectively (Bader 2012).

### 8.2.3 Circulating Biomarkers for Pancreatic Cancer Early Diagnosis

The signature molecules/Biomarkers are the biological molecules often used in bioassays development and their over/underexpression relates to early diagnosis. They are mostly produced by cancerous cells or tumor rafted organs. These can be gene mutated markers, nucleic acids, peptides, fatty acids, glycans, by-products of the cell, small molecules, etc. They are often related to the pathophysiology of cancer cells. The molecules which can differentiate between healthy and defected tumors are referred as putative signature molecules. Biomarkers discovery and validation need time and cancer management; the better the technology used, the better the biomarker-based diagnosis achieved (Hu et al. 2013). The methods and techniques that lead to signature molecule identification preferably depend on the need for discovery. Two things matter: novel biomarker discovery and dosage optimization of the known markers (Goossens et al. 2015). Present scenario, the scaled-up techniques such as bioinformatics, and transcriptomics allow the various forms of samples to understand biomedical markers evaluations. The biomolecules along with secondary metabolites quantification estimation at the laboratory stage and animal model studies lead to novel biomarker discoveries. Advance 3D imaging and morphological processes examination added will boost the naïve discovery in the indicator-biomarker field (Wu and Qu 2015). The diagnostic indicators that arouse from the clinical investigation of genomic approaches are thus known as genetic markers. The whole genome sequencing, Real-time PCR, fluorescence in situ hybridization probes (sometimes said as M-FISH) techniques, etc., are the few genetic tools to identify specific genomic markers. In forensic science, molecular fingerprinting is used for detection, possibly because of microarray (multiplex lab-on-a-chip). The same technique employed for expression profile is used to detect expression biomarkers in cancer cases. Through MALDI-TOF, protein-based new biomarkers discovery is possible because mass spectrometry can analyze small changes in proteomics. Therefore, genetics and proteomics involved techniques allow a specific discovery otherwise there will be a burden on clinical practices in cancer detection. Different PCR-based techniques lead to a faster and more accurate diagnosis. The sensitivity and specificity matter in diagnostics (Scaros and Fisler 2005). An immune-PCR methodology is introduced in prognosis/diagnosis where antibodies/DNA/RNA conjugates and signal amplification detection are used to get specific antigen findings, i.e., immune assays for protein biomarkers examination (Rifai et al. 2006; Niemeyer et al. 2005).

### 8.2.4 The "Bio-Nano" Interface in Cancer Nanotechnology

Nanotechnology is an interdisciplinary and applied field. Its translational outcomes have been proven in many fields such as engineering, molecular biology, physical science, and chemical science-related fields. The nanometer range (1–100 nm) constructs with fruitful properties of optics, magnetism, and topology give a founding rationale in applied research. Solid materials are lacking in these characteristics (McDermed et al. 2012). The branch "Cancer nanotechnology" sets a resonance in the cancer field. National Cancer Institute Alliance for nanoscience/nanomedicine and quantum dots to give momentum in cancer diagnosis and therapeutics (EUR-Lex–32011H0696–EN–EUR-Lex n.d.). In the early diagnosis of adenocarcinoma, cancer nanotechnology sparks light in timely detection, i.e., ease in prognosis and diagnosis because nanosize particles can detect the meager quantity of molecular signals. This is a very important salient feature of nanoscience and provides opportunities for on-time diagnosis in pancreatic cancer patients (EUR-Lex–32011H0696–EN–EUR-Lex n.d.). Several nanoparticle types have been proposed for biomarkers harvesting. Tamburro and his team synthesized multifunctional core-shell NPs to discover invisible biomarkers. Here, core-shell hydrogel potentiates a 10,000-fold amplification of low-molecular-weight macromolecules by expunction albumin and biomolecules (≥30 kilodalton MWt. proteins) (Hartshorn et al. 2019). Smart hydrogel NPs are basically used in biomarkers harvesting (Tamburro et al. 2011). A study was published on nano research that core-shell hydrogel NPs concentrate and preserve labile and low abundance signature molecules (Luchini et al. 2007; Longo et al. 2009) and T.A. Douglas proposed that hydrogel concentrates the antigens of bacteria in a urine sample (Lyme disease) (Fredolini et al. 2008). Through nanotechnology, capturing biomarkers is also employed for early detection and discovery (Douglas et al. 2011). This gained momentum and many studies have been explored based on biomolecules (protein coronas)-NPs conjugates in cancer diagnosis. A protein corona influences the nanobiointerface and therapeutic impact of graphene oxide NPs (Fredolini et al. 2010; Hajipour et al. 2014). Colapicchioni et al. showed that there is a defined link between plasma protein of mammary glands, GIT, pancreas tumor, and personalized protein corona-NPs (Hajipour et al. 2015). Capturing the primary ideas in a diseased state and adjoining nano-biotechniques, disease recognized protein corona sensing array has the specific diagnosis potential and a sensitive platform can be developed (Colapicchioni et al. 2016). Among the fastest growing technologies, NPs enabled blood (NEB) assessment is emerging as an earlier, economical, patient accessible technology for the fastest tumor diagnosis. The NEB clinical examinations are based on protein corona (nano-concentrator) characterization via Zeta-sizer, electrophoresis (micro and 1-D), and SDS-PAGE biotechnological techniques (Caracciolo et al. 2019). A series of biochemical research was performed by Hua and coworkers taking into account the NEB test evaluation. Some are pioneering research on NPs derived-corona-protein (Caputo et al. 2017; Huo et al. 2011). Gold NPs (GNP)-based NEB test was developed for early-stage neoplasm diagnosis with a 50% sensitivity and about 95% specificity to

cancer cells (Huo et al. 2012). Therefore, this represents a valuable upgrade in cancer. In the year 2017, Hua and his team designed and modified a NEB test to understand the active status and acute viral infection (AVI) time period in patients (Zheng et al. 2015). The question becomes more profound and relevant; can modified NEB test resolve the diagnostic issues in early cancer detection? As PDAC cases are huge in number with maximum mortality and asymptomatic behavior of this form of tumor. In a relevant study, liposome-based NEB test differentiates the pancreatic ductal adenocarcinoma patients from healthy persons (Zheng et al. 2017).

## 8.3 Theranostic Nanoparticles

Theragnostic platform (diagnosis/treatment) is the new emerging technology in recent times. The theranostic-designed Q-dots have the ability to deliver therapeutics at the exact location with the advantage of 3D bioimaging (Caputo et al. 2017). Stimuli-responsive smart nanocarrier systems have played a crucial role in theranostic applications to precisely detect and mitigate cancer types (Das et al. 2020c; Bharadwaj et al. 2021; Das et al. 2020a). Two promising events, early diagnosis and surgical resection, increased the survival rate of patients; a study conducted by Cleary and colleagues on PDAC malignancy cases. They concluded that 5-year survival with $31.7 \pm 3.6$ months if prognostic factors are included (Cleary et al. 2004). Sivasankarapillai et al. showed the possibility of image-guided tumor surgery by delivering imaging agents NPs in PDAC cases (Sivasankarapillai et al. 2021). Qi et al. designed and demonstrated fluorophore probe-based bioimaging. They developed indocyanine green (ICG)-loaded hyaluronan NPs for enhancing the fluorescence in pancreatic tumor surgery. They called it "Nano ICG." This gives approximately 8 mm of fluorescence imaging depth in tissues and facilitates visualization of diverse cancer cells. They had taken the homograft orthotopic murine model of PDAC to verify their hypothesis. They had seen greater fluorescence signal from the loaded ICG and this leads to primary tumor diagnosis and also malignant metastasis in comparison to free ICG. This confirms hyaluronic acid NPs potential to target PDAC cells in addition to greater extent imaging in tissue resection (Qi et al. 2018). Mattheolabakis et al. showed that $(C_{14}H_{21}NO_{11})_n$ molecules affinity with hematopoietic CD44 (transmembrane proteoglycan) that are over-produced in pancreatic cancer cells can be used to trigger and accumulates nanoparticles at PDAC sites by the active transport mechanism (Mattheolabakis et al. 2015).

Targeted nanocarriers have shown promising applications in molecular pathway targeting in target-specific cancer therapy (Barkat et al. 2021; Das et al. 2020b; Barkat et al. 2020). Sometimes, the small metastatic sites are present while performing surgery but not visualized due to limited magnification. Therefore, in the year 2018, Hoogstins et al. developed an image-guided surgery by targeting putative SGM-101 (antigen) in PDAC patients. Here, higher resolution in fluorescence is detected by coupling fluorescent moiety to Abs (Hoogstins et al. 2018). This may facilitate identification of the resection margins and quantification of the

residual disease, albeit the clinical benefit still remains to be demonstrated. A combination of magnetic resonance imaging (MRI) with fluorescence imaging, by co-encapsulation of superparamagnetic iron oxide NPs (IONPs) was also proposed in a study conducted by Luo et al. in 2019. The iron oxide NPs and FITC were co-encapsulated into hyaluronic-derived nanoparticles for the specific sensing of hyaluronic acid by cell surface adhesion receptors (CD44) (Luo et al. 2019) and nanoparticles modified with tPA-mediated plasminogen activation molecules with greater binding for galectin-1 (Gal-1), which has higher expression in pancreatic tumor cells. Another proposed model is the image-guided therapy by a combination of magnetic resonance imaging and chemotherapy (DOX) drugs to target antitumor PDAC. This approach was theranostic because high sensing image and therapy, both can be achieved at the same time. Precise monitoring of tumor metastasis and proliferation may be targeted by dexterous methods (Rosenberger et al. 2015). Zhou H and his team researched on the conjugation of magnetic NPs (iron oxide NPs/IONPs) with somatomedin C (insulin-like growth factor-1/IGF-1) that has an affinity to transmembrane receptor IGF1. The IONPs-IGF1 is loaded with DOX drugs. This construct had shown magnificent results in orthotopic preclinical model when given from intravenous route of administration. Moreover, when dosage is given within the tumor, these NPs construct suppress the growth of cancer (66.6%) in comparison to doxorubicin alone (Zhou et al. 2015). A study by Lee and coworkers designed and characterized iron oxide NPs complexed with serine protease (Urokinase/uPA). Drug gemcitabine is encapsulated within nanoconstructs. Through the mechanism of endocytosis, urokinase plasminogen activator receptors were recognized. This method also showed an excellent benefit in both therapy and imaging. Here, 50% inhibition was observed in pancreatic tumor in vivo when performed in orthotopic murine model (Lee et al. 2013).

## 8.4 Conclusion and Future Perspectives

The concluding remark, pancreatic cancer management needs novel strategies. This can be done through theragnostic approaches in medicine. As stated earlier, nanoparticle-based blood tests are commencing the possibilities of early tumor detection with safe consideration. Protein corona-NPs are the new highlighter in biomarker diagnosis. These tests not only detect signature molecules but also a pattern of proteins at a prognostic's stages before tissue resection/surgery. Superior sensitivity and specificity are the foremost factors in nanoparticle-enabled diagnostic tests. In chemotherapy and radiotherapy, safety and efficacy are the firmest factors. Indeed, more and more preclinical, clinical evaluations are needed for relevance in patient compliances. Targeting EGFR pathway genes, i.e., KRAS and others, have shown promising targets in PDAC therapeutics. Fusion strategy in designing Si-RNA NPs/Q-Dots and targeting them to cancerous cells; emerged as momentarily effective in PDACs cases. Some NPs has toxicity profile in vitro but different properties in vivo in terms of stability and toxicity. Therefore, detailed preclinical studies are required to document in vivo route of administration. Cancer

microenvironment/signaling pathways and novel therapies build a greater communication network and greater extent in molecular signal sensing detection and overcome tumor stromal barriers. This will lead to a consequential approach in pancreatic cancer management.

## References

Ansari D, Gustafsson A, Andersson R (2015) Update on the management of pancreatic cancer: surgery is not enough. World J Gastroenterol 21(11):3157–3165. https://doi.org/10.3748/wjg.v21.i11.3157

Bachem MG, Schünemann M, Ramadani M, Siech M, Beger H, Buck A, Zhou S, Schmid-Kotsas A, Adler G (2005) Pancreatic carcinoma cells induce fibrosis by stimulating proliferation and matrix synthesis of stellate cells. Gastroenterology 128(4):907–921. https://doi.org/10.1053/j.gastro.2004.12.036

Bader AG (2012) miR-34–a microRNA replacement therapy is headed to the clinic. Front Genet 3. https://doi.org/10.3389/fgene.2012.00120

Barkat HA, Barkat MA, Taleuzzaman M, Das SS, Rizwanullah M, Hadi HA (2021) Receptor-based combinatorial Nanomedicines: a new Hope for cancer management. In: Kumar S, Rizvi MA, Verma S (eds) *Handbook of research on advancements in cancer therapeutics* (pp. 339–355). IGI Global. https://doi.org/10.4018/978-1-7998-6530-8.ch011

Barkat HA, Das SS, Barkat MA, Beg S, Hadi HA (2020) Selective targeting of cancer signaling pathways with nanomedicines: challenges and progress. Future Oncol 16(35):2959–2979. https://doi.org/10.2217/fon-2020-0198

Bazak R, Houri M, Achy SE, Hussein W, Refaat T (2014) Passive targeting of nanoparticles to cancer: a comprehensive review of the literature. Molecular and Clinical Oncology 2(6):904–908. https://doi.org/10.3892/mco.2014.356

Bharadwaj P, Das SS, Beg S, Rahman M (2021) Formulation and biological stability of nanomedicines in cancer treatment. In: Beg S, Choudhry H, Ahmad FJ, Rahman M, Souto EB (eds) Nanoformulation strategies for cancer treatment-micro and Nano Technologies. Elsevier, pp 277–289. https://doi.org/10.1016/C2019-0-01815-8

Bisht S, Feldmann G, Soni S, Ravi R, Karikar C, Maitra A, Maitra A (2007) Polymeric nanoparticle-encapsulated curcumin ("nanocurcumin"): a novel strategy for human cancer therapy. J Nanobiotechnol 5(1). https://doi.org/10.1186/1477-3155-5-3

Bramhall SR, Schulz J, Nemunaitis J, Brown PD, Baillet M, Buckels JAC (2002) A double-blind placebo-controlled, randomised study comparing gemcitabine and marimastat with gemcitabine and placebo as first line therapy in patients with advanced pancreatic cancer. Br J Cancer 87(2):161–167. https://doi.org/10.1038/sj.bjc.6600446

Burris HA, Moore MJ, Andersen J, Green MR, Rothenberg ML, Modiano MR, Cripps MC, Portenoy RK, Storniolo AM, Tarassoff P, Nelson R, Dorr FA, Stephens CD, von Hoff DD (1997) Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. J Clin Oncol 15(6):2403–2413. https://doi.org/10.1200/jco.1997.15.6.2403

Cabral H, Murakami M, Hojo H, Terada Y, Kano MR, Chung UI, Nishiyama N, Kataoka K (2013) Targeted therapy of spontaneous murine pancreatic tumors by polymeric micelles prolongs survival and prevents peritoneal metastasis. Proc Natl Acad Sci 110(28):11397–11402. https://doi.org/10.1073/pnas.1301348110

Cai L, Michelakos T, Ferrone CR, Zhang L, Deshpande V, Shen Q, DeLeo A, Yamada T, Zhang G, Ferrone S, Wang X (2017) Expression status of folate receptor alpha is a predictor of survival in pancreatic ductal adenocarcinoma. Oncotarget 8(23):37646–37656. https://doi.org/10.18632/oncotarget.16841

Camp ER, Wang C, Little EC, Watson PM, Pirollo KF, Rait A, Cole DJ, Chang EH, Watson DK (2013) Transferrin receptor targeting nanomedicine delivering wild-type p53 gene sensitizes pancreatic cancer to gemcitabine therapy. Cancer Gene Ther 20(4):222–228. https://doi.org/10.1038/cgt.2013.9

Caputo D, Papi M, Coppola R, Palchetti S, Digiacomo L, Caracciolo G, Pozzi D (2017) A protein corona-enabled blood test for early cancer detection. Nanoscale 9(1):349–354. https://doi.org/10.1039/c6nr05609a

Caracciolo G, Safavi-Sohi R, Malekzadeh R, Poustchi H, Vasighi M, Zenezini Chiozzi R, Capriotti AL, Laganà A, Hajipour M, di Domenico M, di Carlo A, Caputo D, Aghaverdi H, Papi M, Palmieri V, Santoni A, Palchetti S, Digiacomo L, Pozzi D et al (2019) Disease-specific protein corona sensor arrays may have disease detection capacity. Nanoscale Horizons 4(5):1063–1076. https://doi.org/10.1039/c9nh00097f

Chandran SP, Natarajan SB, Chandraseharan S, Mohd Shahimi MSB (2017) Nano drug delivery strategy of 5-fluorouracil for the treatment of colorectal cancer. Journal of Cancer Research and Practice 4(2):45–48. https://doi.org/10.1016/j.jcrpr.2017.02.002

Cheng CJ, Bahal R, Babar IA, Pincus Z, Barrera F, Liu C, Svoronos A, Braddock DT, Glazer PM, Engelman DM, Saltzman WM, Slack FJ (2014) MicroRNA silencing for cancer therapy targeted to the tumour microenvironment. Nature 518(7537):107–110. https://doi.org/10.1038/nature13905

Cleary SP, Gryfe R, Guindi M, Greig P, Smith L, Mackenzie R, Strasberg S, Hanna S, Taylor B, Langer B, Gallinger S (2004) Prognostic factors in resected pancreatic adenocarcinoma: analysis of actual 5-year survivors1 1No competing interests declared. J Am Coll Surg 198(5):722–731. https://doi.org/10.1016/j.jamcollsurg.2004.01.008

Colapicchioni V, Tilio M, Digiacomo L, Gambini V, Palchetti S, Marchini C, Pozzi D, Occhipinti S, Amici A, Caracciolo G (2016) Personalized liposome–protein corona in the blood of breast, gastric and pancreatic cancer patients. Int J Biochem Cell Biol 75:180–187. https://doi.org/10.1016/j.biocel.2015.09.002

Cunningham D, Chau I, Stocken DD, Valle JW, Smith D, Steward W, Harper PG, Dunn J, Tudur-Smith C, West J, Falk S, Crellin A, Adab F, Thompson J, Leonard P, Ostrowski J, Eatock M, Scheithauer W, Herrmann R, Neoptolemos JP (2009) Phase III randomized comparison of gemcitabine versus gemcitabine plus Capecitabine in patients with advanced pancreatic cancer. J Clin Oncol 27(33):5513–5518. https://doi.org/10.1200/jco.2009.24.2446

Dai JT, Zhang Y, Li HC, Deng YH, Elzatahry AA, Alghamdi A, Fu DL, Jiang YJ, Zhao DY (2017) Enhancement of gemcitabine against pancreatic cancer by loading in mesoporous silica vesicles. Chin Chem Lett 28(3):531–536. https://doi.org/10.1016/j.cclet.2016.11.008

Danhier F, Feron O, Préat V (2010) To exploit the tumor microenvironment: passive and active tumor targeting of nanocarriers for anti-cancer drug delivery. J Control Release 148(2):135–146. https://doi.org/10.1016/j.jconrel.2010.08.027

Das SS, Alkahtani S, Bharadwaj P, Ansari MT, ALKahtani MD, Pang Z, Hasnain MS, Nayak AK, Aminabhavi TM (2020b) Molecular insights and novel approaches for targeting tumor metastasis. Int J Pharm 585:119556. https://doi.org/10.1016/j.ijpharm.2020.119556

Das SS, Bharadwaj P, Bilal M, Barani M, Rahdar A, Taboada P, Bungau S, Kyzas GZ (2020c) Stimuli-responsive polymeric Nanocarriers for drug delivery, imaging, and Theragnosis. Polymers 12(6):1397. https://doi.org/10.3390/polym12061397

Das SS, Hussain A, Verma PRP, Imam SS, Altamimi MA, Alshehri S, Singh SK (2020d) Recent advances in liposomal drug delivery system of quercetin for cancer targeting: a mechanistic approach. Curr Drug Deliv 17(10):845–860. https://doi.org/10.2174/1567201817666200415112657

Das SS, Verma PRP, Kar S, Singh SK (2020a) Quercetin-loaded Nanomedicine as oncotherapy. In: Rahman M, Beg S, Kumar V, Ahmad FJ (eds) Nanomedicine for bioactives: healthcare applications, 1st edn. Springer, Singapore, pp 155–183. https://doi.org/10.1007/978-981-15-1664-1_5

Diasio RB, Lu Z (1994) Dihydropyrimidine dehydrogenase activity and fluorouracil chemotherapy. J Clin Oncol 12(11):2239–2242. https://doi.org/10.1200/jco.1994.12.11.2239

Douglas TA, Tamburro D, Fredolini C, Espina BH, Lepene BS, Ilag L, Espina V, Petricoin EF, Liotta LA, Luchini A (2011) The use of hydrogel microparticles to sequester and concentrate bacterial antigens in a urine test for Lyme disease. Biomaterials 32(4):1157–1166. https://doi.org/10.1016/j.biomaterials.2010.10.004

El-Zahaby SA, Elnaggar YS, Abdallah OY (2019) Reviewing two decades of nanomedicine implementations in targeted treatment and diagnosis of pancreatic cancer: An emphasis on state of art. J Control Release 293:21–35. https://doi.org/10.1016/j.jconrel.2018.11.013

Erkan M, Hausmann S, Michalski CW, Fingerle AA, Dobritz M, Kleeff J, Friess H (2012) The role of stroma in pancreatic cancer: diagnostic and therapeutic implications. Nat Rev Gastroenterol Hepatol 9(8):454–467. https://doi.org/10.1038/nrgastro.2012.115

EUR-Lex–32011H0696–EN–EUR-Lex (n.d.). Eur-Lex.europa.eu. Retrieved December 7, 2021, from http://data.europa.eu/eli/reco/2011/696/oj

Freag MS, Elnaggar YS, Abdelmonsif DA, Abdallah OY (2016) Layer-by-layer-coated lyotropic liquid crystalline nanoparticles for active tumor targeting of rapamycin. Nanomedicine 11(22): 2975–2996. https://doi.org/10.2217/nnm-2016-0236

Fredolini C, Meani F, Alex Reeder K, Rucker S, Patanarut A, Botterell PJ, Bishop B, Longo C, Espina V, Petricoin EF, Liotta LA, Luchini A (2008) Concentration and preservation of very low abundance biomarkers in urine, such as human growth hormone (hGH), by Cibacron blue F3G-A loaded hydrogel particles. Nano Res 1(6):502–518. https://doi.org/10.1007/s12274-008-8054-z

Fredolini C, Meani F, Luchini A, Zhou W, Russo P, Ross M, Patanarut A, Tamburro D, Gambara G, Ornstein D, Odicino F, Ragnoli M, Ravaggi A, Novelli F, Collura D, D'Urso L, Muto G, Belluco C, Pecorelli S et al (2010) Investigation of the ovarian and prostate cancer Peptidome for candidate early detection markers using a novel nanoparticle biomarker capture technology. AAPS J 12(4):504–518. https://doi.org/10.1208/s12248-010-9211-3

Goossens N, Nakagawa S, Sun X, Hoshida Y (2015) Cancer biomarker discovery and validation. Transl Cancer Res 4(3):256. https://doi.org/10.3978/j.issn.2218-676X.2015.06.04

Hajipour MJ, Laurent S, Aghaie A, Rezaee F, Mahmoudi M (2014) Personalized protein coronas: a "key" factor at the nanobiointerface. Biomater Sci 2(9):1210. https://doi.org/10.1039/c4bm00131a

Hajipour MJ, Raheb J, Akhavan O, Arjmand S, Mashinchian O, Rahman M, Abdolahad M, Serpooshan V, Laurent S, Mahmoudi M (2015) Personalized disease-specific protein corona influences the therapeutic impact of graphene oxide. Nanoscale 7(19):8978–8994. https://doi.org/10.1039/c5nr00520e

Halford S, Yip D, Karapetis C, Strickland A, Steger A, Khawaja H, Harper P (2001) A phase II study evaluating the tolerability and efficacy of CAELYX (liposomal doxorubicin, Doxil) in the treatment of unresectable pancreatic carcinoma. Ann Oncol 12(10):1399–1402. https://doi.org/10.1023/a:1012522120294

Harshita, Barkat MA, Das SS, Pottoo FH, Beg S, Rahman Z (2020) Lipid-based nanosystem as intelligent carriers for versatile drug delivery applications. Curr Pharm Des 26(11):1167–1180. https://doi.org/10.2174/1381612826666200206094529

Hartshorn CM, Russell LM, Grodzinski P (2019) National Cancer Institute Alliance for nanotechnology in cancer–catalyzing research and translation toward novel cancer diagnostics and therapeutics. WIREs Nanomedicine and Nanobiotechnology 11(6). https://doi.org/10.1002/wnan.1570

Hoogstins CES, Boogerd LSF, Sibinga Mulder BG, Mieog JSD, Swijnenburg RJ, van de Velde CJH, Farina Sarasqueta A, Bonsing BA, Framery B, Pèlegrin A, Gutowski M, Cailler F, Burggraaf J, Vahrmeijer AL (2018) Image-guided surgery in patients with pancreatic cancer: first results of a clinical trial using SGM-101, a novel carcinoembryonic antigen-targeting, near-infrared fluorescent agent. Ann Surg Oncol 25(11):3350–3357. https://doi.org/10.1245/s10434-018-6655-7

Hu Q, Jiang Q, Jin X, Shen J, Wang K, Li Y, Xu F, Tang G, Li Z (2013) Cationic microRNA-delivering nanovectors with bifunctional peptides for efficient treatment of PANC-1 xenograft model. Biomaterials 34(9):2265–2276. https://doi.org/10.1016/j.biomaterials.2012.12.016

Hu CMJ, Kaushal S, Cao HST, Aryal S, Sartor M, Esener S, Bouvet M, Zhang L (2010) Half-antibody functionalized lipid−polymer hybrid nanoparticles for targeted drug delivery to carcinoembryonic antigen presenting pancreatic cancer cells. Mol Pharm 7(3):914–920. https://doi.org/10.1021/mp900316a

Huo Q, Colon J, Cordero A, Bogdanovic J, Baker CH, Goodison S, Pensky MY (2011) A facile nanoparticle immunoassay for cancer biomarker discovery. J Nanobiotechnol 9(1):20. https://doi.org/10.1186/1477-3155-9-20

Huo Q, Litherland SA, Sullivan S, Hallquist H, Decker DA, Rivera-Ramirez I (2012) Developing a nanoparticle test for prostate cancer scoring. J Transl Med 10(1). https://doi.org/10.1186/1479-5876-10-44

Janssen HL, Reesink HW, Lawitz EJ, Zeuzem S, Rodriguez-Torres M, Patel K, van der Meer AJ, Patick AK, Chen A, Zhou Y, Persson R, King BD, Kauppinen S, Levin AA, Hodges MR (2013) Treatment of HCV infection by targeting MicroRNA. N Engl J Med 368(18):1685–1694. https://doi.org/10.1056/nejmoa1209026

Jeong SM, Hwang S, Seong RH (2016) Transferrin receptor regulates pancreatic cancer growth by modulating mitochondrial respiration and ROS generation. Biochem Biophys Res Commun 471(3):373–379. https://doi.org/10.1016/j.bbrc.2016.02.023

Kaur S, Kumar S, Momi N, Sasson AR, Batra SK (2013) Mucins in pancreatic cancer and its microenvironment. Nat Rev Gastroenterol Hepatol 10(10):607–620. https://doi.org/10.1038/nrgastro.2013.120

Kim B, Lee C, Lee ES, Shin BS, Youn YS (2016) Paclitaxel and curcumin co-bound albumin nanoparticles having antitumor potential to pancreatic cancer. Asian Journal of Pharmaceutical Sciences 11(6):708–714. https://doi.org/10.1016/j.ajps.2016.05.005

Klein AP (2012) Identifying people at a high risk of developing pancreatic cancer. Nat Rev Cancer 13(1):66–74. https://doi.org/10.1038/nrc3420

Kunnumakkara AB, Guha S, Krishnan S, Diagaradjane P, Gelovani J, Aggarwal BB (2007) Curcumin potentiates antitumor activity of gemcitabine in an Orthotopic model of pancreatic cancer through suppression of proliferation, angiogenesis, and inhibition of nuclear factor-κB–regulated gene products. Cancer Res 67(8):3853–3861. https://doi.org/10.1158/0008-5472.can-06-4257

Lee GY, Qian WP, Wang L, Wang YA, Staley CA, Satpathy M, Nie S, Mao H, Yang L (2013) Theranostic nanoparticles with controlled release of gemcitabine for targeted therapy and MRI of pancreatic cancer. ACS Nano 7(3):2078–2089. https://doi.org/10.1021/nn3043463

Li J, Liu F, Gupta S, Li C (2016) Interventional Nanotheranostics of pancreatic ductal adenocarcinoma. Theranostics 6(9):1393–1402. https://doi.org/10.7150/thno.15122

Li Y, Sarkar FH (2016) MicroRNA targeted therapeutic approach for pancreatic cancer. Int J Biol Sci 12(3):326–337. https://doi.org/10.7150/ijbs.15017

Li J, Wientjes MG, Au JLS (2010) Pancreatic cancer: pathobiology, treatment options, and drug delivery. AAPS J 12(2):223–232. https://doi.org/10.1208/s12248-010-9181-5

Ling H, Fabbri M, Calin GA (2013) MicroRNAs and other non-coding RNAs as targets for anticancer drug development. Nat Rev Drug Discov 12(11):847–865. https://doi.org/10.1038/nrd4140

Lo JT, Chen BH, Lee TM, Han J, Li JL (2010) Self-emulsifying O/W formulations of paclitaxel prepared from mixed nonionic surfactants. J Pharm Sci 99(5):2320–2332. https://doi.org/10.1002/jps.21993

Longo C, Patanarut A, George T, Bishop B, Zhou W, Fredolini C, Ross MM, Espina V, Pellacani G, Petricoin EF, Liotta LA, Luchini A (2009) Core-Shell hydrogel particles harvest, concentrate and preserve labile low abundance biomarkers. PLoS One 4(3):e4763. https://doi.org/10.1371/journal.pone.0004763

Luchini A, Geho DH, Bishop B, Tran D, Xia C, Dufour RL, Jones CD, Espina V, Patanarut A, Zhou W, Ross MM, Tessitore A, Petricoin EF, Liotta LA (2007) Smart hydrogel particles: biomarker harvesting: one-step affinity purification, size exclusion, and protection against degradation. Nano Lett 8(1):350–361. https://doi.org/10.1021/nl072174l

Luo Y, Li Y, Li J, Fu C, Yu X, Wu L (2019) Hyaluronic acid-mediated multifunctional iron oxide-based MRI nanoprobes for dynamic monitoring of pancreatic cancer. RSC Adv 9(19): 10486–10493. https://doi.org/10.1039/c9ra00730j

Macgregor-Das AM, Iacobuzio-Donahue CA (2012) Molecular pathways in pancreatic carcinogenesis. J Surg Oncol 107(1):8–14. https://doi.org/10.1002/jso.23213

Maitra A, Hruban RH (2008) Pancreatic cancer. Annual Review of Pathology: Mechanisms of Disease 3(1):157–188. https://doi.org/10.1146/annurev.pathmechdis.3.121806.154305

Martin L, Li X, Kleiber B, Ellison E, Bloomston M, Zalupski M, Bekaii-Saab T (2012) VEGF remains an interesting target in advanced pancreas cancer (APCA): results of a multi-institutional phase II study of bevacizumab, gemcitabine, and infusional 5-fluorouracil in patients with APCA. Ann Oncol 23(11):2812–2820. https://doi.org/10.1093/annonc/mds134

Mattheolabakis G, Milane L, Singh A, Amiji MM (2015) Hyaluronic acid targeting of CD44 for cancer therapy: from receptor biology to nanomedicine. J Drug Target 23(7–8):605–618. https://doi.org/10.3109/1061186x.2015.1052072

McDermed JE, Sanders R, Fait S, Klem RE, Sarno MJ, Adams TH, Diamandis EP (2012) Nucleic acid detection immunoassay for prostate-specific antigen based on Immuno-PCR methodology. Clin Chem 58(4):732–740. https://doi.org/10.1373/clinchem.2011.170290

Murphy EA, Majeti BK, Barnes LA, Makale M, Weis SM, Lutu-Fuga K, Wrasidlo W, Cheresh DA (2008) Nanoparticle-mediated drug delivery to tumor vasculature suppresses metastasis. Proc Natl Acad Sci 105(27):9343–9348. https://doi.org/10.1073/pnas.0803728105

Neoptolemos JP, Stocken DD, Bassi C, Ghaneh P, Cunningham D, Goldstein D, Padbury R, Moore MJ, Gallinger S, Mariette C, Wente MN, Izbicki JR, Friess H, Lerch MM, Dervenis C, Oláh A, Butturini G, Doi R, Lind PA et al (2010) Adjuvant chemotherapy with fluorouracil plus Folinic acid vs gemcitabine following pancreatic cancer resection. JAMA 304(10):1073. https://doi.org/10.1001/jama.2010.1275

Neoptolemos JP, Stocken DD, Friess H, Bassi C, Dunn JA, Hickey H, Beger H, Fernandez-Cruz L, Dervenis C, Lacaine F, Falconi M, Pederzoli P, Pap A, Spooner D, Kerr DJ, Büchler MW (2004) A randomized trial of Chemoradiotherapy and chemotherapy after resection of pancreatic cancer. N Engl J Med 350(12):1200–1210. https://doi.org/10.1056/nejmoa032295

Niemeyer CM, Adler M, Wacker R (2005) Immuno-PCR: high sensitivity detection of proteins by nucleic acid amplification. Trends Biotechnol 23(4):208–216. https://doi.org/10.1016/j.tibtech.2005.02.006

Nigam P, Waghmode S, Louis M, Wangnoo S, Chavan P, Sarkar D (2014) Graphene quantum dots conjugated albumin nanoparticles for targeted drug delivery and imaging of pancreatic cancer. J Mater Chem B 2(21):3190–3195. https://doi.org/10.1039/c4tb00015c

Ohno SI, Takanashi M, Sudo K, Ueda S, Ishikawa A, Matsuyama N, Fujita K, Mizutani T, Ohgi T, Ochiya T, Gotoh N, Kuroda M (2013) Systemically injected exosomes targeted to EGFR deliver antitumor MicroRNA to breast cancer cells. Mol Ther 21(1):185–191. https://doi.org/10.1038/mt.2012.180

Olive KP, Jacobetz MA, Davidson CJ, Gopinathan A, McIntyre D, Honess D, Madhu B, Goldgraben MA, Caldwell ME, Allard D, Frese KK, DeNicola G, Feig C, Combs C, Winter SP, Ireland-Zecchini H, Reichelt S, Howat WJ, Chang A et al (2009) Inhibition of hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. Science 324(5933):1457–1461. https://doi.org/10.1126/science.1171362

Pai P, Rachagani S, Are C, Batra S (2013) Prospects of miRNA-based therapy for pancreatic cancer. Curr Drug Targets 14(10):1101–1109. https://doi.org/10.2174/13894501113149990181

Patra CR, Bhattacharya R, Wang E, Katarya A, Lau JS, Dutta S, Muders M, Wang S, Buhrow SA, Safgren SL, Yaszemski MJ, Reid JM, Ames MM, Mukherjee P, Mukhopadhyay D (2008)

Targeted delivery of gemcitabine to pancreatic adenocarcinoma using Cetuximab as a targeting agent. Cancer Res 68(6):1970–1978. https://doi.org/10.1158/0008-5472.can-07-6102

Philip PA, Benedetti J, Corless CL, Wong R, O'Reilly EM, Flynn PJ, Rowland KM, Atkins JN, Mirtsching BC, Rivkin SE, Khorana AA, Goldman B, Fenoglio-Preiser CM, Abbruzzese JL, Blanke CD (2010) Phase III study comparing gemcitabine plus Cetuximab versus gemcitabine in patients with advanced pancreatic adenocarcinoma: southwest oncology group–directed intergroup trial S 0205. J Clin Oncol 28(22):3605–3610. https://doi.org/10.1200/jco.2009.25.7550

Qi B, Crawford AJ, Wojtynek NE, Holmes MB, Souchek JJ, Almeida-Porada G, Ly QP, Cohen SM, Hollingsworth MA, Mohs AM (2018) Indocyanine green loaded hyaluronan-derived nanoparticles for fluorescence-enhanced surgical imaging of pancreatic cancer. Nanomedicine 14(3):769–780. https://doi.org/10.1016/j.nano.2017.12.015

Redmond KJ, Wolfgang CL, Sugar EA, Ahn J, Nathan H, Laheru D, Edil BH, Choti MA, Pawlik TM, Hruban RH, Cameron JL, Herman JM (2010) Adjuvant Chemoradiation therapy for adenocarcinoma of the distal pancreas. Ann Surg Oncol 17(12):3112–3119. https://doi.org/10.1245/s10434-010-1200-3

Rifai N, Gillette MA, Carr SA (2006) Protein biomarker discovery and validation: the long and uncertain path to clinical utility. Nat Biotechnol 24(8):971–983. https://doi.org/10.1038/nbt1235

Rosenberger I, Strauss A, Dobiasch S, Weis C, Szanyi S, Gil-Iceta L, Alonso E, González Esparza M, Gómez-Vallejo V, Szczupak B, Plaza-García S, Mirzaei S, Israel L, Bianchessi S, Scanziani E, Lellouche JP, Knoll P, Werner J, Felix K et al (2015) Targeted diagnostic magnetic nanoparticles for medical imaging of pancreatic cancer. J Control Release 214:76–84. https://doi.org/10.1016/j.jconrel.2015.07.017

Ryschich E, Huszty G, Knaebel H, Hartel M, Büchler M, Schmidt J (2004) Transferrin receptor is a marker of malignant phenotype in human pancreatic cancer and in neuroendocrine carcinoma of the pancreas. Eur J Cancer 40(9):1418–1422. https://doi.org/10.1016/j.ejca.2004.01.036

Scaros O, Fisler R (2005) Biomarker technology roundup: from discovery to clinical applications, a broad set of tools is required to translate from the lab to the clinic. BioTechniques 38(4S):S30–S32. https://doi.org/10.2144/05384su01

Shamseddine A, Saleh A, Charafeddine M, Seoud M, Mukherji D, Temraz S, Sibai AM (2014) Cancer trends in Lebanon: a review of incidence rates for the period of 2003–2008 and projections until 2018. Popul Health Metrics 12(1). https://doi.org/10.1186/1478-7954-12-4

Siegel R, Ma J, Zou Z, Jemal A (2014) Cancer statistics, 2014. CA Cancer J Clin 64(1):9–29. https://doi.org/10.3322/caac.21208

Sivasankarapillai V, Das SS, Sabir F, Sundaramahalingam M, Colmenares J, Prasannakumar S, Rajan M, Rahdar A, Kyzas G (2021) Progress in natural polymer engineered biomaterials for transdermal drug delivery systems. Materials Today Chemistry 19:100382. https://doi.org/10.1016/j.mtchem.2020.100382

Tamburro D, Fredolini C, Espina V, Douglas TA, Ranganathan A, Ilag L, Zhou W, Russo P, Espina BH, Muto G, Petricoin EF, Liotta LA, Luchini A (2011) Multifunctional core–shell nanoparticles: discovery of previously invisible biomarkers. J Am Chem Soc 133(47):19178–19188. https://doi.org/10.1021/ja207515j

Thapa RK, Ku SK, Choi HG, Yong CS, Byeon JH, Kim JO (2018) Vibrating droplet generation to assemble zwitterion-coated gold-graphene oxide stealth nanovesicles for effective pancreatic cancer chemo-phototherapy. Nanoscale 10(4):1742–1749. https://doi.org/10.1039/c7nr07603g

Trabulo S, Aires A, Aicher A, Heeschen C, Cortajarena AL (2017) Multifunctionalized iron oxide nanoparticles for selective targeting of pancreatic cancer cells. Biochim Biophys Acta Gen Subj 1861(6):1597–1605. https://doi.org/10.1016/j.bbagen.2017.01.035

Vincent A, Herman J, Schulick R, Hruban RH, Goggins M (2011) Pancreatic cancer. Lancet 378(9791):607–620. https://doi.org/10.1016/s0140-6736(10)62307-0

Wang L, An Y, Yuan C, Zhang H, Liang C, Ding F, Gao Q, Zhang D (2015) GEM-loaded magnetic albumin nanospheres modified with cetuximab for simultaneous targeting, magnetic resonance

imaging, and double-targeted thermochemotherapy of pancreatic cancer cells. Int J Nanomedicine 2507. https://doi.org/10.2147/ijn.s77642

Wu Y, Crawford M, Mao Y, Lee RJ, Davis IC, Elton TS, Lee LJ, Nana-Sinkam SP (2013) Therapeutic delivery of MicroRNA-29b by cationic Lipoplexes for lung cancer. Molecular Therapy–Nucleic Acids 2:e84. https://doi.org/10.1038/mtna.2013.14

Wu L, Qu X (2015) Cancer biomarker detection: recent achievements and challenges. Chem Soc Rev 44(10):2963–2997. https://doi.org/10.1039/c4cs00370e

Yachida S, Iacobuzio-Donahue CA (2009) The pathology and genetics of metastatic pancreatic cancer. Arch Pathol Lab Med 133(3):413–422. https://doi.org/10.5858/133.3.413

Yallapu MM, Ebeling MC, Khan S, Sundram V, Chauhan N, Gupta BK, Puumala SE, Jaggi M, Chauhan SC (2013) Novel curcumin-loaded magnetic nanoparticles for pancreatic cancer treatment. Mol Cancer Ther 12(8):1471–1480. https://doi.org/10.1158/1535-7163.mct-12-1227

Zhang X, Lu J, Huang Y, Zhao W, Chen Y, Li J, Gao X, Venkataramanan R, Sun M, Stolz DB, Zhang L, Li S (2013) PEG-Farnesylthiosalicylate conjugate as a Nanomicellar carrier for delivery of paclitaxel. Bioconjug Chem 24(3):464–472. https://doi.org/10.1021/bc300608h

Zheng T, Finn C, Parrett CJ, Dhume K, Hwang JH, Sidhom D, Strutt TM, Li Sip YY, McKinstry KK, Huo Q (2017) A rapid blood test to determine the active status and duration of acute viral infection. ACS Infectious Diseases 3(11):866–873. https://doi.org/10.1021/acsinfecdis.7b00137

Zheng T, Pierre-Pierre N, Yan X, Huo Q, Almodovar AJ, Valerio F, Rivera-Ramirez I, Griffith E, Decker DD, Chen S, Zhu N (2015) Gold nanoparticle-enabled blood test for early stage cancer detection and risk assessment. ACS Appl Mater Interfaces 7(12):6819–6827. https://doi.org/10.1021/acsami.5b00371

Zhou H, Qian W, Uckun FM, Wang L, Wang YA, Chen H, Kooby D, Yu Q, Lipowska M, Staley CA, Mao H, Yang L (2015) IGF1 receptor targeted Theranostic nanoparticles for targeted and image-guided therapy of pancreatic cancer. ACS Nano 9(8):7976–7991. https://doi.org/10.1021/acsnano.5b01288

# Nanomedicine-Based Combinational Therapy for Breast Cancer

9

Pooja Jain, Nazia Hassan, Uzma Farooq, Sradhanjali Mohapatra, Thomson Santosh Alex, Mahak Fatima, Mohd Aamir Mirza, and Zeenat Iqbal

**Abstract**

Among the malignancies, breast cancer is the most common one and the high mortality rate indicates that the available treatment modalities are not sufficient to cater to this fatal disease. The complex pathophysiology of the disease demands multidrug therapy or combinational drug therapy, in which the two drugs show the nonoverlapping mode of action. Drug combinations such as doxorubicin and cyclophosphamide, bevacizumab and paclitaxel, lapatinib and paclitaxel, etc. are the clinically used combinations for breast cancer treatment where one component either enhances the therapeutic action of another drug or confers less toxicity. Along with combinational drug products, nanomedicine-based products are the promising approach for breast cancer management. Although marketed nanomedicine-based drug-products like Abraxane and Doxil were originally developed for generic anticancer purpose, they have already been exploited with fruitful clinical outcomes as breast cancer adjuvant therapy as well. However, advancements in the molecular level understanding of breast cancer and in-silico techniques have led to the development of novel nanotherapeutic strategies for complete disease amelioration, but still, some issues are there which need to be addressed such as optimal drug proportions and its control over several batches, large scalability, cost, and reproducibility of these nanomedicines during manufacturing to ensure the successful commercialization of nano-combination therapy. This chapter gives an insight into the general pathophysiology of breast cancer and available combinational drug therapy. Further, authors have elaborated on the applications of nano-based combinational

P. Jain · N. Hassan · U. Farooq · S. Mohapatra · T. S. Alex · M. Fatima · M. A. Mirza (✉) · Z. Iqbal (✉)
Department of Pharmaceutics, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi, India
e-mail: zeenatiqbal@jamiahamdard.ac.in



M. Rahman et al. (eds.), *Hormone Related Cancer Mechanistic and Nanomedicines*,
https://doi.org/10.1007/978-981-19-5558-7_9

drug therapy for breast cancer treatment. Researchers may get insight into the clinical trial status and limitations of nano-based combinational drug therapy for breast cancer.

**Keywords**

Nanomedicine · Combinational drug therapy · Clinical trials · Metastatic breast cancer · Nanocarriers

## 9.1 Introduction

In females, one of the prevalent forms of invasive cancer is breast cancer, abnormal growth of breast cells. It is regarded as a serious public health concern worldwide, as it is the second largest cause of cancer mortality after lung/skin cancer. (Momenimovahed and Salehiniya 2019). Breast cancer is often considered gender-specific as it rarely occurs in males, 1% of all breast cancer; however, it is important to be cognizant even for such rare cases (Khattab et al. 2021). Breast cancer is the second most prevalent cancer in the world and the fifth most common cause of cancer-related mortality. It is labelled as the most common cause of death in women due to cancer in developing nations and the second most common cause of cancer death in women in industrialized countries, after lung cancer (Loh et al. 2013). In the previous two decades, the rate of mortality from this malignancy has decreased by 30%, whereas in the last 5-years, overall survival rates have been increased to around 90%. Despite these signals of success, metastatic breast cancer remains a difficult disease to treat. In 2020 itself, worldwide about 2.3 million new instances of breast cancer were identified, with more than 600,000 breast cancer-related death (Pilleron et al. 2021).

Breast cancer originates from the inner lining of lobules or milk ducts of the breast tissue (Weigelt et al. 2010). Various types of tumors arise from the breast tissue, such as the noncancerous benign growth, cysts, generation of scar-like tissue, thickening, lumpiness, and tenderness of breast area (Lang et al. 2021; Patterson et al. 2004).

Despite the prevalence of breast cancer, there has been a decrease in mortality over the years, owing to advances in diagnosis and treatment, which vary greatly among geographic areas. When choosing a therapy, the advantages must outweigh the hazards. A critical concern is there to monitor the doses, medication adherence, dosing regimens, and respective responses to the treatment regimen (Anjum et al. 2017; Howard and Bland 2012). Table 9.1 enlists drugs that have been approved by the FDA in the treatment of breast cancer (Kim et al. 2016; Wang et al. 2004).

Cancer can be thought of as a network of interrelated molecular pathways that can be susceptible to the action of multiple medications at the same time, and this allows a thorough investigation of medication combinations (Foucquier and Guedj 2015). Drug combinations have various advantages, including reduced toxicity, enhanced efficacy, reduced dose at the same or higher level of efficacy, and potential to combat

**Table 9.1** List of drugs approved by FDA in the treatment of breast cancer

| Tamoxifen Citrate | Raloxifene Hydrochloride | Abemaciclib | Anastrozole |
|---|---|---|---|
| Alpelisib | Ado-Trastuzumab Emtansine | Anastrozole | Capecitabine |
| Atezolizumab | Docetaxel | Cyclophosphamide | Doxorubicin hydrochloride |
| Eribulin Mesylate | Everolimus | 5-fluorouracil | Exemestane |
| Gemcitabine hydrochloride | Fulvestrant | Ixabepilone | Goserelin acetate |
| Letrozole | Lapatinib Ditosylate | Methotrexate | Megestrol acetate |
| Olaparib | Palbociclib | Paclitaxel | Neratinib maleate |
| Pembrolizumab | Ribociclib | Pamidronate | Pertuzumab |
| Tamoxifen citrate | Sacituzumab | Thiotepa | Talazoparib |
| Tucatinib | Vinblastine sulfate | Trastuzumab | Toremifene |

**Table 9.2** Combination of Cytotoxic agents approved by FDA

| Combination of Cytotoxic agents approved by FDA | Class of drug |
|---|---|
| Doxorubicin hydrochloride<br>Cyclophosphamide | Anthracycline<br>Alkylating agent |
| Doxorubicin hydrochloride<br>Cyclophosphamide<br>Paclitaxel | Anthracycline<br>Alkylating agent<br>Microtubule damaging agent |
| Doxorubicin hydrochloride<br>Cyclophosphamide<br>5-fluorouracil | Anthracycline<br>Alkylating agent<br>Pyrimidine antagonist |
| Cyclophosphamide<br>5-fluorouracil<br>Methotrexate | Alkylating agent<br>Pyrimidine antagonist<br>Folate antagonist |
| Cyclophosphamide<br>5-fluorouracil<br>Epirubicin | Alkylating agent<br>Pyrimidine antagonist<br>Antibiotic |
| Doxorubicin hydrochloride<br>Cyclophosphamide<br>Docetaxel | Anthracycline<br>Alkylating agent<br>Microtubule damaging agent |

drug resistance from cytotoxic agents (Han et al. 2017). Six medication combinations have been thoroughly studied in breast cancer. Adjuvant therapy with doxorubicin and cyclophosphamide is utilized for the primary, recurring, and metastatic breast cancer treatment (Jones et al. 1975). Docetaxel, doxorubicin, and cyclophosphamide can be used as adjuvant treatment options. In nonmetastatic cancer; a combination of 5-fluorouracil, cyclophosphamide, and or doxorubicin, or a combination of 5-fluorouracil, cyclophosphamide, and methotrexate is being used (Hassan et al. 2010). Combination therapy approved by FDA for treating breast cancer is summarized in Table 9.2 (Yadav et al. 2015).

**Table 9.3** Various nanocarriers for breast cancer treatment

| Type of Breast Cancer | Type of Nanocarrier | Carrier | Reference |
|---|---|---|---|
| Triple-negative breast cancer-EGFR | Nanoparticle—pH and redox-sensitive cationic unimolecular | siRNA | Chen et al. (2017) |
| Triple-negative breast cancer-folate receptor | Micelle | Orlistat | Paulmurugan et al. (2016) |
| Triple-negative breast cancer-CXCR4 | Liposome | siRNA | Guo et al. (2014) |
| HER-2 positive | Modified gold nanoparticles | Radioactive 111-in | Cai et al. (2016) |
| HER-2 positive | Gold sulfide nanoparticle conjugated with HER2 antibody | Gold-gold sulfide | Day et al. (2010) |
| Triple-negative breast cancer-folate receptor | Liposomes conjugated with folate | Derivative of Benzoporphyrin | Sneider et al. (2017) |

Nanomedicine attempts to leverage nanotechnology for a variety of biomedical applications, including disease treatment, diagnosis, molecular imaging, regenerative medicine, and tissue engineering, through the utilization of particles that are 1-100 nm in size. Nanomedicine has been linked to the use of nanoparticles in oncology since the very start (Etheridge et al. 2013). Nanomedicine can be in the form of micelles, nanotubes, nanoparticles, dendrimers, and various others (Table 9.3). These can be made from proteins, lipids, inorganic products, polymers, phospholipids, or a combination of all (Xue et al. 2014; Zhang et al. 2016). In the USA, nanomedicines like Abraxane® and Doxil® are being clinically used for the treatment of breast cancer. Nanomedicine can even be used for cancer detection, theranostic, and tissue repair in breast cancer (Wu et al. 2017). Using nanomedicine, passive and active drug targeting can be done for increasing the drug levels inside the tumor, circulation time of the drug can be increased by PEGylating technique, specificity to target tumor increases, enhancement of endocytosis due to active and passive targeting, control drug release, targeting the tumor microenvironment, and co-delivery of combination chemotherapy (Wu et al. 2017).

## 9.2 Nanotherapy for Breast Cancer

Despite several technical advances, even the most effective breast cancer treatments do not have a high degree of specificity, are invasive in nature, and are marred with severe side effects making them not 100% effective. Owing to this, a pragmatic clinical approach of nanoparticulate-based platforms seems revolutionary as they are rapid and can be employed for detection, diagnosis, treatment, and prevention of breast cancer (Tang et al. 2017). The multivariate functions of nanoparticulate systems are transport across biological barriers to allow prolonged blood circulation time, passive tumor accumulation (enhanced accumulation of drugs in tumors), and active tumor targeting ("Benefits of Nanotechnology for Cancer—National Cancer Institute," 2017). The applicability of nanotherapies to target drug-resistant breast

cancer, triple-negative breast cancer (TNBC), nanoparticle-mediated photothermal ablation, and cancer immunotherapy are trending owing to their promising observations and outcomes which are discussed further (Wu et al. 2017; Tang et al. 2017).

## 9.2.1 Nanoparticulate-Based Systems for Cancer Treatment

### 9.2.1.1 Selection Criteria

The US FDA posits an invasive selection criterion for a clinical application of nanoparticulate-based systems for cancer treatment as presented in Table 9.4. Since their origin, innumerable nanomaterials have been invented but only a few can meet the requirements of the US FDA (Adair et al. 2010).

**Table 9.4** US FDA-approved selection criterion of nanoparticulate-based system for cancer treatment

| Requirements | Inference |
|---|---|
| Nontoxic and biocompatible | The material used for nanoparticulate systems should be inherently nontoxic and biocompatible to ensure patient and environmental safety. |
| Small size | Being nano, the most desired particle size range is 10–200 nm for a wide variety of delivery systems. However, the influence of a particular shape is debatable. |
| Encapsulation efficiency | The prime focus of nanoparticles is the entrapment/encapsulation of active pharmaceutical agents at a desired therapeutic levels (>50 Mol%) within the core. This may salvage unwanted degradation or clearance from blood circulation and facilitates drug release in a suggested manner (controlled/sustained/prolonged, etc.) |
| Colloidal stability | The nanoparticles should be resistant to the physiological conditions, namely, pH/ionic strength/macromolecular interactions/temperature to avoid particle agglomeration and sequestration in organs or membranes. |
| Blood circulation | Being a foreign entity, the nanoparticulate system should follow reasonable circulation time to complete the undersigned function and degrade through a biological process (photo/thermal/biological/chemical). |
| Targeting | The nanoparticulate system should either follow active or passive targeting to ensure accumulation of high concentration of a desired therapeutic within the tumor with maximum efficacy and least side effects, especially in and around healthy tissue is active targeting. Active targeting involves surface receptor molecules and passive targeting involves high permeability and retention effect. |
| Controlled release | The nanoparticulate system should have a trigger mechanism, either biological or extrinsic, to mimic the controlled release of active molecules in tumors. |
| Clearance | A clearance mechanism for the nanoparticle vehicle or carrier is necessary after the completion of a designed task to surpass systemic side effects, biological interference, and toxicity development with the host. |

#### 9.2.1.2 Clinical Modalities

The repercussions of conventional anticancer pharmaceuticals are quite high in terms of nontargeting, systemic toxicity, and other severe side effects (Shapiro and Recht 2001). For long, anthracyclines and taxanes are two mainstay conventional therapeutics used for advanced breast cancer chemotherapy. However, the induction of cardiotoxicity via doxorubicin (anthracycline) and bone marrow suppression, hypersensitivity/cutaneous reactions, and dose-limiting neurotoxicity vis-à-vis paclitaxel and docetaxel (taxanes) have limited their clinical applicability (Tang et al. 2017). The exploration of nanomedicine thus seems important as they offer positive attributes of low degradation of the active component during in vivo transport, low occurrence of adverse effects, enhanced biocompatibility, targeting, elimination, and chemotherapeutic delivery to tumors (Adair et al. 2010). For example, a reported high potential of nanomedicine against breast cancer stem cells, an important factor cited for the initiation, recurrence, and resistance of breast cancer therapies (He et al. 2016). Following is a brief discussion of different forms of the nanoparticulate system in cancer therapy.

Liposomes: Amphiphilic, self-assembling, spherical vesicles having both hydrophilic (aqueous core) and hydrophobic (lipid bilayer) membranes. The small size (typically 50-100 nm) and presence of a membranous bilayer which consists of phospholipids and glycolipids make them biodegradable, thus, supporting the prime requirements for a nanoparticulate system. They are often used for encapsulating hydrophilic chemotherapeutics (Grodzinski and Farrell 2014). Doxil® is the first liposome (50–80 nm) that has been approved by the FDA (Food and Drug Administration) to carry chemotherapy drug Doxorubicin to treat Kaposi's sarcoma, Multiple myeloma, breast, and ovarian cancer. It has been found to significantly slow tumor development, increase progression-free and overall survival rates, and reduce cardiotoxicity (Pillai 2014). Few clinical trials have outlined the combination of Doxil with cyclophosphamide+5-fluorouracil (Rau et al. 2015), cisplatin+infusional fluorouracil (Dellapasqua et al. 2011), cyclophosphamide followed by paclitaxel (Gil-Gil et al. 2015), and targeted therapy such as trastuzumab (Torrisi et al. 2010) for advanced breast cancer treatment. The aforesaid combinations have indicated high treatment efficacy and low cardiotoxicity, but the occurrence of new yet less serious side effects such as mucositis and skin toxicity needs further attention (Ansari et al. 2017). The liposome-based nanomedicine in the treatment of breast cancer is well developed. Sneider et al. have reported a novel liposome formulation for the management of triple-negative breast cancer via photodynamic therapy (Sneider et al. 2017). Qin et al. also have studied chlorotoxin-modified liposomes to target metastatic breast cancer for tumor growth and metastasis (Qin et al. 2014). Unlike, every formulation liposome is marred with some limitations such as physical/chemical instability, low encapsulation efficiency, and affinity to hydrophobic drugs, such as paclitaxel (Wu et al. 2017).

#### 9.2.1.3 Polymeric Nanoparticles or Polymersomes

The nanoparticles are composed of biocompatible and biodegradable polymers (poly lactic-co-glycolic acid, polylactic acid, polyethylene glycol) and polysaccharides

(chitosan, alginate, and pectin). They could further be classified as micelles, dendrimers, and polymer-drug conjugates. Polymeric nanoparticles can encapsulate hydrophilic or hydrophobic medicines, allowing for surface changes and pH-dependent controlled release. They are quite versatile in terms of chemical composition, but degradation as a carrier could be a disadvantage (Hussain et al. 2018).

Micelles: Colloidal particles (~100 nm) which are self-assembled from amphiphilic and biodegradable polymers. They encapsulate hydrophobic drugs for cancer therapy (breast, lung, ovarian) (Oerlemans et al. 2010), ex, polyethylene glycol- poly D L-lactic acid polymeric micelle encapsulating paclitaxel (marketed product: Genexol-PM) ("Current Nanotechnology Treatments—National Cancer Institute," 2017).

Dendrimers: Polymeric macromolecules that are star-shaped or branched, have two or more groups, and are repeated (generations) from a central core. The physicochemical characteristics of a dendrimer are determined by the peripheral surface groups, which may be changed to provide both a charged hydrophilic and lipophilic function. To achieve desirable physical properties, biodistribution, receptor-mediated targeting, and conjugation, dendrimers can be modified in terms of surface groups, interiors, cores, molecular weights, and chemical compositions. A successful study reported ten-fold reduction in tumor size when treated with methotrexate conjugate polyamidoamine (PAMAM) dendrimers as compared to a free form of methotrexate. The few limitations of dendrimers are repetitive steps for synthesis which eventually increase cost especially for large-scale production (Abedi-Gaballu et al. 2018).

#### 9.2.1.4 Inorganic Nanoparticles

Gold nanoparticles, magnetic nanomaterials, carbon nanotubes, silica nanoparticles, and quantum dots are examples of inorganic nanoparticles. Chemical composition, size, form, good stability, ease of functionalization, and better surface-to-volume ratios are among the physicochemical qualities that are exploited. They are primarily proposed for breast cancer predictive oncology and human cancer therapy. They can also be protein-coated like albumin-bound paclitaxel nanoparticles for the treatment of metastatic breast cancer ("Nanobiotechnology for Breast Cancer Treatment | IntechOpen," n.d.).

Gold nanoparticles: small metal particles which are coated with drug fragments. They are one-of-a-kind in terms of oxidation resistance, cell membrane penetration, increased targeting for rapid transport kinetics, long circulatory half-life, increased binding affinity, size-enhanced tumor absorption, and biocompatibility. Gold nanoparticles are used in the diagnosis, biomedical imaging, and therapy of solid breast cancers because of these properties. They're also employed as radiosensitizers, contrast agents, photothermal agents, and medication transporters (Lee et al. 2014). Day et al. have reported the antibody-conjugated gold nanoparticles as multipronged agents for breast cancer treatment and imaging (Day et al. 2010).

Quantum dots: Semiconductor fluorescent nanoparticles having a polymer layer, zinc sulphide, and a stabilizing molecule on the shell and core. They are highly efficient as contrast agents for imaging and drug tracking and as noninvasive probes for targeting disease biomarkers (Hussain et al. 2018).

## 9.2.2 Innovative Nanotherapies

### 9.2.2.1 Nanotherapies for Triple-Negative Breast Cancer (TNBC)

Estrogen receptor, progesterone receptor, and HER2 (transmembrane receptor with tyrosine kinase activity) expression are all absent or poorly expressed in TNBC. TNBC is a difficult condition to treat as it is proliferative, approximately 12–17% of female breast cancer patients, and is marred with poor prognosis, early recurrence, and poor survival rate. Conventionally chemotherapeutics for TNBC treatment include anthracyclines, taxanes, and cisplatin; however, they lack robust outcomes as compared to other breast cancer subtypes. The metastatic nature of TNBC demands a customized therapy and nano targeting could be one approach (Al-Mahmood et al. 2018). Earlier, Alam et al. have proposed a strategic therapy on mice to induce necrotic tumor cell death to inhibit tumor growth using adeno-associated virus type 2 (AAV2). However, the safety of the virus involved posed a big concern. This can be improved by encapsulating the virus in targeted nanocarriers (Alam et al. 2014).

### 9.2.2.2 Nanoparticle-Mediated Photothermal Ablation

Photothermal ablation (PTA) is a noninvasive breast cancer treatment option for individuals who have failed to respond to standard chemotherapies or radiation. When delivered to cancer tissues, the targeted nanoparticle-mediated PTA absorbs near-infrared irradiation and releases thermal energy above the threshold (50 °C) to damage tumor cells through various mechanisms such as membrane conformation/permeability change, cell deformation, and cell cycle dysregulation, ultimately leading to cell death or necrosis. Although the specific mechanism of tissue damage is unknown, a few seconds of irradiation are sufficient to disrupt the plasma membrane integrity of tumor cells without causing detectable harm to nonmalignant cells (Guo et al. 2013). Metallic nanomaterials (gold or silver) or other materials with high photothermal conversion efficiency are used. Metallic nanoparticles have a high photothermal conversion efficiency and substantial absorption in the near-infrared range. The prominent examples include gold nanoshells, gold nanorods, gold nanospheres, and carbon nanotubes (Chen et al. 2019). The potential for biofilm formation and persistent wounds are a key drawback of PTA, as the low metabolic activity of necrotic areas caused by irradiation renders antibiotics, antiseptics, and disinfectants ineffective. The ineffective clearance of metallic nanoparticles could also be concerning (Tang et al. 2017). As a result, new research has explored using organic nanomaterials like conjugated polymeric nanoparticles for PTA to improve biodegradability (Zha et al. 2013).

#### 9.2.2.3 Nanotherapy of Breast Cancer Stem Cells (BCSCs)

Stem cells or tumor-initiating cells are a small fraction of cancer cells capable of self-renewal and differentiation to multiple cancer cell types. These stem cells are tumorigenic as they may initiate the formation of a full tumor. They may also pose drug resistance leading to tumor relapse. The targeting of BCSCs biomarkers via nanomedicines could prevent cancer relapse and metastasis, but not many well-characterized biomarkers for targeting are known. The few most popular ones are CD44, CD133, and DCLK1 receptors. CD44 also has a signalling role in tumor microenvironment for the integration of the growth factors and cytokines (Wu et al. 2017). Gener et al. have investigated PLGA-co-PEG micelles encapsulating paclitaxel and decorated with anti-CD44 antibodies to improve treatment sensitivity against breast/colon cancer stem cells (Gener et al. 2015). Rao et al. have reported an enhanced targeting and elimination of BCSCs by delivering chitosan-decorated doxorubicin nanoparticles to CD44+ cells (Rao et al. 2015). Muntimadugu et al. have proposed CD44 targeted chemotherapy against BCSCs and cancer cells with salinomycin+paclitaxel polymeric nanoparticles surface coated with hyaluronic acid. The use of hyaluronic acid led to a 1.5-fold increase in nanoparticles uptake into the CD44 cells (Muntimadugu et al. 2016). Swaminathan et al. have demonstrated a CD133-targeted paclitaxel delivery to inhibit local tumor recurrence in breast cancer modal when compared to free paclitaxel treatment (Swaminathan et al. 2013). BCSC targeting though seems appealing, their low population and dormant state may require further attention to improve targeting. Recently, Zhao et al. have suggested a novel polymeric nanoparticles formulation double grafted with hyaluronic acid and DCLK1 antibody as a nondrug delivery system for targeting BCSCs. The effect of the dual system was pronounced as compared to only one targeting moiety (Zhao et al. 2016).

#### 9.2.2.4 Nanotherapy to Tackle Drug-Resistant Breast Cancer

A passive targeting approach in which nanocarriers bypass the cell membrane barrier via endocytosis to achieve improved intracellular drug concentration. Drug resistance could be due to ineffective drug concentration, overexpression of glycoproteins, drug-binding sites mutations, mutations of genes, etc. and served as an obstacle in breast cancer treatment. Owing to multiple mechanisms of resistance development, the application of the nano approach could either be single or combinatorial for synergistic and better therapeutic efficacy (Gao et al. 2017; Wu et al. 2017).

## 9.3 Combination Therapies in Breast Cancer Management

Being one of the most prevalent cancer types, breast cancer treatment comprises multidisciplinary approaches such as surgical treatment, radiotherapy, chemotherapy, hormonal therapy, immunotherapy, targeted therapy, neoadjuvant (radiation therapy before surgery), and adjuvant therapy (radiation and/or drug therapy after surgery). An effective breast cancer management approach should produce maximal

therapeutic efficacy, with minimum side effects to ensure an improved quality of life (QoL) which may depend on either single drug treatment, sequential drug treatment, or a combination of treatments.

It has been practically observed that in the case of cancer treatment, the best strategy is the combination of approaches such as surgery, radiation, immune, or chemotherapy. Additionally, surgery or radiation therapy is highly effective against local tumor/cancer. Further, the application of combination therapy depends on the stage, category, and location of cancer. In the case of early stage of breast cancer, surgery can be helpful alone but combination therapy can be preferred depending on the size and the stages (risk of recurrence) of the tumor. It has been reported that cancer drugs are most effective when used in combination. It is necessary that, in combination therapy, the individual drug should have a different mechanism of action, thereby reducing the possibility of cell resistance, and further in drug combination each medication can be used at its optimal dose without producing severe side effects. Furthermore, combination medication therapy is used to alleviate cancer symptoms, especially in advanced malignancies that cannot be treated with a single agent, such as non-small cell lung melanoma, oesophageal, or bladder cancer. Sometimes neoadjuvant therapy is used to shrink a tumor, thereby improving the opportunity for successful tumor removal during operation. Further, adjuvant therapy helps to abolish any remaining cancer cells after surgery.

Each component in a combination therapy (or polychemotherapy) method should have single-agent activity with no cross-resistance, as well as nonoverlapping safety profiles and preclinical synergy data. However, these standards are rarely met. As a result, combination therapy has occasionally failed to meaningfully improve results as compared to the sequential administration of single drugs because their components are delivered at inadequate dosages due to dose-limiting toxicities. Further, sequential or combined administration of antitumor agents remains a controversial approach during metastatic breast cancer management.

### 9.3.1 The Current Status of Combination Therapies in Breast Cancer

Traditionally, combination chemotherapy employed incorporation of an alkylating agent (cyclophosphamide) with antimetabolites (methotrexate and 5-fluorouracil) that effectively reduces the risk of recurrence and used to treat breast cancer (Bonadonna et al. 1976). Furthermore, the prescription to employ many agents rather than a single drug for the treatment of local breast cancer, regardless of lymph node, hormone receptor, or menopausal state, is based on the scant evidence supporting polychemotherapy (Abrams 2001; Mohapatra et al. 2020).

Owing to the advantages like improved efficacy with the reduced dose, minimal drug resistance, and low toxicity, combinational drug therapy has become a traditionally applied method in clinical practice (Anampa et al. 2015; Chou 2006). This is based on the idea that combining numerous medications with diverse biological targets can delay cancer cell mutations in the long run. Additionally, when numerous

medications are designed to target the same biological pathway, they may work together to maximize efficacy and target selectivity (Lee and Nan 2012). In modern medical practice, developments in isolation technique and chemical synthesis, along with cell biology and omics, have served as a major protagonist in the advancement of the combination treatment approaches (Chou 2006; Keith et al. 2005). It results in increased response as compared to single-agent chemotherapy, even if the effect on overall survival is less well-recognized (Telli and Carlson 2009).

In the case of metastatic breast cancer, older, empirically determined medication combinations are no more effective and far more hazardous than monotherapy, affecting quality of life while providing little or no clinical benefit. Sometimes combination therapy may be chosen over sequential therapy to meet the patient's need for urgent intervention. Furthermore, sequential therapy provides for the most effective distribution of a single drug, potentially lowering the risk of toxicity, particularly in the case of frail or elderly patients who cannot withstand the side effects of combination therapies.

Hypothetically, the combination treatment is supposed to be the promising approach based on the nonoverlapping mechanism of action of the individual drug in combination. However, practically it is not completely possible. The current combination regimen in metastatic breast cancer leads to moderate enhanced efficacy with some adverse effects which might reduce the overall effectiveness. A few clinically used combination regimens for metastatic breast cancer management are included in Table 9.5. Traditionally in a combination regimen, individual anticancer medicaments are taken as a physical mix without modifying the pharmacokinetic behavior, and hence, eliminated independently. Thus, they produce simultaneous increases in therapeutic effectiveness as well as adverse effects which is less helpful. An improved strategy such as combining molecularly targeted agents is helpful but is not patient-compliant. This leads to the development of novel methods by incorporating nanotechnology with combination treatment for the management of cancer.

The above-discussed challenges encouraged researchers to investigate novel approaches by incorporating nanotechnology with combination anticancer treatment. The potential theory is that by simultaneously delivering multiple drugs via a carrier-mediated drug delivery system, the combination system may be able to create synergistic anticancer benefits with enhanced pharmacology and reduced individual drug-related toxicity. This can be shown by the following Fig. 9.1.

### 9.3.2 Novel Approaches for Combination Drug Delivery in Breast Cancer Management

Currently, the new technique of delivering multiple drugs simultaneously via a carrier-mediated drug delivery system is widely used, and it has a number of advantages over traditional drug delivery via physical mixing. This novel approach of drug combinations helps to enhance the drug-carrying capacity; increases the circulation half-life; increases the active targeting and accumulation at the tumor site;

**Table 9.5** Combination regimens for metastatic breast cancer frequently used in clinical practice

| Drug combination regimen | Advantages | Disadvantages | Reference |
|---|---|---|---|
| Doxorubicin + cyclophosphamide | ↑ response rate (RR) | No substantial alteration in survival/progression time, ↑ treatment linked toxicity, & ↓ quality of life | Bonneterre (1991); Joensuu et al. (1998); Lee and Nan (2012) |
| Epirubicin + fluorouracil | | | |
| Epirubicin + cyclophosphamide | | | |
| Doxorubicin + paclitaxel | ↑ response rate and progression-free survival | ↑ hematologic toxicity, cardiotoxicity | Bria et al. (2005); Burzykowski et al. (2008) |
| Capecitabine + docetaxel | ↑ time to progression, response rate, and overall survival | ↑ nonhematologic toxicity (stomatitis, diarrhoea, and hand-foot syndrome) | Albain et al. (2008); Chan et al. (2009); O'Shaughnessy et al. (2002) |
| Cyclophosphamide + methotrexate + fluorouracil | ↑ response rate, relapse-free survival, and overall survival | Rapid bone loss | Ackland et al. (2001); Bonadonna et al. (1995); Hainsworth (1997) |
| Doxorubicin + cyclophosphamide+ Trastuzumab | ↑ response rate, progression-free survival, and overall survival | Hematologic toxicity & cardiomyopathy | Marty et al. (2005); Robert et al. (2006) |
| Trastuzumab + other chemotherapy (Vinorelbine, docetaxel, paclitaxel, platinum compounds, Capecitabine, and gemcitabine) | ↑ response rate and progression-free survival | ↑ hematologic toxicity | Bartsch et al. (2007) |
| Paclitaxel + bevacizumab | ↑ progression-free survival | ↑ toxicity (bleeding, hypertension, nasal septum perforation, proteinuria, heart failure, thromboembolic event, mortality) | Miller et al. (2007) |
| Paclitaxel + Lapatinib | ↑ response rate, time to progression, and progression-free survival | ↑ toxicity (toxicity from chemotherapy characterized by diarrhoea, skin rash, nausea, pruritis) | Cameron et al. (2010) |
| Cisplatin + Erotinib + gemcitabine | Well-tolerated | No survival assistance | Gatzemeier et al. (2007) |
| Iniparib + carboplatin + gemcitabine | ↑ progression-free survival and ↑ overall survival | Neutropenia, thrombocytopenia, anemia,leukopenia, fatigue or asthenia | O'Shaughnessy et al. (2011) |

(continued)

**Table 9.5** (continued)

| Drug combination regimen | Advantages | Disadvantages | Reference |
|---|---|---|---|
| Lapatinib + Trastuzumab | ↑ progression-free survival and overcome trastuzumab resistance | Patient noncompliance with additive toxicity | Blackwell et al. (2010) |
| Pertuzumab+ Trastuzumab+ plus docetaxel/ hyaluronidase-zzxf | ↑ response rate | Alopecia, nausea, diarrhoea, anemia, & asthenia | Richard et al. (2016) |
| Palbociclib+Fulvestrant | ↑ progression-free survival and ↑ response rate | Neutropenia, leucopenia & fatigue | Turner et al. (2018) |

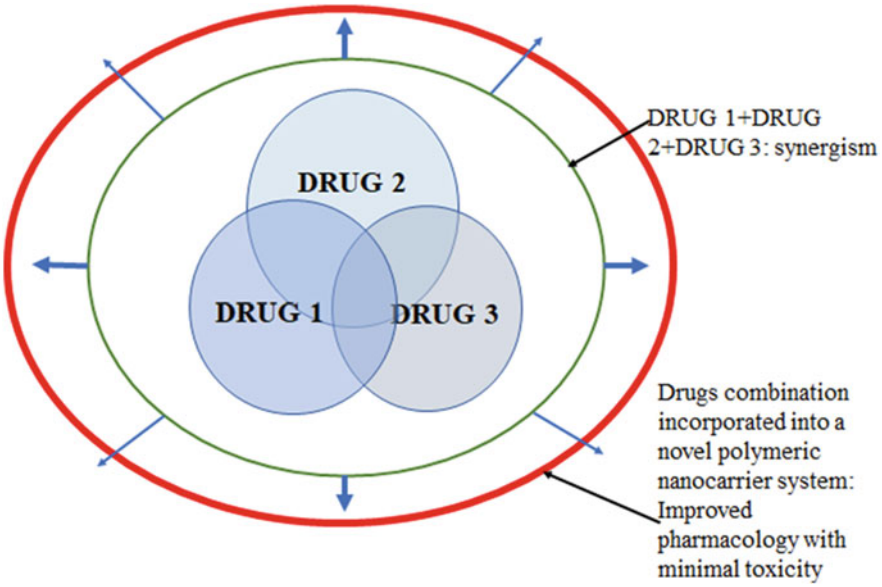



**Fig. 9.1** Drug combinations utilizing a carrier-mediated drug delivery system

has the potential to circumvent the multidrug resistance mechanisms by endocytotic uptake; reduces nonspecific uptake; controls the pharmacokinetics of individual drug; capability to carry and transport varieties of diagnostics and therapeutic agents through a single carrier system, that would act in a controlled manner; enhanced drug stability and biocompatibility; reduces the overall dose, its frequency, and toxicity, and ultimately enhances the efficacy and patient compliance thus pragmatically used in breast cancer management.

Among novel approaches, nanotechnology in cancer plays a vital role with several applications such as in the diagnosis, treatment, to understand the cancer progression and identification of cancer biomarkers, etc. They can deliver both hydrophilic and hydrophobic drugs to the targeted site along with the delivery of small molecular drugs, peptides, or antibodies. Further, the small size of the particle adds value to their penetration capacity and hence efficiency. Nanoparticles can combine several therapeutic agents that belong to different classes within the same system to achieve the desired objective in breast cancer treatment. Nano-combinations of drugs help to control the release characteristic and altered pharmacokinetics and biodistribution of individual drugs in such a manner that would not be possible with conventional formulations of free drugs. Practically, it has been found that nano-combination chemotherapy produces comparatively better outcomes and an enhanced survival than that of single-drug therapy in both preclinical and clinical investigations. During the last few years, nanotechnology has become an important area of research in medicine and has contributed to clinical therapeutics significantly. Liposomes and polymeric nanoparticles, in particular, have made medication delivery safer and more efficient for a variety of treatments. Few other nanoparticulate systems include nanoemulsions, dendrimers, carbon nanoparticles, inorganic nanoparticles, carbon nanotubes, quantum dots, water-soluble polymer-drug conjugates, etc. Currently, theranostic nanocarriers made up of metallic/magnetic nanoparticles have gained much attention owing to their ability to perform concurrent functions at targeted sites. In addition to the above, some other interesting systems have been exploited in breast cancer to improve the antitumor immunogenicity such as phage nanoparticles, virus-like nanoparticles, extracellular vehicles, etc. (Bahreyni et al. 2020). The following Fig. 9.2 depicts the several possible combinations of cytotoxic therapies (such as chemotherapy, photothermal, and photodynamic) with biologics and immunotherapies using nanoparticles that are used in clinical applications for cancer management (Jadia et al. 2016). Irrespective of all these stratagems, cancer management is still a hard nut to crack. However, several investigations are carried out for nano-combination treatments, using multiple drug loading tactics through nanoparticles to target various cancer hallmarks; but it is important to select the right combination to target and schedule the specific therapies for better outcomes. Table 9.6 enlists various combinational drug delivery approaches using the novel delivery systems for metastatic breast cancer management.

However, nanotechnology is expanding its stake in cancer drug applications, but has several limitations specified in the development of nano-combinations which decrease its scalability. The main disadvantage related to nanoparticles is their cell-mediated clearance by the reticuloendothelial system which may stimulate the production of cytokines such as interleukins, interferons, and tumor necrosis factor that leads to local inflammation (Desai 2012; von Roemeling et al. 2017). This nonspecific uptake to normal tissues results in a higher dose for anticancer drugs and hence increases the toxic effects. This can be minimized by altering the surface chemistry to prevent serum protein adsorption/absorption on the nanoparticle surface. PEGylation suspends the opsonization (Choi et al. 2009), by promoting the

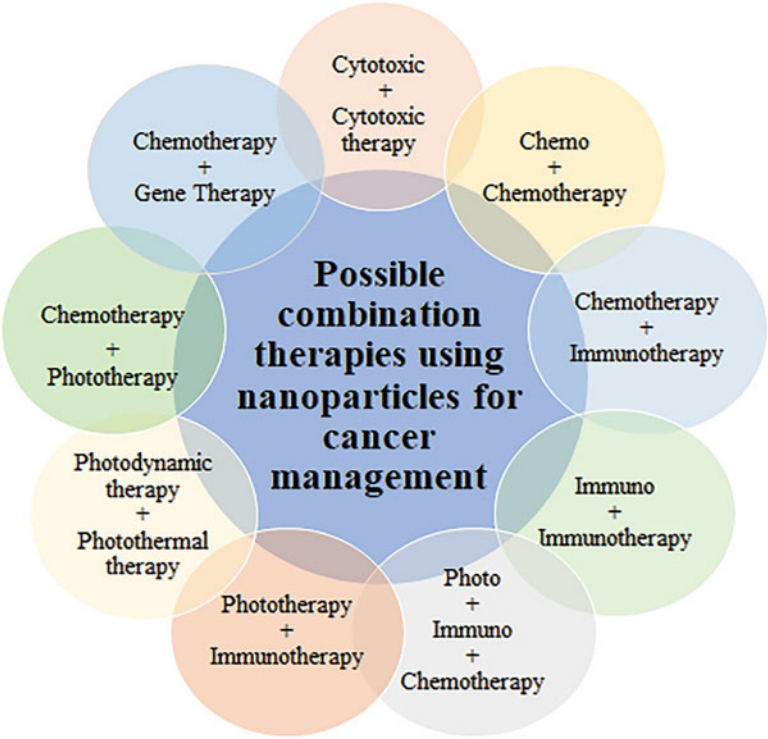



**Fig. 9.2** Possible combination therapies using nanoparticles for cancer management

stronger bonds between polyethylene glycol subunits and water molecules, thereby forming a hydrating layer that can block the absorption/adsorption of protein (Schöttler et al. 2016). Further, the coating of nanoparticles with surfactants facilitates receptor-mediated transport by specifically adsorbing serum proteins (Niewoehner et al. 2014), which further helps to cross the blood-brain barrier and to treat brain cancer/tumor. Furthermore, the optimal drug proportions and its control over several batches, large scalability, cost, and reproducibility of these nanomedicines during manufacturing are the challenges that need to be addressed to ensure the successful commercialization of nano-combination therapy.

## 9.4 Clinical Trials on Combinational Nano Therapy for Breast Cancer

Based on the promising results of in vitro and in vivo studies, studies on human subjects were conducted across the globe and some of them are listed in table 9.7.

**Table 9.6** Combinational drug delivery approaches using the novel delivery system for metastatic breast cancer management

| Drug combination | Carrier composition | Status | Reference |
|---|---|---|---|
| Quercetin + vincristine | PEG-liposome | In vivo | Wong and Chiu (2011) |
| siRNA + doxorubicin | Cationic, anionic PEG-liposome | In vivo | Chen et al. (2010) |
| Lonidamine + paclitaxel | PEG-PLGA | In vivo | Milane et al. (2011) |
| Paclitaxel + Interleukin-12 or siRNA | Poly(N-methyldietheneamine sebacate)-co-[(cholesteryl oxocarbonylamido ethyl) methyl bis(ethylene) ammonium bromide] sebacate P(MDS-co-CES) | In vivo | Wang et al. (2006) |
| Doxorubicin + Wortmannin | PEG-block-poly(N-hexyl stearate l-aspartamide) | In vitro | Bae et al. (2007) |
| Aminoglutethimide + doxorubicin | N-(2-hydroxypropyl) methacrylamide (HPMA) | In vitro | Greco et al. (2007, 2005) |
| Trastuzumab + PKI166 | N-(2-hydroxypropyl) methacrylamide copolymer | In vitro | Lee et al. (2011) |
| Doxorubicin + Mitomycin C | Polymer-lipid hybrid nanoparticles | In vitro/ *clinical* | Prasad et al. (2013); Shuhendler et al. (2010); Zhang et al. (2016) |
| Paclitaxel+ rapamycin+17-AAG (Triolimus) | Poly(ethyleneglycol)-block-poly (lactic-acid; PEG-PLA) micelles | In vitro & in vivo | Hasenstein et al. (2012) |
| Hyaluronic acid ligand modified paclitaxel | 1,2-distearoyl phosphatideylethanolamine | In vitro *and* in vivo | Yu et al. (2016) |
| Docetaxel+ Trastuzumab | Nanostructured lipid carriers having fatty amines stearyl amine/ dodecyl amine/spermine | In vitro | Varshosaz et al. (2018) |
| Gemcitabine + gadolinium | Magnetic resonance imaging contrast agent, with self-assembled nanoparticles | In vivo | Li et al. (2016) |
| Thechnetium-99 m + Galium-68 | Trastuzu-mab and diethylene-triamine-pentaacetic acid-conjugated iron oxide nanoparticles | In vitro & in vivo | de Souza Albernaz et al. (2018) |
| Vincristine +verapamil | Poly(lactic-co-glycolic acid) nanoparticle Co-encapsulating | In vitro | Song et al. (2009) |
| Paclitaxel+ thioridazine and + HY19991 | Biodegradable polymer+ zinc phthalocyanine photosensitizer | Ex vivo | Marrache et al. (2013) |
| Doxorubicin+ T780 | Bovine serum albumin | In vivo | Bahreyni et al. (2020) |
| Indocyanine green+ doxorubicin, a + CpG | Surface-functionalized modified copper sulfide nanoparticles | In vivo | Wang et al. (2019) |

**Table 9.7** Clinical trials on combinational nano therapy for breast cancer

| S. No | Title | Clinical trial identifier | Study detail | Studied Drug |
|---|---|---|---|---|
| 1. | Combination for triple-negative breast cancer | NCT03289819 | This is a multicenter, phase II, a one-arm, open-label neoadjuvant study in TNBC patients. | Pembrolizumab, nab-paclitaxel, Epirubicin, cyclophosphamide |
| 2. | Combination for breast cancer | NCT03417544 | This is a phase II clinical trial to test the safety and effectiveness of an investigational intervention. | Atezolizumab, Pertuzumab, Trastuzumab |
| 3. | Combination chemotherapy for locally advanced and metastatic breast cancer | NCT00001239 | This is a phase II trial to find the best chemotherapy regimen for advanced breast cancer patients. | FLAC with GM-CSF |
| 4. | Comparison of drug combination with hormonal therapy versus combination chemotherapy in advanced or metastatic breast cancer | NCT03839823 | In patients with hormone receptor-positive/HER2-negative inoperable locally advanced or metastatic breast cancer, a phase II randomized research compared the combination of Ribociclib plus goserelin acetate with hormonal therapy vs physician-selected chemotherapy. | Docetaxel / Capecitabine / Vinorelbinepaclitaxel / gemcitabineRibociclibLetrozole OR AnastrozoleGoserelin |
| 5. | Combination chemotherapy with or without trastuzumab for metastatic breast cancer treatment | NCT00004888 | Phase II trial to evaluate the efficacy of combination chemotherapy with or without trastuzumab in metastatic breast cancer patients. | Drug: Pegylated liposomal doxorubicin hydrochloridedocetaxeltrastuzumab |

(continued)

**Table 9.7** (continued)

| S. No | Title | Clinical trial identifier | Study detail | Studied Drug |
|---|---|---|---|---|
| 6. | Bevacizumab and combination chemotherapy with or without Darbepoetin Alfa in treating women with stage III breast canceration chemotherapy in patients with lymph node-positive breast cancer | NCT00119262 | This phase II trial is designed to evaluate the effectiveness of bevacizumab in combination with chemotherapy on patients undergoing surgery for breast cancer that has metastasis to the lymph nodes. | Doxorubicin hydrochlorideDrucyclophosphamidebevacizumabpaclitaxelfilgrastimpegfilgrastimradiation therapytamoxifen citratedrug: Aromatase inhibition therapy |
| 7. | Combination chemotherapy with or without Darbepoetin Alfa in treating women with stage III breast cancer | NCT00309920 | In patients with stage III breast cancer, a randomized clinical trial was conducted to compare the effectiveness of combination chemotherapy with darbepoetin alfa vs combination chemotherapy alone. | Darbepoetin alfacyclophosphamidedocetaxeldoxorubicin hydrochloride epirubicin hydrochloridefluorouracil |
| 8. | Combination chemotherapy in treating patients with early stage breast cancer that has been removed by surgery (TACT2) | NCT00301925 | A randomized phase III trial was conducted to examine the efficacy of different combination chemotherapy regimens in patients with early-stage breast cancer who underwent surgery. | Pegfilgrastimcapecitabinecyclophosphamideepirubicin hydrochloridefluorouracilmethotrexate |
| 9. | Combination chemotherapy in treating women with breast cancer | NCT00003519 | Women with breast cancer who have undergone surgery to remove the tumor were | Cyclophosphamidedocetaxeldoxorubicin hydrochloride |

| | | | | |
|---|---|---|---|---|
| | | | enrolled in a randomized phase III trial comparing two chemotherapy regimen combinations. | |
| 10. | Combination chemotherapy to treat stage I, stage II, or stage IIIA breast cancer and positive axillary lymph nodes | NCT00003782 | Randomized phase III trial which compared evaluate the combinations of chemotherapy in treating women who underwent surgery for stage I, stage II, or stage IIIA breast cancer with positive axillary lymph nodes. | Cyclophosphamidedocetaxel doxorubicin |
| 11. | Combination chemotherapy in patience with locally advanced breast cancer | NCT00310089 | This is a randomized clinical trial to study the effectiveness of AZD2171 with combination chemotherapy works in the patience of locally advanced breast cancer. | FilgrastimPegfilgrastimcediranib maleatecyclophosphamidedocetaxeldoxorubicin hydrochloride |
| 12. | Combination chemotherapy in treating women with stage IIIB or stage IV breast cancer | NCT00003352 | Women with stage IIIB or stage IV breast cancer will be treated in a phase II trial using combination of doxorubicin, cyclophosphamide, and docetaxel. | CyclophosphamideTaxotereAdriamycin |
| 13. | Combination chemotherapy in treating patients with breast cancer | NCT00003088 | In patients with stage II or stage IIIA breast cancer, a randomized phase III trial will compare single-drug therapy to combination chemotherapy. | Cyclophosphamidedoxorubicin hydrochloridepaclitaxel |

(continued)

**Table 9.7** (continued)

| S. No | Title | Clinical trial identifier | Study detail | Studied Drug |
|---|---|---|---|---|
| 14. | Trastuzumab and chemotherapy followed by surgery and combination chemotherapy in locally advanced breast cancer patients | NCT00009997 | Phase I trial to study the effectiveness of drug combination after surgery and in patients with advanced breast cancer. | Trastuzumabcyclophosphamidedoxorubicin hydrochloridepaclitaxeltamoxifen citrate |

## 9.5 Limitations of Nanotherapy for Breast Cancer

Angiogenesis is actively involved in tumor growth so that the blood vessels can continuously supply oxygen and nutrients to the cancer cells. For the treatment of cancer, antiangiogenic drug molecules are either alone or in combination with cytotoxic drugs. However, the mechanism of their combinational therapies is not clear, with few studies revealing their limitations (Casanovas 2012). According to research reports, antiangiogenic agents pharmacologically act to prevent the delivery of cytotoxic drugs to the targeted area, and hence lead to the reduced therapeutic effect of other anticancer drugs, thereby enhancing the tumor growth. As we know, angiogenesis inhibitors act on vascular endothelial growth factor (VEGF) that efficiently prevents tumor aggressive cell growth (Conley et al., 2012).

As per vascular normalization theory, an antiangiogenesis drug abnormally develops changes in structure and function of blood vessels in tumors and behaves as normal functional blood vessels having better blood flow and allows more systemic delivery of cytotoxic drugs on the cancer cell (Luchino et al. 2013). By using radiolabelled docetaxel with PET (positron emission tomography) imaging technology, an inhibitor of VEGF in combination with drugs (known as bevacizumab) exhibits fast and sustained delivery of docetaxel on the targeted tumor, but it reduces the permeation of docetaxel as shown in Fig. 9.3. Bevacizumab facilitates high glucose uptake to the cancer cell due to the development of a normal vascular system (Hurwitz et al. 2005).

Another limitation of antiangiogenesis drugs is related to the high adaptability of tumors. Initially, it was believed that no resistance will be developed for antiangiogenesis drugs, as these drugs target blood vessels instead of tumor cells. But later on, studies revealed that antiangiogenesis drugs could also develop resistance (Kabbinavar et al. 2005). To clarify this problem, Conley and coworkers studied mice (animal models) by the implantation of human breast cancer cell lines and further treated with antiangiogenic drugs. During treatment, certain cancer cells are deposited to activate the enzyme aldehyde dehydrogenase similar to progenitor cells, which can initiate cancer by reinjection in another animal (mice) (Sarah J. Conley et al. 2012).

Treatment-related issues can be resolved by the addition of drugs that can induce hypoxia or inhibit the Akt/β-catenin pathway with an antiangiogenesis agent. Hypoxia (deficiency of oxygen) in cancer activates the hypoxia-response program and the Akt/β-catenin pathway regulates the growth of cells and adheres in between the cells (Van der Veldt et al. 2012). It was observed that drugs can suppress the tumor cells due to hypoxia or deactivate the Akt/β-catenin pathway. In order to control the cancer spreadability, semaphorin proteins can also be targeted with the drug molecules (Conley et al., 2012).

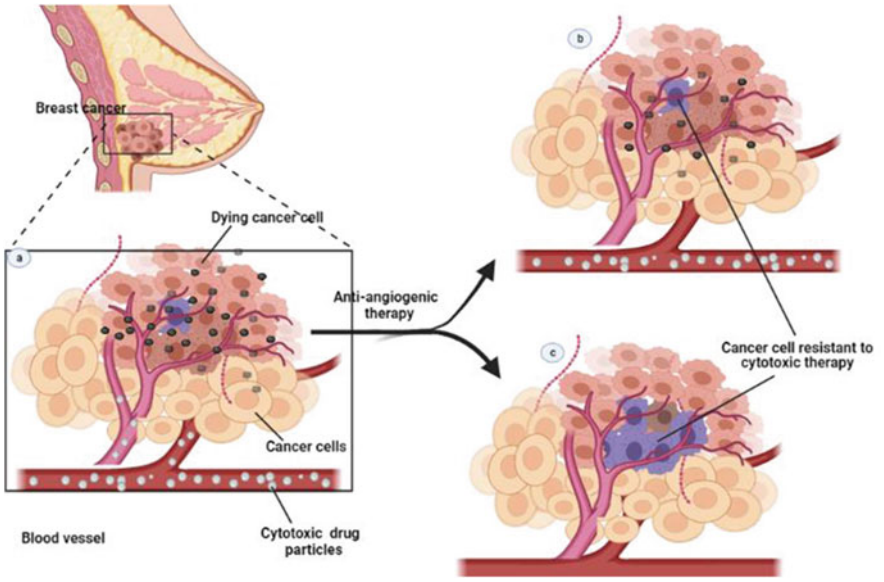



**Fig. 9.3** Limitations of anticancer therapies. (**a**) Cancer is treated with antiangiogenesis either single or along with cytotoxic agents that prevent cell growth. In this, some cancer cells are resistant to cytotoxic drugs (more aggressive than other cancer cells) and can be capable to spread cancer in other organs. (**b**) Low cytotoxic drug distribution and efficacy due to limited blood vessels (**c**) In the tumor under hypoxia, antiangiogenic treatment can induce the deposition of more aggressive cells and high spread ability

## 9.6 Conclusion

The high mortality rate associated with breast cancer is due to its heterogeneous nature. If cancer metastasized, then despite the availability of various standard chemotherapeutic agents, the effective treatment options become very less. Owing to the complex pathophysiology, single drug treatment is not sufficient for breast cancer treatment, hence combination therapy is most suitable. But, in most cases, systemic drug delivery is not effective, and despite the combinational therapy, chances of patient recovery are very less. Therefore, we are in urgent need of novel treatment modalities to tackle this deadly disease. And nanomedicine is a broad umbrella, underneath which various vehicular systems are present to cater to complex diseases. The past few decades have witnessed substantial progress in nanomedicines development. FDA-approved nanomedicines are available in the market for cancer patients; however, for breast cancer, no specific product is available but many of them are in different phases of the clinical trial.

But before exploiting nano vehicles for breast cancer treatment, nanoformulations-associated toxicity and immunogenicity should be carefully evaluated. And the emphasis should be given to drug pharmacology,

pharmacokinetics, formulation materials, toxicity, and nano-formulation type. Altogether, it is hoped that, in near future, we will be able to successfully treat breast cancer with nano medicinal products.

**Acknowledgments** Author, Pooja Jain is thankful to CSIR for providing financial assistance in the form of SRF [09/0591(11905)/2021-EMR-I].

## References

Abedi-Gaballu F, Dehghan G, Ghaffari M, Yekta R, Abbaspour-Ravasjani S, Baradaran B, Dolatabadi JEN, Hamblin MR (2018) PAMAM dendrimers as efficient drug and gene delivery nanosystems for cancer therapy. Appl Mater Today 12:177–190. https://doi.org/10.1016/j.apmt.2018.05.002

Abrams JS (2001) Adjuvant therapy for breast cancer–results from the USA consensus conference. Breast Cancer Tokyo Jpn 8:298–304. https://doi.org/10.1007/BF02967528

Ackland SP, Anton A, Breitbach GP, Colajori E, Tursi JM, Delfino C, Efremidis A, Ezzat A, Fittipaldo A, Kolaric K, Lopez M, Viaro D, HEPI 013 study group (2001) Dose-intensive epirubicin-based chemotherapy is superior to an intensive intravenous cyclophosphamide, methotrexate, and fluorouracil regimen in metastatic breast cancer: a randomized multinational study. J Clin Oncol Off J Am Soc Clin Oncol 19:943–953. https://doi.org/10.1200/JCO.2001.19.4.943

Adair JH, Parette MP, Altınoglu EI, Kester M (2010) Nanoparticulate alternatives for drug delivery. ACS Nano 4:4967–4970

Alam S, Bowser BS, Israr M, Conway MJ, Meyers C (2014) Adeno-associated virus type 2 infection of nude mouse human breast cancer xenograft induces necrotic death and inhibits tumor growth. Cancer Biol Ther 15:1013–1028. https://doi.org/10.4161/cbt.29172

Albain KS, Nag SM, Calderillo-Ruiz G, Jordaan JP, Llombart AC, Pluzanska A, Rolski J, Melemed AS, Reyes-Vidal JM, Sekhon JS, Simms L, O'Shaughnessy J (2008) Gemcitabine plus paclitaxel versus paclitaxel monotherapy in patients with metastatic breast cancer and prior anthracycline treatment. J Clin Oncol Off J Am Soc Clin Oncol 26:3950–3957. https://doi.org/10.1200/JCO.2007.11.9362

Al-Mahmood S, Sapiezynski J, Garbuzenko OB, Minko T (2018) Metastatic and triple-negative breast cancer: challenges and treatment options. Drug Deliv Transl Res 8:1483–1507. https://doi.org/10.1007/s13346-018-0551-3

Anampa J, Makower D, Sparano JA (2015) Progress in adjuvant chemotherapy for breast cancer: an overview. BMC Med 13:195. https://doi.org/10.1186/s12916-015-0439-8

Anjum F, Razvi N, Masood MA (2017) Breast cancer therapy: a mini review. MOJ Drug Dev Ther 1:00006

Ansari L, Shiehzadeh F, Taherzadeh Z, Nikoofal-Sahlabadi S, Momtazi-Borojeni AA, Sahebkar A, Eslami S (2017) The most prevalent side effects of pegylated liposomal doxorubicin monotherapy in women with metastatic breast cancer: a systematic review of clinical trials. Cancer Gene Ther 24:189–193. https://doi.org/10.1038/cgt.2017.9

Bae Y, Diezi TA, Zhao A, Kwon GS (2007) Mixed polymeric micelles for combination cancer chemotherapy through the concurrent delivery of multiple chemotherapeutic agents. J Control Release Off J Control Release Soc 122:324–330. https://doi.org/10.1016/j.jconrel.2007.05.038

Bahreyni A, Mohamud Y, Luo H (2020) Emerging nanomedicines for effective breast cancer immunotherapy. J Nanobiotechnology 18:180. https://doi.org/10.1186/s12951-020-00741-z

Bartsch R, Wenzel C, Altorjai G, Pluschnig U, Rudas M, Mader RM, Gnant M, Zielinski CC, Steger GG (2007) Capecitabine and trastuzumab in heavily pretreated metastatic breast cancer. J Clin Oncol Off J Am Soc Clin Oncol 25:3853–3858. https://doi.org/10.1200/JCO.2007.11.9776

Benefits of Nanotechnology for Cancer–National Cancer Institute [WWW Document] (2017). URL https://www.cancer.gov/nano/cancer-nanotechnology/benefits. Accessed 7.18.21

Blackwell KL, Burstein HJ, Storniolo AM, Rugo H, Sledge G, Koehler M, Ellis C, Casey M, Vukelja S, Bischoff J, Baselga J, O'Shaughnessy J (2010) Randomized study of Lapatinib alone or in combination with trastuzumab in women with ErbB2-positive, trastuzumab-refractory metastatic breast cancer. J Clin Oncol Off J Am Soc Clin Oncol 28:1124–1130. https://doi.org/10.1200/JCO.2008.21.4437

Bonadonna G, Brusamolino E, Valagussa P, Rossi A, Brugnatelli L, Brambilla C, De Lena M, Tancini G, Bajetta E, Musumeci R, Veronesi U (1976) Combination chemotherapy as an adjuvant treatment in operable breast cancer. N Engl J Med 294:405–410. https://doi.org/10.1056/NEJM197602192940801

Bonadonna G, Valagussa P, Moliterni A, Zambetti M, Brambilla C (1995) Adjuvant cyclophosphamide, methotrexate, and fluorouracil in node-positive breast cancer: the results of 20 years of follow-up. N Engl J Med 332:901–906. https://doi.org/10.1056/NEJM199504063321401

Bonneterre J (1991) A prospective randomized trial comparing epirubicin monochemotherapy to two fluorouracil, cyclophosphamide, and epirubicin regimens differing in epirubicin dose in advanced breast cancer patients. The French Epirubicin study group. J Clin Oncol Off J Am Soc Clin Oncol 9:305–312. https://doi.org/10.1200/JCO.1991.9.2.305

Bria E, Giannarelli D, Felici A, Peters WP, Nisticò C, Vanni B, Cuppone F, Cognetti F, Terzoli E (2005) Taxanes with anthracyclines as first-line chemotherapy for metastatic breast carcinoma. Cancer 103:672–679. https://doi.org/10.1002/cncr.20757

Burzykowski T, Buyse M, Piccart-Gebhart MJ, Sledge G, Carmichael J, Lück H-J, Mackey JR, Nabholtz J-M, Paridaens R, Biganzoli L, Jassem J, Bontenbal M, Bonneterre J, Chan S, Basaran GA, Therasse P (2008) Evaluation of tumor response, disease control, progression-free survival, and time to progression as potential surrogate end points in metastatic breast cancer. J Clin Oncol Off J Am Soc Clin Oncol 26:1987–1992. https://doi.org/10.1200/JCO.2007.10.8407

Cai Z, Chattopadhyay N, Yang K, Kwon YL, Yook S, Pignol J-P, Reilly RM (2016) 111In-labeled trastuzumab-modified gold nanoparticles are cytotoxic in vitro to HER2-positive breast cancer cells and arrest tumor growth in vivo in athymic mice after intratumoral injection. Nucl Med Biol 43:818–826. https://doi.org/10.1016/j.nucmedbio.2016.08.009

Cameron D, Casey M, Oliva C, Newstat B, Imwalle B, Geyer CE (2010) Lapatinib plus capecitabine in women with HER-2-positive advanced breast cancer: final survival analysis of a phase III randomized trial. Oncologist 15:924–934. https://doi.org/10.1634/theoncologist.2009-0181

Casanovas O (2012) Limitations of therapies exposed. Nature 484:44–46

Chan S, Romieu G, Huober J, Delozier T, Tubiana-Hulin M, Schneeweiss A, Lluch A, Llombart A, du Bois A, Kreienberg R, Mayordomo JI, Antón A, Harrison M, Jones A, Carrasco E, Vaury AT, Frimodt-Moller B, Fumoleau P (2009) Phase III study of gemcitabine plus docetaxel compared with capecitabine plus docetaxel for anthracycline-pretreated patients with metastatic breast cancer. J Clin Oncol Off J Am Soc Clin Oncol 27:1753–1760. https://doi.org/10.1200/JCO.2007.15.8485

Chen G, Wang Y, Xie R, Gong S (2017) Tumor-targeted pH/redox dual-sensitive unimolecular nanoparticles for efficient siRNA delivery. J. Control Release Off J Control Release Soc 259:105–114. https://doi.org/10.1016/j.jconrel.2017.01.042

Chen J, Ning C, Zhou Z, Yu P, Zhu Y, Tan G, Mao C (2019) Nanomaterials as photothermal therapeutic agents. Prog Mater Sci 99:1–26. https://doi.org/10.1016/j.pmatsci.2018.07.005

Chen Y, Bathula SR, Li J, Huang L (2010) Multifunctional nanoparticles delivering small interfering RNA and doxorubicin overcome drug resistance in cancer. J Biol Chem 285:22639–22650. https://doi.org/10.1074/jbc.M110.125906

Choi HS, Ipe BI, Misra P, Lee JH, Bawendi MG, Frangioni JV (2009) Tissue–and organ-selective biodistribution of NIR fluorescent quantum dots. Nano Lett 9:2354–2359. https://doi.org/10.1021/nl900872r

Chou T-C (2006) Theoretical basis, experimental design, and computerized simulation of synergism and antagonism in drug combination studies. Pharmacol Rev 58:621–681. https://doi.org/10.1124/pr.58.3.10

Conley SJ, Gheordunescu E, Kakarala P, Newman B, Korkaya H, Heath AN, Clouthier SG, Wicha MS (2012a) Antiangiogenic agents increase breast cancer stem cells via the generation of tumor hypoxia. Proc Natl Acad Sci 109:2784–2789

Conley SJ, Gheordunescu E, Kakarala P, Newman B, Korkaya H, Heath AN, Clouthier SG, Wicha MS (2012) Antiangiogenic agents increase breast cancer stem cells via the generation of tumor hypoxia. Proc Natl Acad Sci U S A 109:2784–2789. https://doi.org/10.1073/pnas.1018866109

Current Nanotechnology Treatments–National Cancer Institute [WWW Document] (2017). URL https://www.cancer.gov/nano/cancer-nanotechnology/current-treatments Accessed 7.18.21

Day ES, Bickford LR, Slater JH, Riggall NS, Drezek RA, West JL (2010) Antibody-conjugated gold-gold sulfide nanoparticles as multifunctional agents for imaging and therapy of breast cancer. Int J Nanomedicine 5:445–454. https://doi.org/10.2147/ijn.s10881

de Souza Albernaz M, Toma SH, Clanton J, Araki K, Santos-Oliveira R (2018) Decorated superparamagnetic iron oxide nanoparticles with monoclonal antibody and diethylene-Triamine-Pentaacetic acid labeled with Thechnetium-99m and Galium-68 for breast cancer imaging. Pharm Res 35:24. https://doi.org/10.1007/s11095-017-2320-2

Dellapasqua S, Mazza M, Rosa D, Ghisini R, Scarano E, Torrisi R, Maisonneuve P, Viale G, Cassano E, Veronesi P, Luini A, Goldhirsch A, Colleoni M (2011) Pegylated liposomal doxorubicin in combination with low-dose metronomic cyclophosphamide as preoperative treatment for patients with locally advanced breast cancer. Breast 20:319–323. https://doi.org/10.1016/j.breast.2011.02.014

Desai N (2012) Challenges in development of nanoparticle-based therapeutics. AAPS J 14:282–295. https://doi.org/10.1208/s12248-012-9339-4

Etheridge ML, Campbell SA, Erdman AG, Haynes CL, Wolf SM, McCullough J (2013) The big picture on nanomedicine: the state of investigational and approved nanomedicine products. Nanomedicine Nanotechnol Biol Med 9:1–14. https://doi.org/10.1016/j.nano.2012.05.013

Foucquier J, Guedj M (2015) Analysis of drug combinations: current methodological landscape. Pharmacol Res Perspect 3:e00149. https://doi.org/10.1002/prp2.149

Gao M, Xu Y, Qiu L (2017) Sensitization of multidrug-resistant malignant cells by liposomes co-encapsulating doxorubicin and chloroquine through autophagic inhibition. J Liposome Res 27:151–160. https://doi.org/10.1080/08982104.2016.1185731

Gatzemeier U, Pluzanska A, Szczesna A, Kaukel E, Roubec J, De Rosa F, Milanowski J, Karnicka-Mlodkowski H, Pesek M, Serwatowski P, Ramlau R, Janaskova T, Vansteenkiste J, Strausz J, Manikhas GM, Von Pawel J (2007) Phase III study of erlotinib in combination with cisplatin and gemcitabine in advanced non-small-cell lung cancer: the Tarceva lung cancer investigation trial. J Clin Oncol Off J Am Soc Clin Oncol 25:1545–1552. https://doi.org/10.1200/JCO.2005.05.1474

Gener P, Gouveia LP, Sabat GR, de Sousa Rafael DF, Fort NB, Arranja A, Fernández Y, Prieto RM, Ortega JS, Arango D, Abasolo I, Videira M, Schwartz S (2015) Fluorescent CSC models evidence that targeted nanomedicines improve treatment sensitivity of breast and colon cancer stem cells. Nanomedicine Nanotechnol Biol Med 11:1883–1892. https://doi.org/10.1016/j.nano.2015.07.009

Gil-Gil MJ, Bellet M, Morales S, Ojeda B, Manso L, Mesia C, Garcia-Martínez E, Martinez-Jáñez N, Melé M, Llombart A, Pernas S, Villagrasa P, Blasco C, Baselga J (2015) Pegylated liposomal doxorubicin plus cyclophosphamide followed by paclitaxel as primary chemotherapy in elderly or cardiotoxicity-prone patients with high-risk breast cancer: results of the phase II CAPRICE study. Breast Cancer Res Treat 151:597–606. https://doi.org/10.1007/s10549-015-3415-2

Greco F, Vicent MJ, Gee S, Jones AT, Gee J, Nicholson RI, Duncan R (2007) Investigating the mechanism of enhanced cytotoxicity of HPMA copolymer-dox-AGM in breast cancer

cells. J. Control Release Off J Control Release Soc 117:28–39. https://doi.org/10.1016/j.jconrel.2006.10.012

Greco F, Vicent MJ, Penning NA, Nicholson RI, Duncan R (2005) HPMA copolymer-aminoglutethimide conjugates inhibit aromatase in MCF-7 cell lines. J Drug Target 13:459–470. https://doi.org/10.1080/10611860500383788

Grodzinski P, Farrell D (2014) Future opportunities in cancer nanotechnology–NCI strategic workshop report. Cancer Res 74:1307–1310. https://doi.org/10.1158/0008-5472.CAN-13-2787

Guo P, You J-O, Yang J, Jia D, Moses MA, Auguste DT (2014) Inhibiting metastatic breast cancer cell migration via the synergy of targeted, pH-triggered siRNA delivery and chemokine axis blockade. Mol Pharm 11:755–765. https://doi.org/10.1021/mp4004699

Guo Y, Zhang Z, Kim D-H, Li W, Nicolai J, Procissi D, Huan Y, Han G, Omary RA, Larson AC (2013) Photothermal ablation of pancreatic cancer cells with hybrid iron-oxide core gold-shell nanoparticles. Int J Nanomedicine 8:3437–3446. https://doi.org/10.2147/IJN.S47585

Hainsworth JD (1997) Mitoxantrone, 5-fluorouracil and high-dose leucovorin (NFL) in the treatment of metastatic breast cancer: randomized comparison to cyclophosphamide, methotrexate and 5-fluorouracil (CMF) and attempts to improve efficacy by adding paclitaxel. Eur J Cancer Care (Engl) 6:4–9. https://doi.org/10.1111/j.1365-2354.1997.tb00318.x

Han K, Jeng EE, Hess GT, Morgens DW, Li A, Bassik MC (2017) Synergistic drug combinations for cancer identified in a CRISPR screen for pairwise genetic interactions. Nat Biotechnol 35:463–474. https://doi.org/10.1038/nbt.3834

Hasenstein JR, Shin H-C, Kasmerchak K, Buehler D, Kwon GS, Kozak KR (2012) Antitumor activity of Triolimus: a novel multidrug-loaded micelle containing paclitaxel, rapamycin, and 17-AAG. Mol Cancer Ther 11:2233–2242. https://doi.org/10.1158/1535-7163.MCT-11-0987

Hassan MSU, Ansari J, Spooner D, Hussain SA (2010) Chemotherapy for breast cancer (review). Oncol Rep 24:1121–1131. https://doi.org/10.3892/or_00000963

He L, Gu J, Lim LY, Yuan Z-X, Mo J (2016) Nanomedicine-mediated therapies to target breast cancer stem cells. Front Pharmacol 7:313. https://doi.org/10.3389/fphar.2016.00313

Howard JH, Bland KI (2012) Current management and treatment strategies for breast cancer. Curr Opin Obstet Gynecol 24:44–48. https://doi.org/10.1097/GCO.0b013e32834da4b1

Hurwitz HI, Fehrenbacher L, Hainsworth JD, Heim W, Berlin J, Holmgren E, Hambleton J, Novotny WF, Kabbinavar F (2005) Bevacizumab in combination with fluorouracil and leucovorin: an active regimen for first-line metastatic colorectal cancer. J Clin Oncol 23:3502–3508

Hussain Z, Khan JA, Murtaza S (2018) Nanotechnology: an emerging therapeutic option for breast cancer. Crit Rev Eukaryot Gene Expr 28:163–175. https://doi.org/10.1615/CritRevEukaryotGeneExpr.2018022771

Jadia R, Scandore C, Rai P (2016) Nanoparticles for effective combination therapy of cancer. Int J Nanotechnol Nanomedicine 1. http://www.opastonline.com/wp-content/uploads/2016/10/nanoparticles-for-effective-combination-therapy-of-cancer-ijnn-16-003.pdf

Joensuu H, Holli K, Heikkinen M, Suonio E, Aro AR, Hietanen P, Huovinen R (1998) Combination chemotherapy versus single-agent therapy as first–and second-line treatment in metastatic breast cancer: a prospective randomized trial. J Clin Oncol Off J Am Soc Clin Oncol 16:3720–3730. https://doi.org/10.1200/JCO.1998.16.12.3720

Jones SE, Durie BG, Salmon SE (1975) Combination chemotherapy with adriamycin and cyclophosphamide for advanced breast cancer. Cancer 36:90–97. https://doi.org/10.1002/1097-0142(197507)36:1<90::aid-cncr2820360104>3.0.co;2-h

Kabbinavar FF, Hambleton J, Mass RD, Hurwitz HI, Bergsland E, Sarkar S (2005) Combined analysis of efficacy: the addition of bevacizumab to fluorouracil/leucovorin improves survival for patients with metastatic colorectal cancer. J Clin Oncol 23:3706–3712

Keith CT, Borisy AA, Stockwell BR (2005) Multicomponent therapeutics for networked systems. Nat Rev Drug Discov 4:71–78. https://doi.org/10.1038/nrd1609

Khattab A, Kashyap S, Monga DK (2021) Male breast cancer, in: stat pearls. StatPearls Publishing, Treasure Island (FL)

Kim S, Thiessen PA, Bolton EE, Chen J, Fu G, Gindulyte A, Han L, He J, He S, Shoemaker BA, Wang J, Yu B, Zhang J, Bryant SH (2016) PubChem substance and compound databases. Nucleic Acids Res 44:D1202–D1213. https://doi.org/10.1093/nar/gkv951

Lang DK, Sood A, Kaur R, Arora R, Behl T (2021) Focus on the multimodal role of biomarkers in breast cancer. Curr Pharm Des 27. https://doi.org/10.2174/1381612827999210111185213

Lee J, Chatterjee DK, Lee MH, Krishnan S (2014) Gold nanoparticles in breast cancer treatment: promise and potential pitfalls. Cancer Lett 347:46–53. https://doi.org/10.1016/j.canlet.2014.02.006

Lee JH, Nan A (2012) Combination drug delivery approaches in metastatic breast cancer. J Drug Deliv 2012:915375. https://doi.org/10.1155/2012/915375

Lee JH, Sabnis G, Brodie A, Nan A, (2011) In vitro evaluation of HER2 targeted N-(2-hydroxypropyl) methacrylamide (HPMA) copolymer conjugates in combination treatment with tyrosine kinase inhibitor (PKI-166)

Li L, Tong R, Li M, Kohane DS (2016) Self-assembled gemcitabine-gadolinium nanoparticles for magnetic resonance imaging and cancer therapy. Acta Biomater 33:34–39. https://doi.org/10.1016/j.actbio.2016.01.039

Loh SY, Packer T, Chinna K, Quek KF (2013) Effectiveness of a patient self-management programme for breast cancer as a chronic illness: a non-randomised controlled clinical trial. J Cancer Surviv Res Pract 7:331–342. https://doi.org/10.1007/s11764-013-0274-x

Luchino J, Hocine M, Amoureux M-C, Gibert B, Bernet A, Royet A, Treilleux I, Lécine P, Borg J-P, Mehlen P (2013) Semaphorin 3E suppresses tumor cell death triggered by the plexin D1 dependence receptor in metastatic breast cancers. Cancer Cell 24:673–685

Marrache S, Tundup S, Harn DA, Dhar S (2013) Ex vivo programming of dendritic cells by mitochondria-targeted nanoparticles to produce interferon-gamma for cancer immunotherapy. ACS Nano 7:7392–7402. https://doi.org/10.1021/nn403158n

Marty M, Cognetti F, Maraninchi D, Snyder R, Mauriac L, Tubiana-Hulin M, Chan S, Grimes D, Antón A, Lluch A, Kennedy J, O'Byrne K, Conte P, Green M, Ward C, Mayne K, Extra J-M (2005) Randomized phase II trial of the efficacy and safety of trastuzumab combined with docetaxel in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer administered as first-line treatment: the M77001 study group. J Clin Oncol Off J Am Soc Clin Oncol 23:4265–4274. https://doi.org/10.1200/JCO.2005.04.173

Milane L, Duan Z, Amiji M (2011) Pharmacokinetics and biodistribution of lonidamine/paclitaxel loaded, EGFR-targeted nanoparticles in an orthotopic animal model of multi-drug resistant breast cancer. Nanomedicine Nanotechnol Biol Med 7:435–444. https://doi.org/10.1016/j.nano.2010.12.009

Miller K, Wang M, Gralow J, Dickler M, Cobleigh M, Perez EA, Shenkier T, Cella D, Davidson NE (2007) Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. N Engl J Med 357:2666–2676. https://doi.org/10.1056/NEJMoa072113

Mohapatra S, Iqbal Z, Ahmad S, Kohli K, Farooq U, Padhi S, Kabir M, Panda AK (2020) Menopausal remediation and quality of life (QoL) improvement: insights and perspectives. Endocr Metab Immune Disord Drug Targets 20:1624–1636. https://doi.org/10.2174/1871530320666200730225830

Momenimovahed Z, Salehiniya H (2019) Epidemiological characteristics of and risk factors for breast cancer in the world. Breast Cancer Dove Med Press 11:151–164. https://doi.org/10.2147/BCTT.S176070

Muntimadugu E, Kumar R, Saladi S, Rafeeqi TA, Khan W (2016) CD44 targeted chemotherapy for co-eradication of breast cancer stem cells and cancer cells using polymeric nanoparticles of salinomycin and paclitaxel. Colloids Surf B Biointerfaces 143:532–546. https://doi.org/10.1016/j.colsurfb.2016.03.075

Nanobiotechnology for Breast Cancer Treatment | IntechOpen [WWW Document] (n.d.). URL https://www.intechopen.com/books/breast-cancer-from-biology-to-medicine/nanobiotechnology-for-breast-cancer-treatment. Accessed 7.18.21

Niewoehner J, Bohrmann B, Collin L, Urich E, Sade H, Maier P, Rueger P, Stracke JO, Lau W, Tissot AC, Loetscher H, Ghosh A, Freskgård P-O (2014) Increased brain penetration and potency of a therapeutic antibody using a monovalent molecular shuttle. Neuron 81:49–60. https://doi.org/10.1016/j.neuron.2013.10.061

Oerlemans C, Bult W, Bos M, Storm G, Nijsen JFW, Hennink WE (2010) Polymeric micelles in anticancer therapy: targeting, imaging and triggered release. Pharm Res 27:2569–2589. https://doi.org/10.1007/s11095-010-0233-4

O'Shaughnessy J, Miles D, Vukelja S, Moiseyenko V, Ayoub J-P, Cervantes G, Fumoleau P, Jones S, Lui W-Y, Mauriac L, Twelves C, Van Hazel G, Verma S, Leonard R (2002) Superior survival with capecitabine plus docetaxel combination therapy in anthracycline-pretreated patients with advanced breast cancer: phase III trial results. J Clin Oncol Off J Am Soc Clin Oncol 20:2812–2823. https://doi.org/10.1200/JCO.2002.09.002

O'Shaughnessy J, Osborne C, Pippen JE, Yoffe M, Patt D, Rocha C, Koo IC, Sherman BM, Bradley C (2011) Iniparib plus chemotherapy in metastatic triple-negative breast cancer. N Engl J Med 364:205–214. https://doi.org/10.1056/NEJMoa1011418

Patterson JA, Scott M, Anderson N, Kirk SJ (2004) Radial scar, complex sclerosing lesion and risk of breast cancer. Analysis of 175 cases in Northern Ireland. Eur J Surg Oncol J Eur Soc Surg Oncol Br Assoc Surg Oncol 30:1065–1068. https://doi.org/10.1016/j.ejso.2004.08.004

Paulmurugan R, Bhethanabotla R, Mishra K, Devulapally R, Foygel K, Sekar TV, Ananta JS, Massoud TF, Joy A (2016) Folate receptor-targeted polymeric micellar Nanocarriers for delivery of Orlistat as a repurposed drug against triple-negative breast cancer. Mol Cancer Ther 15:221–231. https://doi.org/10.1158/1535-7163.MCT-15-0579

Pillai G (2014) Nanomedicines for cancer therapy: an update of FDA approved and those under various stages of development. SOJ Pharm Pharm, Sci, p 1

Pilleron S, Soto-Perez-de-Celis E, Vignat J, Ferlay J, Soerjomataram I, Bray F, Sarfati D (2021) Estimated global cancer incidence in the oldest adults in 2018 and projections to 2050. Int J Cancer 148:601–608. https://doi.org/10.1002/ijc.33232

Prasad P, Shuhendler A, Cai P, Rauth AM, Wu XY (2013) Doxorubicin and mitomycin C co-loaded polymer-lipid hybrid nanoparticles inhibit growth of sensitive and multidrug resistant human mammary tumor xenografts. Cancer Lett 334:263–273. https://doi.org/10.1016/j.canlet.2012.08.008

Qin C, He B, Dai W, Zhang H, Wang X, Wang J, Zhang X, Wang G, Yin L, Zhang Q (2014) Inhibition of metastatic tumor growth and metastasis via targeting metastatic breast cancer by chlorotoxin-modified liposomes. Mol Pharm 11:3233–3241. https://doi.org/10.1021/mp400691z

Rao W, Wang H, Han J, Zhao S, Dumbleton J, Agarwal P, Zhang W, Zhao G, Yu J, Zynger DL, Lu X, He X (2015) Chitosan-decorated doxorubicin-encapsulated nanoparticle targets and eliminates tumor reinitiating cancer stem-like cells. ACS Nano 9:5725–5740. https://doi.org/10.1021/nn506928p

Rau K-M, Lin Y-C, Chen Y-Y, Chen J-S, Lee K-D, Wang C-H, Chang H-K (2015) Pegylated liposomal doxorubicin (Lipo-dox®) combined with cyclophosphamide and 5-fluorouracil is effective and safe as salvage chemotherapy in taxane-treated metastatic breast cancer: an open-label, multi-center, non-comparative phase II study. BMC Cancer 15:423. https://doi.org/10.1186/s12885-015-1433-4

Richard S, Selle F, Lotz J-P, Khalil A, Gligorov J, Soares DG (2016) Pertuzumab and trastuzumab: the rationale way to synergy. An Acad Bras Cienc 88(Suppl 1):565–577. https://doi.org/10.1590/0001-3765201620150178

Robert N, Leyland-Jones B, Asmar L, Belt R, Ilegbodu D, Loesch D, Raju R, Valentine E, Sayre R, Cobleigh M, Albain K, McCullough C, Fuchs L, Slamon D (2006) Randomized phase III study of trastuzumab, paclitaxel, and carboplatin compared with trastuzumab and paclitaxel in women with HER-2-overexpressing metastatic breast cancer. J Clin Oncol Off J Am Soc Clin Oncol 24:2786–2792. https://doi.org/10.1200/JCO.2005.04.1764

Schöttler S, Becker G, Winzen S, Steinbach T, Mohr K, Landfester K, Mailänder V, Wurm FR (2016) Protein adsorption is required for stealth effect of poly(ethylene glycol)–and poly (phosphoester)-coated nanocarriers. Nat Nanotechnol 11:372–377. https://doi.org/10.1038/nnano.2015.330

Shapiro CL, Recht A (2001) Side effects of adjuvant treatment of breast cancer. N Engl J Med 344: 1997–2008. https://doi.org/10.1056/NEJM200106283442607

Shuhendler AJ, Cheung RY, Manias J, Connor A, Rauth AM, Wu XY (2010) A novel doxorubicin-mitomycin C co-encapsulated nanoparticle formulation exhibits anti-cancer synergy in multi-drug resistant human breast cancer cells. Breast Cancer Res Treat 119:255–269. https://doi.org/10.1007/s10549-008-0271-3

Sneider A, Jadia R, Piel B, VanDyke D, Tsiros C, Rai P (2017) Engineering remotely triggered liposomes to target triple negative breast cancer. Onco Targets Ther 2:1–13. https://doi.org/10.7150/oncm.17406

Song XR, Cai Z, Zheng Y, He G, Cui FY, Gong DQ, Hou SX, Xiong SJ, Lei XJ, Wei YQ (2009) Reversion of multidrug resistance by co-encapsulation of vincristine and verapamil in PLGA nanoparticles. Eur J Pharm Sci Off J Eur Fed Pharm Sci 37:300–305. https://doi.org/10.1016/j.ejps.2009.02.018

Swaminathan SK, Roger E, Toti U, Niu L, Ohlfest JR, Panyam J (2013) CD133-targeted paclitaxel delivery inhibits local tumor recurrence in a mouse model of breast cancer. J. Control Release Off J Control Release Soc 171:280–287. https://doi.org/10.1016/j.jconrel.2013.07.014

Tang X, Loc WS, Dong C, Matters GL, Butler PJ, Kester M, Meyers C, Jiang Y, Adair JH (2017) The use of nanoparticulates to treat breast cancer. Nanomed. 12:2367–2388. https://doi.org/10.2217/nnm-2017-0202

Telli ML, Carlson RW (2009) First-line chemotherapy for metastatic breast cancer. Clin Breast Cancer 9(Suppl 2):S66–S72. https://doi.org/10.3816/CBC.2009.s.007

Torrisi R, Cardillo A, Cancello G, Dellapasqua S, Balduzzi A, Ghisini R, Luini A, Veronesi P, Viale G, Goldhirsch A, Colleoni M (2010) Phase II trial of combination of pegylated liposomal doxorubicin, cisplatin, and infusional 5-fluorouracil (CCF) plus trastuzumab as preoperative treatment for locally advanced and inflammatory breast cancer. Clin Breast Cancer 10:483–488. https://doi.org/10.3816/CBC.2010.n.064

Turner NC, Slamon DJ, Ro J, Bondarenko I, Im S-A, Masuda N, Colleoni M, DeMichele A, Loi S, Verma S, Iwata H, Harbeck N, Loibl S, André F, Puyana Theall K, Huang X, Giorgetti C, Huang Bartlett C, Cristofanilli M (2018) Overall survival with Palbociclib and Fulvestrant in advanced breast cancer. N Engl J Med 379:1926–1936. https://doi.org/10.1056/NEJMoa1810527

Van der Veldt AA, Lubberink M, Bahce I, Walraven M, de Boer MP, Greuter HN, Hendrikse NH, Eriksson J, Windhorst AD, Postmus PE (2012) Rapid decrease in delivery of chemotherapy to tumors after anti-VEGF therapy: implications for scheduling of anti-angiogenic drugs. Cancer Cell 21:82–91

Varshosaz J, Davoudi MA, Rasoul-Amini S (2018) Docetaxel-loaded nanostructured lipid carriers functionalized with trastuzumab (Herceptin) for HER2-positive breast cancer cells. J Liposome Res 28:285–295. https://doi.org/10.1080/08982104.2017.1370471

von Roemeling C, Jiang W, Chan CK, Weissman IL, Kim BYS (2017) Breaking down the barriers to precision cancer Nanomedicine. Trends Biotechnol 35:159–171. https://doi.org/10.1016/j.tibtech.2016.07.006

Wang D-Y, Fulthorpe R, Liss SN, Edwards EA (2004) Identification of estrogen-responsive genes by complementary deoxyribonucleic acid microarray and characterization of a novel early estrogen-induced gene: EEIG1. Mol Endocrinol Baltim Md 18:402–411. https://doi.org/10.1210/me.2003-0202

Wang R, He Z, Cai P, Zhao Y, Gao L, Yang W, Zhao Y, Gao X, Gao F (2019) Surface-functionalized modified copper sulfide nanoparticles enhance checkpoint blockade tumor immunotherapy by Photothermal therapy and antigen capturing. ACS Appl Mater Interfaces 11:13964–13972. https://doi.org/10.1021/acsami.9b01107

Wang Y, Gao S, Ye W-H, Yoon HS, Yang Y-Y (2006) Co-delivery of drugs and DNA from cationic core-shell nanoparticles self-assembled from a biodegradable copolymer. Nat Mater 5: 791–796. https://doi.org/10.1038/nmat1737

Weigelt B, Geyer FC, Reis-Filho JS (2010) Histological types of breast cancer: how special are they? Mol Oncol 4:192–208. https://doi.org/10.1016/j.molonc.2010.04.004

Wong M-Y, Chiu GNC (2011) Liposome formulation of co-encapsulated vincristine and quercetin enhanced antitumor activity in a trastuzumab-insensitive breast tumor xenograft model. Nanomedicine Nanotechnol Biol Med 7:834–840. https://doi.org/10.1016/j.nano.2011.02.001

Wu D, Si M, Xue H-Y, Wong H-L (2017) Nanomedicine applications in the treatment of breast cancer: current state of the art. Int J Nanomedicine 12:5879–5892. https://doi.org/10.2147/IJN.S123437

Xue HY, Liu S, Wong HL (2014) Nanotoxicity: a key obstacle to clinical translation of siRNA-based nanomedicine. Nanomed 9:295–312. https://doi.org/10.2217/nnm.13.204

Yadav B, Wennerberg K, Aittokallio T, Tang J (2015) Searching for drug synergy in complex dose-response landscapes using an interaction potency model. Comput Struct Biotechnol J 13:504–513. https://doi.org/10.1016/j.csbj.2015.09.001

Yu D, Li W, Zhang Y, Zhang B (2016) Anti-tumor efficiency of paclitaxel and DNA when co-delivered by pH responsive ligand modified nanocarriers for breast cancer treatment. Biomed Pharmacother Biomedecine Pharmacother 83:1428–1435. https://doi.org/10.1016/j.biopha.2016.08.061

Zha Z, Yue X, Ren Q, Dai Z (2013) Uniform polypyrrole nanoparticles with high photothermal conversion efficiency for photothermal ablation of cancer cells. Adv Mater Deerfield Beach Fla 25:777–782. https://doi.org/10.1002/adma.201202211

Zhang RX, Cai P, Zhang T, Chen K, Li J, Cheng J, Pang KS, Adissu HA, Rauth AM, Wu XY (2016) Polymer-lipid hybrid nanoparticles synchronize pharmacokinetics of co-encapsulated doxorubicin-mitomycin C and enable their spatiotemporal co-delivery and local bioavailability in breast tumor. Nanomedicine Nanotechnol Biol Med 12:1279–1290. https://doi.org/10.1016/j.nano.2015.12.383

Zhao Y, Geng S, Li Y, Hou X, Liu Y, Lin F-H, Yao L, Tian W (2016) A novel double-targeted nondrug delivery system for targeting cancer stem cells. Int J Nanomedicine 11:6667–6678. https://doi.org/10.2147/IJN.S116230

# Nanoliposomal System for Breast Cancer Therapy

10

Gulam Mustafa, Mohammad Zaki Ahmad, Mohammed Aslam, Anuj Garg, and Javed Ahmad

**Abstract**

In the midst of a plethora of nanomaterial-based drug delivery platforms, liposomes have been predominantly successful with several products conceded into clinical applications. Liposomes are well-established and effective drug delivery systems, extensively used in cancer therapy including breast cancer. In this chapter nanoliposomes designed with the breast cancer targeting feature and drug release triggering functions are emphasized. The chapter also highlights the recent advances in the nanoliposomes-based therapeutic system for breast cancer, including gene and theranostic delivery perspectives. The challenges associated with the development of liposome-based products for future clinical settings are also discussed.

**Keywords**

Liposome · Nanoliposomes · Breast cancer · Theranostic · Challenges · Clinical translation

G. Mustafa
College of Pharmacy (Boys), Al-Dawadmi Campus, Shaqra University, Riyadh, Saudi Arabia

M. Z. Ahmad · J. Ahmad (✉)
Department of Pharmaceutics, College of Pharmacy, Najran University, Najran, Saudi Arabia

M. Aslam
Faculty of Pharmacy, Al Hawash Private University, Homs, Syria

A. Garg
Institute of Pharmaceutical Research, GLA University, Mathura, India



M. Rahman et al. (eds.), *Hormone Related Cancer Mechanistic and Nanomedicines*,
https://doi.org/10.1007/978-981-19-5558-7_10

## 10.1 Introduction

Subsequent to skin cancer, breast cancer (BC) is the most common cancer diagnosed in women in the United States. Breast cancer can occur in both men and women, but it's far more common in women. BC is also one of the leading causes of cancer-related deaths. Every year, one million women are diagnosed with breast cancer, with approximately one new case diagnosed every 18 seconds (Bray et al. 2018). It has been estimated that the breast carcinomas cases could rise to 2.50 million by 2025, with 768,646 people dying as a result of this disease. An in-depth study of BC pathophysiology at the molecular level confirms the heterogeneous nature of this carcinoma. If detected in its early stage, then BC can be cured. The metastatic progression is the most challenging aspect in the alleviation of BC. In the current scenario, the therapeutic intervention has been drastically improved as reflected in increased patient survival rates. However, an advanced form of breast cancer that has spread to other organs could not be cured with current chemotherapeutics. BC is usually characterized by uninhibited and excessive cell growth that affects other nearby healthy tissues and organs (Nosrati et al. 2018). Nonetheless, breast cancer alleviation is very difficult to deal with despite the tremendous efforts and development in this field. Until now, chemotherapy, in addition to surgery, has been the foremost strategy to treat BC. There are serious concerns with most of the chemotherapeutic drugs such as they exert a toxic effect on normal cells and tissues (Zhu et al. 2016). In addition, multidrug resistance caused by chemotherapeutics drugs could lead to failure of treatment upon the recurrence of carcinomas (Lu et al. 2016). At the present, therapeutic intervention of BC lays stress upon biologically directed therapies, personalized treatment, and de-escalation of chemotherapy. Although the 5-year survival rate of advanced or metastasized BC is low (28%), the primary goal of targeted therapy is to prolong survival, control symptoms, and reduce cytotoxic drug toxicity, thereby improving the quality of life of BC patients (Harbeck et al. 2019).

Studies at the molecular level revealed that mutated genes' expression substantially contributes to the development and progression of BC (Yang et al. 2018). Therefore, gene therapy is a promising approach that can revolutionize the BC treatment paradigm (Cardoso et al. 2012). Nevertheless, gene therapy is also very challenging owing to the issue of the safety and efficient delivery of therapeutic genes or gene-regulating products into the nucleus of mammalian cells.

Various drug and gene delivery systems, including viral and nonviral vectors, are being explored currently (Huo et al. 2017; Li et al. 2019; Maggio et al. 2020). Liposomes (ZununiVahed et al. 2017), polymeric (Zuris et al. 2015; Chen et al. 2017), and inorganic nanomaterials (Ma et al. 2015) are examples of nonviral vectors. The clinical application of viral vectors is hampered due to safety concerns and cargo size limitations. On the other hand, liposomes are well-established potential nanocarriers for drug/gene delivery with high loading capacity, ease of preparation, and excellent physiological compatibility (Zylberberg et al. 2017, Zuris et al. 2015; Chen et al. 2017).

Liposomes are established and absolute nanomaterials for drug/gene delivery (Pattni et al. 2015; Zylberberg et al. 2017). The predominant merits of liposomes are high loading capacity, convenient preparation, and excellent biocompatibility (Zuris et al. 2015; Wang et al. 2017a, b, c). Liposomes are chiefly composed of phospholipid molecules having hydrophobic tails and hydrophilic heads, forming the amphiphilic vesicle structures in aqueous solutions. Structurally, liposomes are alienated into small unilamellar vesicles (~ 100 nm) and large unilamellar vesicles (200–800 nm) with a single bilayer, and multilamellar vesicles (500–5000 nm) containing multiple bilayers. Owing to their amphipathic nature, liposomes can successfully encapsulate both hydrophilic and hydrophobic compounds (Olusanya et al. 2018a, b; Yang et al. 2021).

The mitigation of adverse effects of anticancer drugs for the patients can be accomplished via targeted liposomes (Federman and Denny 2010). The liposomes' surface can be adapted by apt ligands to target the specific receptors of BC or its microenvironment to attain selective delivery. Triggering is one more approach that allows to control the local dose of the drug and, for example, initiate the drug release at a certain time point after accumulation of a required dose, depending upon the sensitivity of the tumor (Moussa et al. 2015). Nonetheless, liposomes represent a promising nanoparticle-based delivery system in cancer therapy including breast cancer.

In this chapter, we first discuss characteristics of breast cancer followed by recent achievements in liposome-based drug delivery for therapeutic intervention in breast cancer. The key challenges that need to be addressed to improve the utility of liposomes in clinical settings are also discussed.

## 10.2 Breast Cancer Characteristics and Novel Targets

The molecular mechanisms involved in BC pathophysiology, invasion, and metastasis are explicitly studied (Allred et al. 2001). The profound understanding of molecular events in BC resulted in novel targeted therapies. The different targets and receptors expressed on breast cancer cells along with their endogenous ligands and drugs that bind to them are summarized in Table 10.1.

Nonhormonal targets regulate the process of cell progression and mobility, cellular communications between cancer cells, and the microenvironment of the tumor. Hormonal targets primarily regulate cell differentiation. Steroid receptors and nonsteroid receptors are among the possible hormone targets. Signal transduction mediators, cell-cycle mediators, and angiogenesis mediators are the three categories of nonhormonal targets. There are five different types of steroid hormone receptors that have been implicated in the pathophysiology of breast cancer. Estrogen receptors (ERs), progesterone receptors (PRs), androgen receptors (ARs), glucocorticoid receptors (GRs), and mineralocorticoid receptors (MCRs) are some of them. The importance of estrogen receptors and progesterone receptors in breast cancer upregulation is well established. Vitamin D's biological actions are mediated by the Vitamin D receptor (VDR) (Haussler et al. 1998), which plays a key role in the

**Table 10.1** Different class of targets and receptors

| Target class | Receptor | Natural ligand | Synthetic ligand |
|---|---|---|---|
| Steroids | Estrogen receptor | Estrogen | Tamoxifen |
| | Progesterone receptor | Progesterone | Medroxyprogesterone acetate |
| | Androgen receptor | Dihydrotestosterone | Hydroxyflutamide |
| | Glucocorticoid receptor | Cortisol | CP-409069 |
| Nonsteroids | Thyroid receptor | Tri-iodothyronine | – |
| | Vitamin D receptor | 1a,25-(OH)2 D3 | 1a-(OH)2 D5, EB1089 |
| | Retinoic acid receptor | Retinoic acid | All-trans retinoic acid |
| | Retinoic-X receptor | Retinoic acid | All-trans retinoic acid, 9-cis retinoic acid |
| | Peroxisome proliferators activated receptor | 15-deoxy-D12,14-Prostaglandin | Troglitazone |
| | Farnesoid X-activated Receptor | Bile acid | SR-45023A |
| | Constitutive active receptor | Phenobarbital | None |
| Signal transduction Modulators Cell-cycle and apoptosis Modulation | Endothelial growth factor Receptor (EGFR) | Endothelial growth factors | Gefitinib, Erlotinib |
| | HER2/neu | – | Trastuzumab |
| | c-kit Ras family | Kit ligand, steel factor | Imatinib mesylate p53 - CP-31398 |
| Angiogenesis modulation | VEGF receptor | Growth factors | Bevacizumab, Imatinib |
| | PDGF receptor | PDGF | Tanomastat |
| | Integrins | – | RGD peptide |
| | Matrix metalloproteinases | MMP substrate peptide | Anti-MT1-MMP |

regulation of cell growth and differentiation. The VDRs are ligand-dependent nuclear receptors that regulate gene expression. In about 80–90% of breast cancer cases, VDR expression has been identified (Colston et al. 1989). Cellular retinol-binding proteins control the actions of every member of this family (Mangelsdorf et al. 1995). During breast carcinogenesis, RAR loss has been observed (Ariga et al. 2000). Thyroid hormones (tri-iodothyronine (T3) and the prohormone thyroxin) have also been studied in the context of breast cancer. T3 receptors are abundant in normal mammary epithelial cells, but how T3 acts on mammary epithelial cells at the cellular or molecular level is still unknown. The nuclear receptor superfamily includes three members (Tontonoz et al. 1994). PPARγ are transcription factors that belong to the nuclear receptor superfamily (Selliti et al. 1983). PPARγ is found primarily in adipose tissue and is expressed in both primary and metastatic breast cancers. Changes in gene expression occur when PPARγ is activated in breast cancer cells (Mueller et al. 1998). Natural prostaglandin (PGJ2) and synthetic antidiabetic

thiazolidinediones are PPARγ ligands (Schwartz 1997). Hence, PPARγ serves as an interesting target for the prevention and treatment of breast cancer.

Signal transduction moderators perform as a messenger to control the cell cycle and communication between the cell's intracellular and extracellular compartments (Aaronson 1991). The constituents of signal transduction could be categorized into cell surface receptors, growth factors, and intracellular signaling pathways.

Breast cancer has also been linked to members of the human EGFR family, particularly HER1 and HER2 (Bacus et al. 1994). HER2 is established as a promising therapeutic target for metastatic breast cancer as its activation results in neo-angiogenesis (Cobleigh et al. 1999; Viloria Petit et al. 1997).

The C-kit receptor tyrosine kinase expresses structural similarities with the macrophage colony-stimulating factor and the PDGF receptor (PDGFR) (Qiu et al. 1988). The appearance of c-Kit is critical for the maintenance of normal hematopoiesis as well as several other functions. Several studies have shown that c-Kit is expressed in both malignant and benign breast epithelia (Matsuda et al. 1993; DiPaola et al. 1997). Cancers of mesenchymal origin like human breast carcinoma have been linked to the PDGFR (Lokker et al. 2002; Bhardwaj et al. 1996). Ras is a membrane-bound GTP/GDP-binding (G) protein that converts signals from the cell surface to the nucleus (Valencia et al. 1991). Breast cancer has also been shown to regulate the expression of Rho C and RAC1. Rho C expression may also be a determinant of recurrence in axillary negative node breast cancer in patients, even in lesions just under 1 cm in length (Kleer et al. 2002).

The ability to regulate cell proliferation and apoptosis is crucial for the growth and development of cancer cells. Cyclins and CDKs have been linked to cell-cycle control (Sherr and Roberts 1999). TNF and the TNF-receptor family are two components of apoptotic cell death. Cell-cycle control and apoptosis are impossible without p53 working properly. The activation of p53 is essential for cells to respond effectively to stress stimuli like DNA damage and hypoxia (Giaccia and Kastan 1998). Growth arrest, senescence, or apoptosis are examples of the response (Asker et al. 1999). There is a link between p53 abnormalities and more aggressive tumors, early metastasis, and lower overall survival (Pharoah et al. 1999).

Angiogenesis is the formation of blood vessel networks to transport nutrients and oxygen to the tumor cells, as well as waste products. Many proangiogenic and antiangiogenic factors are involved in angiogenesis that can be used as molecular targets in the treatment of breast cancer. (Carmeliet 2000). Vascular endothelium cells are activated by angiogenesis factors such as b-FGF and VEGF, which secrete and activate MMPs and plasminogen activators. There are several factors involved in the expression of VEGF, a vascular endothelium cell-specific mitogen, which tends to increase tumor growth and angiogenesis. When VEGF binds to its receptor proteins, recognized as VEGF receptors 1 (Flt-1) and 2 (KDR/fek-1), signaling pathways that control cellular functions involved in new blood vessel formation are initiated (Ullrich and Schlessinger 1990). Small molecules with tyrosine kinase inhibitory activity or neutralizing antibodies (rhuMab-VEGF) are explored in the testing of BC for therapeutic VEGF targeting. Several proteinases, including MMPs, serine, and cysteine proteinases, are critical in the progression of cancer (Fox et al.

2001). High levels of uPA in the primary tumor have been found to have prognostic significance, including axillary node-negative breast cancer (Malmstrom et al. 2001). It has been discovered that uPA system antagonists impede tumorigenesis (Rabbani and Gladu 2002). In the integrin family, there are transmembrane subunit pairs and that is chosen from at least 16a and 8a subunits to form more than 20 heterodimeric receptors on the cell surface. Integrins a3 1 and a6 4 have been linked to the invasion and spread of mammary carcinoma (Mercurio et al. 2001). When it comes to cancer metastasis and invasion, specific modulators may be used to target members of the interleukin (IL) family, particularly a6 4 (and possibly a3 1). Another intriguing component of the angiogenesis process involves prostaglandins, which appear to be involved in cell proliferation, migration, and the ability to form new vessels (Rozic et al. 2001).

## 10.3 Different Types of Nanoliposomes-Based Drug Delivery in Breast Cancer

Liposomes represent an ideal approach with desirable characteristics prerequisite for targeted delivery of anticancer agents in BC therapeutic intervention. Examples of commercially available liposomes include Doxil® (PEGylated liposomal doxorubicin; Centocor Ortho Biotech Inc., USA), DaunoXome® (non-PEGylated liposomal daunorubicin; Diatos, France), and Myocet® (non-PEGylated liposomal doxorubicin; Sopherion Therapeutics, USA) (Misra et al. 2010). Liposomes can efficiently incorporate both lipophilic and hydrophilic compounds within the lipid bilayer and aqueous core phase, respectively. Liposomes possess good biocompatibility and a high drug loading capacity. Liposomes can be easily customized to achieve desirable attributes such as prolong blood circulation time and receptor-mediated site-specific dissemination (Wang et al. 2017a, b, c).

Recently, a plethora of research studies are being carried out to explore different aspects of liposomal-based therapeutic delivery to cancer cells. Functionalized liposomes are being explicitly explored at present to reap the benefits of biochemical and physiological differences between normal and cancerous tissue. The most promising strategy in tumor therapy is active targeting combined with a conjunction of other strategies, such as the stimuli-responsive targeting approach. Liposomes are transplanted with a variety of targeting ligands including peptides, dendrimers, and monoclonal antibodies employing apt surface engineering technology. Surface functionalization has played a remarkable role in the development of a liposomal delivery system for efficient targeting, endocytosis, and producing an optimal therapeutic response. Additionally, other advantages of liposomes include avoiding lysosomal degradation, high accumulation at the tumor site, and stimuli-responsive drug release at the desired location. Moreover, simultaneous diagnostic and therapeutic functions can be incorporated in liposomes (Durymanov et al. 2015). A typical structure of liposomes with their advantages is shown in Fig. 10.1.

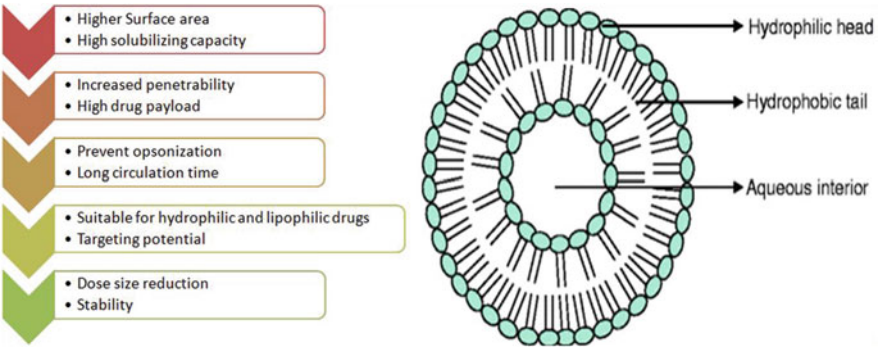



**Fig. 10.1** Typical structure of conventional liposomes with advantages of nanoliposomes

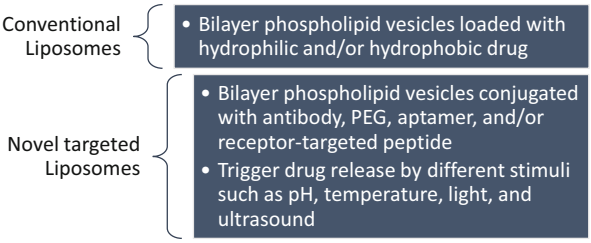



**Fig. 10.2** Characteristic attributes of conventional and novel surface-engineered or stimuli-responsive liposomes

***Conventional Liposomes*** It is constituted of phospholipid bilayers (Fig. 10.1) and, when injected intravenously, are prone to be engulfed by the reticuloendothelial system (RES), resulting in short circulation times.

They are destroyed by phagocytes because opsonin recognizes them as foreign substances.

To enhance circulation time, the outer layer of the liposomes was coated with a hydrophilic polymer, namely polyethylene glycol (PEG), and termed as PEGylated or stealth liposomes (Hatakeyama et al. 2013). It improved the electrostatic repulsive between the liposomes and serum components. Stealth liposomes prevent opsonization and hence, extended circulation half-life. Caelyx® (liposomal doxorubicin; Merck & Co., Whitehouse Station, New Jersey, USA) is an indicator of the effective use of stealth liposomes in the chemotherapeutic agent. Figure 10.2 highlights the basic characteristic attributes of conventional and novel surface-engineered targeted liposomes.

***Surface-engineered Liposomes*** A diverse range of therapeutics, together with bioactives, have demonstrated potential anticancer efficacy via multiple mechanisms, including angiogenesis, tumor growth, invasion, and metastasis suppression. Despite their potential antitumor activity, they showed limited applications in cancer therapy (Liu et al. 2020; Feng et al. 2017). Perhaps the key reason is that their low hydrophobicity results in poor cellular uptake and devastated

physicochemical stability. Furthermore, in animals, several bioactive constituents undergo a series of reactions that transform them into water-soluble metabolites, which are then excreted in urine and bile, and some drugs are also excreted in their original form. Because of their hydrophilic nature, they generally show low serum protein binding and are rapidly cleared up by the reticuloendothelial system (RES) (Zhan et al. 2014). Novel liposomal formulations are being developed that are surface-modified with a low molecular weight lipid conjugate and can be used in place of PEG. The formulations had very good stability characteristics in ion- and protein-rich mediums. Adsorption of proteins to the liposomal surface did not affect the cellular interaction. The limitation of PEGylation includes its potential to cause impaired cellular interactions, allergic reactions to be triggered, and an increase in IgM production after repeated dosing.

***Multifunctional Liposomes*** The multifunctional liposomes transport therapeutic molecules with excellent targeting and imaging properties. Liposomes with a single functionality face numerous challenges, and multifunctional liposomes have the potential to solve these problems. The formulation of nanoscale liposomes with a wide range of functions was made possible by combining surface-functionalization and modification techniques. Liposomes with two ligands, such as two peptides (Yuan et al. 2015), two ligands, and two anticancer drugs (Zhang et al. 2017), targeting ligand and an imaging agent (Erdogan and Torchilin 2017; Portnoy et al. 2011; Al-Jamal et al. 2008), have been reported by various studies (Fig. 10.3).

Liposomes containing iron-oxide or metal have therapeutic and imaging properties in one package. The imaging in core and ligand on the liposome surface of a multifunctional carrier are decorated. HER-2 overexpressing breast cancer was imaged using an immunoliposome-encapsulated nitroxide sensor developed by Burks and coworkers (Burks et al. 2010). These multifunctional liposomes have been shown to preferentially generate an intracellular EPR signal in the cells overexpressed with HER-2. High nitroxide concentrations resulted in greatly reduced EPR spectral transmissions and endocytosis of liposomes produced by a cell-activated contrast-generating technique.

***Stimuli-responsive Liposomes*** The slow release of drugs from liposomes could be attributed to passive diffusion as the main mechanism of release from them. Stimuli-responsive liposomes can modulate the release rate of encapsulated drugs. Liposomes do not release their contents unless structural changes are induced by an endogenous or exogenous stimulus. These liposomes provide rapid release of drugs at the desired target sites and reduce the risk of the emergence of MDR tumors. Endogenous stimuli include pathological changes in the target cancer tissues, such as reduced pH (Karanth and Murthy 2007), overexpression of specific enzymes, and abundance of reducing agents (Deshpande et al. 2013).

***Temperature-sensitive liposomes*** Encapsulated bioactives are released near the liposome's phase transition temperature, where the lipid membrane transitions

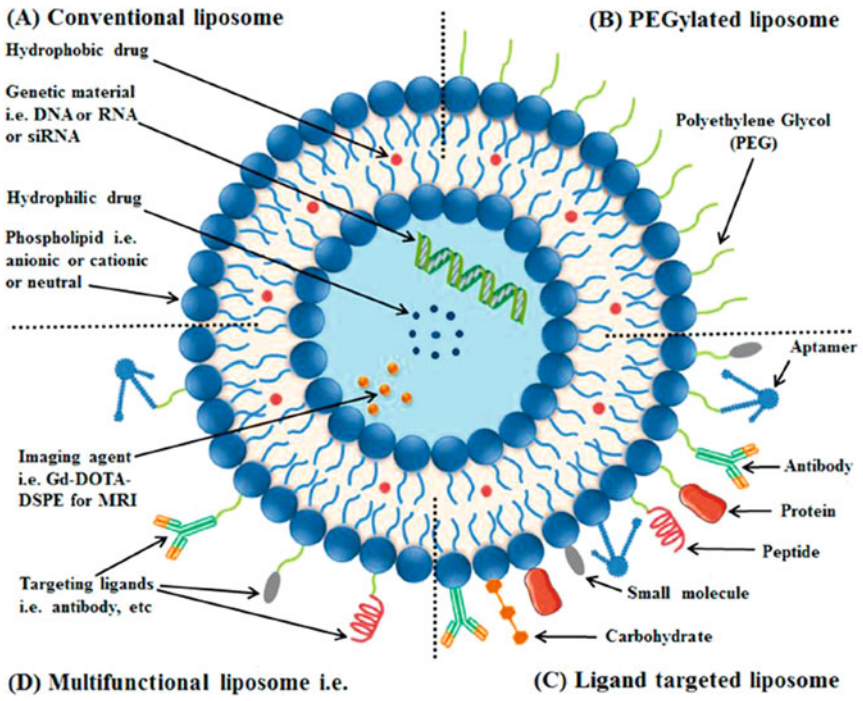



**Fig. 10.3** Liposomes and characteristics of its different types: Conventional liposomes are made of phospholipids (**a**); PEGylated/stealth liposomes contain a layer of polyethylene glycol (PEG) at the surface of liposomes (**b**); targeted liposomes contain a specific targeting ligand to target a cancer site (**c**); and multifunctional such as theranostic liposomes, which can be used for diagnosis and treatment of breast tumors (**d**). (Reproduced from Riaz et al. 2018)

from its gel to liquid crystal phase (Yatvin et al. 1978). Lipid bilayer transient pores are created by the incorporation of lysolipids into the liposomal membrane. These transient pores allow the rapid release of the encapsulants. Hyperthermia is the medical term for heating a tumor to a temperature that is slightly higher than normal (40–43 °C). Hyperthermia increases liposome uptake by two to three times (Leopold et al. 1992). Celsion Corporation has developed a thermoresponsive liposomal formulation of DOX, i.e., ThermoDox® for the treatment of various cancers, including breast cancer. ThermoDox®, when given intravenously and combined with hyperthermia, exhibited a potentially inhibiting effect on tumor growth.

***Enzyme-Responsive Liposomes*** Several enzyme concentrations are elevated in several pathological conditions, including cancer. Enzyme-responsive liposomes were developed using this approach (de la Rica et al. 2012; Yan and Boyd 2007). Secreted phospholipase A2 (sPLA2) levels increase substantially of various inflammatory conditions, atherosclerosis, and cancer. Prostate, breast, and pancreatic cancer exhibited elevated levels of sPLA2 (Dennis et al. 2011; Dong et al. 2006;

Graff et al. 2001). Hence, sPLA2 responsive liposomes loading Doxorubicin were formulated with 1,2-distearyl-sn-glycero-3-phosphocholine (DSPC), 1,2-distearyl-sn-glycero-3-phosphoethanolamine (DSPE), cholesterol, and DSPE-PEG. The developed enzyme responsive liposomes exhibited a higher (2.5 times) decrease in tumor growth than conventional liposomes in a mouse model of prostate cancer. MMPs play a significant role in tumor growth, invasion, and metastasis (Curran and Murray 2000; Chambers and Matrisian 1997). Zhu et al. (2012) described enzyme MMP responsive liposomes comprising two kinds of components: TATp-PEG −1,2-dioctadecanoyl-sn-glycero-3-phosphoethanolamine and mAb 2C5-PEG-MMP2 cleavable peptide-1,2-dioleoyl-sn-glycero-3-phosphoethanolamine to target hepatocellular tumor; PEG was attached to the phospholipid DOPE via an MMP-2 cleavable peptide linker. These MMP-sensitive peptides act as a linkage between the lipids and the polymer. The conjugated liposomes with polymer prevent liposome uptake. The peptide is cleaved when it comes into contact with MMPs at the target site, resulting in the dissociation of the polymer and finally uptake of liposomes. Liposomes' galactose ligand is protected from cellular uptake by the PEG group, which forms a barrier on their surface. MMP-2 overexpression in HCC, on the other hand, cleaves the linker and releases PEG. The exposed galactose ligand then binds to HCC cells' asialoglycoprotein receptors. Gal-PEG-DOPE (0.5 percent) liposome uptake was significantly enhanced in the presence of over 5 mcg/mL hMMP-2 in HepG2 cells, according to Terada et al. (Terada et al. 2006). An elevated level of a serine protease known as urokinase plasminogen activator (uPA) is reported in several human cancers, including breast, colon, bladder, and ovarian (Liu et al. 2001). Tumor angiogenesis, progression, and metastasis are aided by uPA (Duffy 2002). In peptides containing the consensus sequence Ser-Gly-Arg-Ser-Ala, the enzyme cleaves the Arg-Ser bond. Liposomes encapsulating uPA-cleavable peptides could thus discharge their encapsulated payload while they come into contact with uPA at tumor sites. It was discovered by Basel et al. that uPA-responsive liposomes in a hyperosmotic medium can be stabilized with a copolymer cage on their surface (Basel et al. 2011).

***pH-sensitive Liposomes*** Because of lactic acid production and ATP hydrolysis, the pH around the tumor cells is lower than healthy tissue (Gerweck and Seetharaman 1996). The endosomal or lysosomal partitions of tumor cells have low pH, which can be utilized to construct pH-responsive liposomal delivery models. The pH-sensitive liposomes systems are fabricated for extracellular or endosomal/lysosomal triggered release. The pH-responsive liposomes have been optimized so that they can transform at various stages between pH 7.4 and 5.0 to efficiently deliver anticancer agents at target sites. If pH is low because of pathological conditions like inflammation, infection, or malignancy, the pH-sensitive liposomes become soft and leaky (Torchilin et al. 1993). The pH-sensitive liposomes are intricately fabricated to circumvent the obstacles such as recognition and endocytosis by the RES, so that they could efficiently release their anticancer agents in the endosome and partially into the cytosol (Budker et al. 1996). Antitumor drugs, antigens, antisense oligonucleotides, and plasmid DNA have been delivered in vitro cytoplasmically

using these methods (Legendre et al. 1992). Liposomal systems encoded with specific ligands can bind to and be taken up by cells, allowing for pH-mediated intracellular drug delivery (Harel and Kato 2007). Accumulation in low pH compartments, such as endosomes and lysosomes, can be utilized to trigger drug release after internalization in tumor cells. Karve and associates reported pH-sensitive immunoliposomes encapsulating DOX for site-specific delivery (Karve et al. 2009). The study demonstrated that pH-sensitive liposomes prevent the growth of cancer cells in a much better way than simple immunoliposomes, and hence, could potentially enhance the therapeutic prospect via liposomal chemotherapy.

## 10.4 Advances in Nanoliposomes-Based Drug Delivery for Therapeutic Intervention in Breast Cancer

Cationic liposomes are being investigated as a possible efficient method for gene delivery. The complex formed between encapsulated plasmids DNA (Kaneda et al. 1989) and cationic lipids of liposomes is termed as lipoplex (Alino et al. 1996; Crystal 1995). The lipoplex can be fused with the plasma or endosome membrane to transport into cells (Sharma and Sharma 1997). The lipid composition, lipid/DNA ratio, and particle size of the liposome–DNA complex greatly impact their transfection efficiency (Zou et al. 2000). The limitation of cationic liposomes is their poor specificity. This limitation can be addressed by modifying cationic liposomes with cell-specific targeting moieties. For direct cytosolic delivery of liposomes, several cell-penetrating peptides have been implicated. For breast cancer gene therapy, antiangiogenesis strategies involve nonviral vectors (Feldman and Libutti 2000). Angiostatin and endostatin gene therapy is one way to deliver angiogenic polypeptide inhibitors. Previously, liposomes complexed with plasmids encoding angiostatin (PCI-Angio) or endostatin (PCIEndo) exhibited successful inhibition of angiogenesis in BC cells (Chen et al. 1999). Such liposomal delivery of antiangiogenic genes could represent promising therapeutic prospects. The wild-type p53 gene was also included in a liposome–plasmid complex used to treat naked mice injected with breast carcinoma cells (Lesoon-Wood et al. 1995). Xu et al. investigated a cationic immunoliposome tagged with single-chain antibody Fv fragment (scFv) for systemic p53 tumor suppressor gene therapy for treating BC (Xu et al. 2001). The scFv-tagged immunolipoplexes significantly improved transfection efficiency and extended the animals' survival time. However, the scFv-targeted immunoplex's expression was found to be low. A new expression strategy for anti-TfRscFv was reported by Xu and colleagues (Xu et al. 2002). In this, the scFv was covalently coupled to the liposome via a cysteine at the 3′ end of the protein and a maleimide group on the liposome. According to the findings, the immunological activity and targeting ability of the scFv were not affected by this conjugation. The scFv-cys-targeted tumor cells with a cationic liposome–DNA complex (lipoplex) markedly improved transfection efficiencies in BC models. BC cells overexpress the human HER-2/neu oncogene, and research has shown that the

E1A gene acts as a tumor inhibitor gene by reducing HER-2/neu transcription. tgDCC-E1A, a stable E1A lipid complex, was developed by Yoo and coworkers using cationic liposomes and plasmid DNA encoding E1A (Yoo et al. 2001). In accordance with the results of preliminary tests carried out on animals as well as on humans, researchers moved forward with clinical trials to see if delivering the E1A gene via liposomes would be safe and would have an effect on tumor response when administered to cancer patients with an advanced form of BCs. Recent advancements in the use of liposomes as a diagnostic tool underscore the use of imaging techniques and the recognition of diverse molecular targets. There are numerous medical imaging techniques that make use of liposomes as nanomedicines. Some of these methodologies encompass ultrasound, nuclear imaging, fluorescence, and magnetic resonance.

Fluorescence imaging is the most commonly used diagnostic tool. Imagining the position of the biomolecule, enzyme activity as well as gene expression is also possible in living cells or tissues. Functionalized quantum dot–liposome (f-QD-L) was developed by encompassing quantum dots by PEG (QD) for cancer diagnosis (Chen et al. 2000). There has been a lot of interest in NIR fluorescence imaging because of the low photon absorption and scattering (Min et al. 2014). Rare-earth-doped nanoparticles are one material under consideration for use in NIR fluorescence (Eliseeva and Bunzli 2010; Bunzli 2010). Using a liposome-nanoparticle hybrid, Soga and coworkers found that it has a strong NIR fluorescence component.

Radiofrequency pulses are commonly used in MRI for whole-body imaging (Sun et al. 2008). Liposomes can encapsulate a wide range of contrast agents like fluorophores, enabling efficient implementation and controlled release of probes for better image analysis (Kamaly et al. 2009; Soenen et al. 2011). Studies have been reported in which liposomes were concomitantly loaded with the MRI contrast agent Gd-DTPA and doxorubicin (DOX) (Tagami et al. 2011). The simultaneous delivery of Gd-DTPA and DOX allows controlled drug release in a tumor's microenvironment where localized heating could potentially trigger the release of a drug. Another paramagnetic MRI contrast agent is ferrimagnetic iron oxide (FMIO) nanoparticles, which have been used in the preparation of liposomal MRI probes (Mikhaylov et al. 2011). An external magnetic field was used to target the liposomal FMIO nanoparticle cluster at tumors and the tumor microenvironment.

Medical ultrasound, which is defined as sound waves with frequencies greater than 20,000 Hz, is another commonly used noninvasive diagnostic imaging technique. (Cheng et al. 2010) Ultrasound imaging is performed by directing ultrasound pulses into tissue and measuring the echoes caused by the tissue at various reflection angles. Contrast agents for ultrasound imaging, like MRI, have the ability to label specific tissue types or tumors. Acoustic liposomes (ALs), which contain perfluoropropane gas, can be used as ultrasound imaging probes (Deckers and Moonen 2010). For passive tumor tissue localization, high-permeability/high-retention acoustic liposomes can be used because of their small diameter (100 nm) and high retention effect (90% retention). With the help of high-frequency ultrasound (HF-US), acoustic liposomes can be used to test both drug delivery efficiency and antitumor efficacy (Ferrara et al. 2009). Using HF-US imaging, a cisplatin-loaded

AL was evaluated for its antitumor effects (Kodama et al. 2011). Nuclear imaging is another noninvasive imaging technique that uses small molecule radioactive tracers (Srivatsan and Chen 2014). PET (positron emission tomography) works by detecting gamma rays emitted from the destruction of positrons of radioactive materials. SPECT (single-photon emission computed tomography) is able to detect gamma rays directly emitted from isotopes such as 99mTc (Rahmim and Zaidi 2008). Various research studies have been reported indicating that liposomes can encapsulate radionuclide tracers inside their aqueous compartment or within a chemically engineered lipid bilayer (Henriksen et al. 2015; Ogawa et al. 2014; Seo et al. 2008; Petersen et al. 2011). Ferrara and colleagues designed temperature-sensitive liposomes using a combination of PET and fluorescence imaging (Paoli et al. 2010). They used 18F- or 64Cu-labeled lipids in liposome preparations, which embody the fluorophore Alexa Fluor 750 as a hydrophilic model drug. The tumor fluorescence images correlate well with PET and ex vivo fluorescence images when using a stable liposome formulation. Hence, a combination of PET and optical imaging techniques could be very beneficial for alleviating tumors.

188Re-labeled DSPC-based liposomal doxorubicin was fabricated and evaluated their targeting efficiency and antitumor effects in a C26murine tumor model by SPECT imaging (Chang et al. 2010). In in vivo micro, SPECT/CT imaging was used to assess the liposomes tumor targeting by measuring their biodistribution and pharmacokinetics. The bimodal radiochemotherapeutic 188Re-liposome-DOX showed greater tumor inhibition and a longer median survival time than either single-functionalized 188Re-liposomes or liposome-DOX.

## 10.5 Challenges in Translation of Nanoliposomes-Based Drug Delivery in Clinical Settings

The advancements in the development of liposome delivery systems are progressing at a fast pace, in light of the demand for the new stratagems for breast cancer therapy. However, a well-built understanding or road map on the design of the new liposome formulation for BC is lacking somewhere. Selection of the targeting and triggering modalities depends on the molecular subtypes of the tumor and the ongoing conventional treatments. Although the conventional liposome-encapsulated chemotherapeutic drug-based formulation is being frequently used in the clinical practice in BC treatment, still there are countless obstacles in the clinical implementation of these novel and advance versions of liposome formulations (Yang et al. 2021). With triggerable liposomes, the triggering mechanisms need to be further explored while designing such liposome formulations. For example, the selection of the phospholipid component for light-triggered liposomes needs to be in accordance with desired photo-induced mechanisms. In a photochemical pathway, for instance, photooxidative reaction, unsaturated phospholipids should be used in the formulation (Yang et al. 2021). Furthermore, active ingredients employed in the triggerable liposome formulation should be optimized to weigh up their benefits and risks to healthy tissues (Yang et al. 2021). From the perspective of the clinical applications,

the far-reaching development of liposome technology will ultimately benefit BC patients. Recent studies confirmed that various liposome constructs loaded with drugs can reduce the intensities of cardiotoxicity, address drug resistance, and improve the overall drug release profile. Modification of the liposome surface with targeting ligands offers surplus opportunities for designing site-specific therapy and curtailing the nonspecific effect of conventional chemotherapeutic drugs. The new genera liposomes with triggering features also allow efficient control of payload release and thus substantially augmenting the therapeutic outcomes for BC patients. Advance forms of liposome formulations could magnify the assortment of drug delivery options for the treatments of BC by efficaciously addressing the critical problems of drug toxicity and limited therapeutic effects.

## 10.6 Conclusion

The liposomes represent a promising approach as efficient and targeted delivery of anticancer agents to various tumor sites in BC alleviation. The advantages of encapsulating the drug in the nanoliposomal carrier are improved solubilization of the drug, increased half-life, prolonged circulation, selective accessibility of the drug to the target site, and substantially overcoming of multidrug resistance. The advanced and modified versions of liposomes as discussed in this chapter curtail the highly desirable characteristic features of tumor cell recognition and internalizing that could contribute substantially to the therapeutic intervention of BC. Precise molecular targeting can be accomplished with these smart generations of nanoliposomes, besides enhanced pharmacokinetic and biodistribution of anticancer agents. The integrated approaches such as theranostic liposomes or antibody-targeted ones can be efficiently employed for targeting small molecule drugs as well as biological agents with anticancer activity and can provide a new landscape in the liposomes-based BC therapeutic front. Earlier, PEGylated liposomes have addressed critical pharmacological concerns accompanied with the conventional drug delivery system, such as disruption by blood lipoproteins, uptake by RES, and rapid clearance from blood circulation. Now PEGylated liposomes are approved by regulatory authorities and have successfully reached the market. However, the clinical success of PEGylated liposomes is hampered by some grave limitations, such as a lack of tumor cell specificity. Therefore, to increase the target specificity and the amount of released therapeutic agent at the tumor site, stimuli-sensitive liposomes and multifunctional carriers such as theranostic liposomes have been designed. The increasing complexity of advanced versions of liposomal formulation needs rigorous in vitro and in vivo preclinical studies for their translation to the clinics. Certainly, recent research studies demonstrate that new generation liposomes could be a viable anticancer therapeutic tool in the treatment of BC.

## References

Aaronson SA (1991) Growth factors and cancer. Science 254:1146–1153
Alino SF, Bobadilla M, Crespo J, Lejarreta M (1996) Human a1-antitrypsin gene transfer to in vivo mouse hepatocytes. Hum Gene Ther 7:531–536
Al-Jamal W, Al-Jamal K, Bomans P, Frederik P, Kostarelos K (2008) Functionalized-quantum-dot-liposome hybrids as multimodal nanoparticles for cancer. Small 4(9):1406–1415
Allred DC, Mohsin S, K, Fuqua S.A. (2001) Histological and biological evolution of human premalignant breast disease. Endocr Relat Cancer 8:47–61
Ariga N, Moriya T, Suzuki T, Kimura M, Ohuchi N, Sasano H (2000) Retinoic acid receptor and retinoid X receptor in ductal carcinoma in situ and intraductal proliferative lesions of the human breast. Jap J Cancer Res 91:1169–1176
Asker C, Wiman KG, Selivanova G (1999) p53-induced apoptosis as a safeguard against cancer. Biochem Biophys Res Comm 265:1–6
Bacus SS, Zelnick CR, Plowman G, Yarden Y (1994) Expression of the erbB-2family of growth factor receptors and their ligands in breast cancers. Implication for tumor biology and clinical behavior. Am J Clin Pathol 102:S13–S24
Basel MT, Shrestha TB, Troyer DL, Bossmann SH (2011) Protease-sensitive, polymer-caged liposomes: a method for making highly targeted liposomes using triggered release. ACS Nano 5:2162–2175
Bhardwaj B, Klassen J, Cossette N et al (1996) Localization of platelet-derived growth factor a receptor expression in the periepithelial stroma of human breast carcinoma. Clin Cancer Res 2: 773–782
Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A (2018) Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68:394–424
Budker V, Gurevich V, Hagstrom JE, Bortzov F, Wolff JA (1996) pH sensitive, cationic liposomes: a new synthetic virus-like vector. Nat Biotechnol 14:760–764
Bunzli JCG (2010) Lanthanide luminescence for biomedical analyses and imaging. Chem Rev 110: 2729–2755
Burks SR, Macedo LF, Barth ED et al (2010) Anti-HER2 immunoliposomes for selective delivery of electron paramagnetic resonance imaging probes to HER2-overexpressing breast tumor cells. Breast Cancer Res Treat 124:121–131
Cardoso F, Harbeck N, Fallowfield L, Kyriakides S, Senkus E, Group E.G.W (2012) Locally recurrent or metastatic breast cancer: ESMO clinical practice guidelines for diagnosis, treatmentand follow-up. Ann Oncol 23(7):11–19
Carmeliet P (2000) Mechanisms of angiogenesis and arteriogenesis. Nature Med 6:389–395
Chambers AF, Matrisian LM (1997) Changing views of the role of matrix metalloproteinases in metastasis. J Natl Cancer Inst 89:1260–1270
Chang YJ, Chang CH, Yu CY, Chang TJ, Chen LC, Chen MH et al (2010) Therapeutic efficacy and micro SPECT/CT imaging of re-188-DXR-liposome in a C26 murine colon carcinoma solid tumor model. Nucl Med Biol 37:95–104
Chen J, Wu W, Tahir SK et al (2000) Down-regulation of survivin by antisense oligonucleotides increases apoptosis, inhibits cytokines and anchorage-independent growth. Neoplasia 2000(2): 235–241
Chen QR, Kuma D, Stass SA, Mixson AJ (1999) Liposomes complexed to plasmids encoding angiostatin and endostatin inhibit breast cancer in nude mice. Cancer Res 59:3308–3312
Chen WJ, Deng W, Goldys EM (2017) Light-triggerableliposomes for enhanced endolysosomal escape and gene silencingin PC12 cells. Mol Ther-Nucl Acids 7:366–377
Cheng HD, Shan J, Ju W, Guo YH, Zhang L (2010) Automated breast cancer detection and classification using ultrasound images: A survey. Pattern Recogn 43:299–317

Cobleigh MA, Vogel CL, Tripathy D et al (1999) Multinational study of the efficacy and safety of humanized anti-HER2antibody in women who have HER2-overexpressing breast cancer that has progressed after therapy for metastatic disease. J Clin Oncol 17:2639–2648
Colston KW, Berger U, Coombes RC (1989) Possible role for vitamin D in controlling breast cancer cell proliferation. Lancet 28:188–191
Crystal RG (1995) Transfer of genes to humans: early lesions and obstacles to success. Science 270: 404–410
Curran S, Murray GI (2000) Matrix metalloproteinases: molecular aspects of their roles in tumour invasion and metastasis. Eur J Cancer 36:1621–1630
de la Rica R, Aili D, Stevens MM (2012) Enzyme-responsive nanoparticles for drug release and diagnostics. Adv Drug Deliv Rev 64:967–978
Deckers R, Moonen CTW (2010) Ultrasound triggered, image guided, local drug delivery. J Control Release 148:25–33
Dennis EA, Cao J, Hsu YH, Magrioti V, Kokotos G (2011) Phospholipase A2 enzymes: physical structure, biological function, disease implication, chemical inhibition, and therapeutic intervention. Chem Rev 111:6130–6185
Deshpande P, Biswas S, Torchilin V (2013) Current trends in the use of liposomes for tumor targeting. Nanomedicine 8(9):1509–1528
DiPaola RS, Kuczynski W, Onodera K et al (1997) Evidence for a functional kit receptor in melanoma, breast, and lung carcinoma cells. Cancer Gene Ther 4:176–182
Dong Q, Patel M, Scott KF, Graham GG, Russell PJ, Sved P (2006) Oncogenic action of phospholipase A2 in prostate cancer. Cancer Lett 240:9–16
Duffy MJ (2002) Urokinase-type plasminogen activator: a potent marker of metastatic potential in human cancers. Biochem Soc Trans 30:207–210
Durymanov MO, Rosenkranz AA, Sobolev AS (2015) Current approaches for improving intratumoralaccumulation and distribution of nanomedicines. Theranostics 5:1007–1020
Eliseeva SV, Bunzli JCG (2010) Lanthanide luminescence for functional materials and bio-sciences. Chem Soc Rev 39:189–227
Erdogan S, Torchilin VP (2017) Gadolinium-loaded polychelating polymer-containing tumor-targeted liposomes. In: Methods in molecular biology (Clifton, N.J.), vol 1522. Springer, Berlin, Germany, pp 179–192
Federman N, Denny CT (2010) Targeting liposomes toward novel pediatric anticancer therapeutics. Pediatr Res 67(5):514–519
Feldman AL, Libutti SK (2000) Progress in antiangiogenic gene therapy. Cancer 89:1181–1194
Feng T, Wei Y, Lee R, Zhao L (2017) Liposomal curcumin and its application in cancer. Int J Nanomedicine 12:6027–6044
Ferrara KW, Borden MA, Zhang H (2009) Lipid-shelled vehicles: engineering for ultrasound molecular imaging and drug delivery. Acc Chem Res 42:881–892
Fox SB, Taylor M, Grondahl-Hansen J, Kakolyris S, Gatter KC, Harris AL (2001) Plasminogen activator inhibitor-1 as a measure of vascular remodeling in breast cancer. J Pathol 195:236–243
Gerweck LE, Seetharaman K (1996) Cellular pH gradient in tumor versus normal tissue: potential exploitation for the treatment of cancer. Cancer Res 56:1194–1198
Giaccia AJ, Kastan MB (1998) The complexity of p53 modulation: emerging patterns from divergent signals. Genes Dev 12:2973–2983
Graff JR, Konicek BW, Deddens JA, Chedid M, Hurst BM, Colligan B, Neubauer BL, Carter HW, Carter JH (2001) Clin Cancer Res 7:3857–3861
Harbeck N, Penault-Llorca F, Cortes J, Gnant M, Houssami N, Poortmans P, Ruddy K, Tsang J, Cardoso F (2019) Breast cancer Nat Rev Dis Primers 5:66
Harel YE, Kato Y (2007) Intracellular delivery of nanocarriers for cancer therapy. Curr Nanosci 3: 329–338
Hatakeyama H, Akita H, Harashima H (2013) The polyethylene glycol dilemma: advantage and disadvantage of PEGylation of liposomes for systemic genes and nucleic acids delivery to tumors. Biol Pharm Bull 36:892–899

Haussler MR, Whitfield GK, Haussler CA et al (1998) The nuclear vitamin D receptor: biological and molecular regulatory properties revealed. J Bone Min Res 13:325–349

Henriksen JR, Petersen AL, Hansen AE, Frankaer CG, Harris P, Elema DR et al (2015) Remote loading of 64Cu2+ into liposomes without the use of ion transport enhancers. ACS Appl Mater Interfaces 7:22796–22806

Huo WY, Zhao GN, Yin JG, Ouyang X, Wang YN, Yang CH, Wang BJ, Dong PX, Wang ZX, Watari H, Chaum E, Pfeffer LM, Yue JM (2017) Lentiviral CRISPR/Cas9 vector mediated miR-21gene editing inhibits the epithelial to mesenchymal transition inovarian cancer cells. J Cancer 8(1):57–64

Kamaly N, Kalber T, Thanou M, Bell JD, Miller AD (2009) Folate receptor targeted bimodal liposomes for tumor magnetic resonance imaging. Bioconjug Chem 20:648–655

Kaneda Y, Iwai K, Uchida T (1989) Increased expression of DNA cointroduced with nuclear protein in adult rat liver. Science 243:375–378

Karanth H, Murthy R (2007) pH-sensitive liposomes-principle and application in cancer therapy. J Pharm Pharmacol 59(4):469–483

Karve S, Alaouie A, Zhou Y, Rotolo J, Sofou S (2009) The use of pH-triggered leaky heterogeneities on rigid lipid bilayers to improve intracellular trafficking and therapeutic potential of targeted liposomal immunochemotherapy. Biomaterials 30:6055–6064

Kleer CG, van Golen KL, Zhang Y, Wu ZF, Rubin MA, Merajver SD (2002) Characterization of RhoC expression in benign and malignant breast disease: a potential new marker for small breast carcinomas with metastatic ability. Am J Pathol 160:579–584

Kodama T, Tomita N, Yagishita Y, Horie S, Funamoto K, Hayase T et al (2011) Volumetric and Angiogenic evaluation of antitumor effects with acoustic liposome and high-frequency ultrasound. Cancer Res 71:6957–6964

Legendre JY, Szoka FC Jr (1992) Delivery of plasmid DNA into mammalian cell lines using pH-sensitive liposomes: comparison with cationic liposomes. Pharm Res 9:1235–1242

Leopold KA, Dewhirst MW, Samulski TV et al (1992) Relationship among tumor temperature, treatment time, and histopathological outcome using preoperative hyperthermia with radiation in soft tissue sarcomas. Int J Radiat Oncol Biol Phys 22:989–998

Lesoon-Wood LA, Kim WH, Kleinman HK, Weintraub BD, Mixson AJ (1995) Systemic gene therapy with p53 reduces growth and metastases of a malignant human breast cancer in nude mice. Hum Gene Ther 6:395–405

Li A, Lee CM, Hurley AE, Jarrett KE, De Giorgi M, Lu WQ, Balderrama KS, Doerfler AM, Deshmukh H, Ray A, Bao G, Lagor WR (2019) A self-deleting AAV-CRISPR system forin vivo genome editing. MolTher-Meth Clin D 12:111–122

Liu S, Bugge TH, Leppla SH (2001) Targeting of tumor cells by cell surface urokinase plasminogen activator-dependent anthrax toxin. J Biol Chem 276:17976–17984

Liu Y, Xie X, Hou X, Shen J, Shi J, Chen H, He Y, Wang Z, Feng N (2020) Functional oral nanoparticles for delivering silibinin and cryptotanshinone against breast cancer lung metastasis. J Nanobiotechnology 18(1):83

Lokker NA, Sullivan CM, Hollenbach SJ, Israel MA, Giese NA (2002) Platelet-derived growth factor (PDGF) autocrine signalling regulates survival and mitogenic pathways in glioblastoma cells: evidence that the novel PDGF-C and PDGF-D ligands may play a role in the development of brain tumors. Cancer Res 62:3729–3735

Lu C, Shieh G, Wang C, Su B, Su Y, Chen Y et al (2016) Chemotherapeutics-induced Oct4 expression contributes to drug resistance and tumor recurrence in bladder cancer. Oncotarget 8(19):30844–30858

Ma PA, Xiao HH, Li CX, Dai YL, Cheng ZY, Hou ZY, Lin J (2015) Inorganic nanocarriers for platinum drug delivery. Mater Today 18(10):554–564

Maggio I, Zittersteijn HA, Wang Q, Liu J, Janssen JM, Ojeda IT, van der Maarel SM, Lankester AC, Hoeben RC, Goncalves MAFV (2020) Integrating gene delivery and gene-editing technologiesby adenoviral vector transfer of optimized CRISPRCas9components. Gene Ther 27:209–225

Malmstrom P, Bendahl PO, Boisen P, Brunner N, Idvall I, Ferno M (2001) S-phase fraction and urokinase plasminogen activator are better markers for distant recurrences than Nottingham prognostic index and histologic grade in a prospective study of premenopausal lymph node-negative breast cancer. J Clin Oncol 19:2010–2019

Mangelsdorf DJ, Thummel C, Beato M et al (1995) The nuclear receptor superfamily: the second decade. Cell 83:835–839

Matsuda R, Takahashi T, Nakamura S et al (1993) Expression of the c-kit protein in human solid tumors and in corresponding fetal and adult normal tissues. Am J Pathol 142:339–346

Mercurio AM, Bachelder RE, Chung J et al (2001) Integrin laminin receptors and breast carcinoma progression. J Mammary Gland Biol Neoplasia 6:299–309

Mikhaylov G, Mikac U, Magaeva AA, Itin VI, Naiden EP, Psakhye I et al (2011) Ferri-liposomes as an MRI-visible drug-delivery system for targeting tumours and their microenvironment. Nat Nanotechnol 6:594–602

Min YZ, Li JM, Liu F, Padmanabhan P, Yeow EKL, Xing BG (2014) Recent advance of biological molecular imaging based on lanthanide-doped Upconversion-luminescent nanomaterials. Nano 4:129–154

Misra R, Acharya S, Sahoo S (2010) Cancer nanotechnology: application of nanotechnology in cancer therapy. Drug Discov Today 15(19–20):842–850

Moussa HG, Martins AM, Husseini GA (2015) Review on triggered liposomal drug delivery with a focus on ultrasound. Curr Cancer Drug Tar 15(4):282–313

Mueller EF, Sarraf P, Tontonoz P et al (1998) Terminal differentiation of human breast cancer through PPAR. Mol Cell 1:465–470

Nosrati H, Salehiabar M, Manjili H, Danafar H, Davaran S (2018) Preparation of magnetic albumin nanoparticles via a simple and one-pot desolvation and co-precipitation method for medical and pharmaceutical applications. Int J Biol Macromol 108:909–915

Ogawa M, Umeda IO, Kosugi M, Kawai A, Hamaya Y, Takashima M et al (2014) Development of in-111-labeled liposomes for vulnerable atherosclerotic plaque imaging. J Nucl Med 55:115–120

Olusanya TOB, Haj Ahmad RR, Ibegbu DM, Smith JR, Elkordy AA (2018a) Liposomal drug delivery systems and anticancer drugs. Molecules Molecules 23(4):907

Olusanya TOB, Ahmad RRH, Ibegbu DM, Smith JR, Elkordy AA (2018b) Liposomal drug delivery systems and anticancer drugs. Molecules 23(4):907

Paoli EE, Kruse DE, Seo JW, Zhang H, Kheirolomoom A, Watson KD et al (2010) An optical and micro PET assessment of thermally-sensitive liposome biodistribution in the Met-1 tumor model: importance of formulation. J Control Release 143:13–22

Pattni BS, Chupin VV, Torchilin VP (2015) New developments in liposomal drug delivery. Chem Rev 115(19):10938–10966

Petersen AL, Binderup T, Rasmussen P, Henriksen JR, Elema DR, Kjaer A et al (2011) 64Cu loaded liposomes as positron emission tomography imaging agents. Biomaterials 32:2334–2341

Pharoah PDP, Day NE, Caldas C (1999) Somatic mutations in the p53 gene and prognosis in breast cancer: a meta-analysis. Br J Cancer 80:1968–1973

Portnoy E, Lecht S, Lazarovici P, Danino D, Magdassi S (2011) Cetuximab-labeled liposomes containing near-infrared probe for in vivo imaging. Nanomedicine 7(4):480–488

Qiu F, Ray P, Brown K et al (1988) Primary structure of c-kit: relationship with the CSF-1/PDGF receptor kinase family–oncogenic activation of v-kit involves deletion of extracellular domain and C terminus. EMBO J 7:1003–1011

Rabbani SA, Gladu J (2002) Urokinase receptor antibody can reduce tumor volume and detect the presence of occult tumor metastases in vivo. Cancer Res 62:2390–2397

Rahmim A, Zaidi H (2008) PET versus SPECT: strengths, limitations and challenges. Nucl Med Commun 29:193–207

Riaz MK, Riaz MA, Zhang X, Lin C, Wong KH, Chen X, Zhang G, Lu A, Yang Z (2018) Surface functionalization and targeting strategies of liposomes in solid tumor therapy: A review. Int J Mol Sci 19(1):195

Rozic JH, Chakraborty C, Lala PK (2001) Cyclooxygenase inhibitors retard murine mammary tumor progression by reducing tumor cell migration, invasiveness and angiogenesis. Int J Cancer 93:497–506

Schwartz MA (1997) Integrins, oncogenes, and anchorage independence. J Cell Biol 139:575–578

Selliti D, Tseng Y, Latham K (1983) Nuclear thyroid hormone receptors in C3H/HeN mouse mammary glands and spontaneous tumors. Cancer Res 43:1030–1038

Seo JW, Zhang H, Kukis DL, Meares CF, Ferrara KWA (2008) Novel method to label preformed liposomes with 64Cu for positron emission tomography (PET) imaging. Bioconjug Chem 19: 2577–2584

Sharma A, Sharma US (1997) Liposomes in drug delivery: progress and limitations. Int J Pharm 154:123–140

Sherr C, Roberts JM (1999) CDK inibitors: positive and negative regulators of G1-phase progression. Genes Dev 13:1501–1512

Soenen SJ, VandeVelde G, Ketkar-Atre A, Himmelreich U, De Cuyper M (2011) Magnetoliposomes as magnetic resonance imaging contrast agents. Wiley Interdiscip Rev Nanomed Nanobiotechnol 3:197–211

Srivatsan A, Chen XY (2014) Recent advances in nanoparticle-based nuclear imaging of cancers. Emerging Applications of Molecular Imaging to Oncol 124:83–129

Sun C, Lee JSH, Zhang MQ (2008) Magnetic nanoparticles in MR imaging and drug delivery. Adv Drug Deliver Rev 60:1252–1265

Tagami T, Foltz WD, Ernsting MJ, Lee CM, Tannock IF, May JP et al (2011) MRI monitoring of intratumoral drug delivery and prediction of the therapeutic effect with a multifunctional thermosensitive liposome. Biomaterials 32:6570–6578

Terada T, Iwai M, Kawakami S, Yamashita F, Hashida M (2006) J Control Release 111:333–342

Tontonoz P, Hu E, Graves RA, Budavari A, Spiegelman BM (1994) mPPAR tissue-specific regulator of an adipocyte enhancer. Genes Dev 8:1224–1234

Torchilin VP, Zhou F, Huang L (1993) pH-sensitive liposomes. J Liposome Res 3:201–205

Ullrich A, Schlessinger J (1990) Signal transduction by receptors with tyrosine kinase activity. Cell 61:203–212

Valencia A, Chardin P, Wittinghofer A, Sander C (1991) The ras protein family: evolutionary tree and role of conserved amino acids. Biochemist 30:4637–4648

Viloria Petit AM, Rak J, Hung MC et al (1997) Neutralizing antibodies against epidermal growth factor and ErbB-2/neu receptor tyrosine kinases down-regulate vascular endothelial growth factor production by tumor cells in vitro and in vivo: angiogenic implications for signal transduction therapy of solid tumors. Am J Pathol 151:1523–1530

Wang HX, Li M, Lee CM, Chakraborty S, Kim HW, Bao G, Leong KW (2017a) CRISPR/Cas9-based genome editing fordisease modeling and therapy: challenges and opportunities fornonviral delivery. Chem Rev 117(15):9874–9906

Wang M, Zuris JA, Meng FT, Rees H, Sun S, Deng P, Han Y, Gao X, Pouli D, Wu Q, Georgakoudi I, Liu DR, Xu QB (2017b) Efficient delivery of genome-editing proteins using bioreducible lipid nanoparticles. Proc Natl Acad Sci U S A 113(11):2868–2873

Wang X, Wang Q, Liu Z, Zheng X (2017c) Preparation, pharmacokinetics and tumour-suppressive activity of berberine liposomes. J Pharm Pharmacol 69(6):625–632.2

Xu L, Huang C, Huang W, Tang WH, Rait A, Yin YZ (2002) Systemic tumor-targeted gene delivery by anti-transferrin receptor scFv-immunoliposomes. Mol Cancer Therap 1:337–346

Xu L, Tang WH, Huang CC, Alexander W, Xiang LM, Pirollo KF (2001) Systemic p53 gene therapy of cancer with immunolipoplexes targeted by anti-transferrin receptor scFv. Mol Med 7: 726–738

Yan C, Boyd DD (2007) Regulation of matrix metalloproteinase gene expression. J Cell Physiol 211:19–26

Yang B, Song BP, Shankar S, Guller A, Deng W (2021 Jul) Recent advances in liposome formulations for breast cancer therapeutics. Cell Mol Life Sci 78(13):5225–5243

Yang HT, Jaeger M, Walker A, Wei D, Leiker K, Tao WT (2018) Break breast cancer addiction by CRISPR/Cas9 genome editing. J Cancer 9(2):219–231

Yatvin MB, Weinstein JN, Dennis WH, Blumenthal R (1978) Design of liposomes forenhanced local release of drugs byhyperthermia. Science 202:1290–1293

Yoo GH, Hung MC, Lopez-Berestein G, LaFollette S, Ensley JF, Carey M (2001) Phase I trial of intratumoral liposome E1A gene therapy in patients with recurrent breast and head and neck cancer. Clin Cancer Res 7:1237–1245

Yuan D, Zong T, Gao H, He Q (2015) Cell penetrating peptide TAT and brain tumor targeting peptide T7 dual modified liposome preparation and in vitro targeting evaluation. Yao XueXueBao 50:104–110

Zhan S, Guo W, Shao Q, Fan X, Li Z, Cheng Y (2014) A pharmacokinetic and pharmacodynamic study of drug–drug interaction between ginsenoside Rg1, ginsenoside Rb1 and schizandrin after intravenous administration to rats. J Ethnopharmacol 152(2):333–339

Zhang Y, Zhai M, Chen Z, Han X, Yu F, Li Z et al (2017) Dual-modified liposome codelivery of doxorubicin and vincristine improve targeting and therapeutic efficacy of glioma. Drug Deliv 24(1):1045–1055

Zhu D, Tao W, Zhang H, Liu G, Wang T, Zhang L et al (2016) Docetaxel (DTX)-loaded polydopamine-modified TPGS-PLA nanoparticles as a targeted drug delivery system for the treatment of liver cancer. Acta Biomater 30:144–154

Zhu L, Kate P, Torchilin VP (2012) Matrix metalloprotease 2-responsive multifunctional liposomal nanocarrier for enhanced tumor targeting. ACS Nano 6:3491–3498

Zou Y, Zong G, Ling YH, Perez-Soler R (2000) Development of cationic liposome formulations for intratracheal gene therapy of early lung cancer. Cancer Gene Ther 7:683–696

ZununiVahed S, Salehi R, Davaran S, Sharifi S (2017) Liposome-based drug co-delivery systems in cancer cells. Mater Sci Eng C Mater Biol Appl 71:1327–1341

Zuris JA, Thompson DB, Shu Y, Guilinger JP, Bessen JL, Hu JH, Maeder ML, Joung JK, Chen ZY, Liu DR (2015) Cationic lipidmediateddelivery of proteins enables efficient protein-basedgenome editing in vitro and in vivo. Nat Biotechnol 33(1):73–80

Zylberberg C, Gaskill K, Pasley S, Matosevic S (2017) Engineeringliposomal nanoparticles for targeted gene therapy. Gene Ther 24(8):441–452

# Conventional to Nanotherapeutic Strategies against Triple-Negative Breast Cancer

11

Srushti Tambe, Sabya Sachi Das, Neeru Singh, Priya Ranjan Prasad Verma, Purnima Amin, and Sandeep Kumar Singh

**Abstract**

Triple-negative breast cancer (TNBC) is an aggressive form of breast cancer that lacks the expression of the progesterone receptor, estrogen receptor, and human epidermal growth factor receptor-2. In the past several decades, the absence of specific targets resulted in a shortage of innovative treatments, and chemotherapy remained the dominant treatment option. However, TNBC has a lower survival rate and an increased likelihood of recurrence and metastasis compared to the other subtypes of breast cancer, resulting in the development of resistance to chemotherapy. The use of nanotechnology enables the attachment of several targeting moieties, controlled release, site-specific targeting, and small size (nanometric) as well as active and passive targeting, which offers significant

Srushti Tambe and Sabya Sachi Das contributed equally with all other contributors.

S. Tambe · P. Amin
Department of Pharmaceutical Science and Technology, Institute of Chemical Technology, Mumbai, India

S. S. Das
Department of Pharmaceutical Sciences and Technology, Birla Institute of Technology, Ranchi, Jharkhand, India

School of Pharmaceutical and Population Health Informatics, DIT University, Dehradun, Uttarakhand, India

N. Singh
Department of Biomedical Laboratory Technology, University Polytechnic, Birla Institute of Technology, Ranchi, Jharkhand, India

P. R. P. Verma · S. K. Singh (✉)
Department of Pharmaceutical Sciences and Technology, Birla Institute of Technology, Ranchi, Jharkhand, India
e-mail: sandeep.singh@bitmesra.ac.in



M. Rahman et al. (eds.), *Hormone Related Cancer Mechanistic and Nanomedicines*,
https://doi.org/10.1007/978-981-19-5558-7_11

potential to address the unmet needs of TNBC treatments. This chapter discusses the conventional treatment approaches, novel target therapies, their molecular mechanisms, and recent advancements in nanotherapeutic-based treatments. Overall, this chapter aims to lay a roadmap for the researchers currently working in the field for their ongoing and future research. Nonetheless, the panorama of therapy options for TNBC is rapidly shifting toward a more individualized approach, with encouraging expectations resulting from this evolution.

**Keywords**

Triple-negative breast cancer · Receptor-based mechanism · Nanotechnology · Targeted drug delivery · Controlled drug release

## 11.1 Introduction

Breast cancer is the most common kind of cancer among females, aged 45 to 55 years and the world's second major cause of cancer-related death (DeSantis et al. 2019). An estimated 1 to 1.3 million new instances of breast cancer are identified each year around the world and triple-negative breast cancer (TNBC) accounts for around 15-20% of them (Anders and Carey 2008). TNBC is characterized as tumors with triplet negative staining for the progesterone receptor, estrogen receptor, and human epidermal growth factor receptor 2 (HER2) as determined by immunohistochemistry or the lack of its gene amplification, as determined by fluorescence in situ hybridization technique (Das et al. 2020b). The three key breast cancer indicators include a lack of progesterone and estrogen receptor expression, as well as HER2 (also known as ERBB2) amplification or overexpression (Gluz et al. 2009). TNBC patients are frequently diagnosed late, with a severe histological grade, and therefore hormonal or targeted treatment is ineffective because of the lack of early detection biomarkers and unambiguous therapeutic targets. As a result, an extensive effort has been made to decipher the underlying mechanisms for this heterogeneous attribute and to identify therapeutically relevant target molecules for TNBC. Numerous attempts have centered on categorizing TNBC molecular features into subtypes with distinct disease histories and similar sensitivity patterns to chemotherapy or novel treatments. Since TNBC represents a significant clinical issue, rapid development of new diagnostic therapeutic targets and biomarkers are of paramount importance and should be prioritized for extensive research.

Over 95% of TNBCs (invasive mammary carcinomas that are undefined, or invasive ductal carcinomas) show no distinguishing histological traits; however, there are additional subtypes that have been identified (Weigelt and Reis-Filho 2009). The classically reported medullary carcinoma, which has been identified as a subgroup within TNBC15 by gene-expression profiling, is atypical (0.4–1%)

(Bertucci et al. 2006) and is characterized by pronounced lymphoplasmacytic infiltration and a favorable prognosis when compared to other subtypes (Huober et al. 2012). Other subtypes with distinct morphologies, such as adenoid cystic carcinoma (Wetterskog et al. 2012; Weigelt and Reis-Filho 2009), fibromatosis-like spindle-cell metaplastic carcinomas, and adenosquamous carcinoma, are uncommon (1%) and far less severe, capable of only local recurrence, which should be taken into account for adjuvant therapy (Wetterskog et al. 2012). Adenoid cystic carcinoma is a genomically different subtype defined by a less occurrence of copy-number defects and a specific chromosomal translocation t(6;9) (q22–23;p23–24), which leads to the MYB–NFIB fusion gene, which is found in 90% of patients with this TNBC subtype (Wetterskog et al. 2012). Furthermore, based on transcriptomic and genomic data, various attempts have been undertaken to construct a molecular categorization of TNBC. Lehmann et al. (Lehmann et al. 2011) defined six novel TNBC subtypes based on gene-expression profiles to better dissect TNBC-specific tumor heterogeneity: two mesenchymal-related subtypes including mesenchymal and mesenchymal stem-like (M and MSL, respectively), two basal-like-related subtypes including basal-like 1, and basal-like 2 (BL1 and BL2, respectively)], one luminal subtype (LAR), and lastly one immunomodulatory subtype (IM) (Lehmann et al. 2011). TNBCs are classified as LAR subtype tumors by immunohistochemistry, although they are genetically and histologically identical to luminal A breast cancer subtype. Since the TNBC of the LAR subtype is defined by androgen receptor (AR) expression in the context of a luminal-like expression profile, it can be treated with AR-targeting agents, similar to prostate cancer therapy. Some TNBC subtypes are interestingly connected to histological types: MSL and M tumors overlap with metaplastic breast cancer, IM tumors overlap with medullary breast cancer, and LAR tumors with apocrine tumors (Lehmann et al. 2011). TNBC can also be classified based on the intrinsic PAM50 subtyping into basal-like, followed by HER2-enriched normal-like, luminal B, and luminal A accounting for 80.6%, 10.2%, 4.7%, 3.5%, and 1.1% occurrences, respectively. The convergence between the TNBC type and PAM50 classification inferred that, apart from MSL and LAR type of tumors, the majority of the other TNBC subtypes were grouped as basal-like tumors namely BL1, BL2, IM, and M, accounting for 99%, 95%, 84%, and 97%, respectively, by PAM50 analysis. MSL TNBCs were classified as basal-like in 50% of cases, normal-like in 28% of cases, and luminal B tumors in 14% of cases. Finally, within the LAR subtype, tumors were mostly classified as HER2 enriched or luminal B, accounting for 74% and 14%, respectively. In another study, TNBC was classified using integrated genomic and transcriptome data to create 10 integrative clusters (IntClust) of breast cancer (Curtis et al. 2012). Basal-like breast cancers (BLBCs) in the IntClust 4 subgroup exhibit substantial Jessner lymphocytic infiltration, a powerful immunological and inflammatory sign, and minimal copy-number aberrations ('CNA-devoid' subgroup), thus patients in this grouping have a good prognosis (Curtis et al. 2012). IntClust 10 BLBCs, on the other hand, exhibit a high level of genomic instability and commonly show severe chromosomal abnormalities including chromosome 5 deletion, 8q gain, 10p gain, or 12p gain (Curtis et al. 2012).

Patients dread TNBC since there are no site-specific treatments and the illness has a dismal prognosis. In fact, the treatment approaches are much more complicated depending on the drug sensitivity, with drug sensitivity being determined not just by the target pathway's degree of expression or mutational status, but also by the state of upstream, downstream, and parallel pathways. Furthermore, there are likely to be distinct treatment methods for different subclassifications of TNBCs. The following section sheds light on the conventional and evolving therapeutic interventions for the treatment of TNBC.

## 11.2 Conventional Approaches for the Treatment of TNBC

There are no specific therapeutic options for TNBC at this time, thus surgery, radiation, and chemotherapy are the mainstays of treatment. TNBC, like other kinds of breast cancer, is best controlled locally with surgical excision by mastectomy, followed by radiotherapy [breast-conserving therapy (BCT)] (Yagata et al. 2011). Radiation of the chest wall following mastectomy (if necessary) and of the breast and surrounding region following BCT is also a viable option (Yagata et al. 2011). Furthermore, chemotherapy is the only systemic treatment for TNBC that has been approved due to the lack of a specific target owing to the heterogeneous nature of TNBC. Anthracyclines, taxanes, and platinum-based drugs (carboplatin and cisplatin) are among the current therapies for TNBC. Conventional treatment approaches for TNBCs are shown in Table 11.1. The Japan Breast Cancer Society's evidence-based medicine (EBM) medication therapy recommendations propose anthracycline (doxorubicin and epirubicin) or taxane-based regimens (docetaxel, paclitaxel), or a combination of the two, as standard chemotherapy for TNBC (Shimoi et al. 2020). Other systemic therapeutic alternatives with greater selectivity and efficacy are now being investigated, including medications that target the cytoskeleton, molecular-directed targeted therapies, vascular system, cell migration, and high proliferation gene pathways, vaccine, and immunotherapy.

TNBC is a vascularized malignancy with high levels of VEGF (vascular endothelial growth factor) in the intratumoral space (Linderholm et al. 2009). In TNBC, the elevated levels of VEGF are an unfavorable prognostic factor, laying the groundwork for clinically testing VEGF receptor (VEGFR) inhibiting agents. The angiogenesis blockers, also known as VEGF inhibitors, prevent tumor neovasculature from growing. Bevacizumab (Avastin), an anti-VEGF humanized monoclonal antibody, has consistently increased PFS (progression-free survival) and response rate in HER2-negative BC when given along with the first-line chemotherapy (Miles et al. 2013). Bevacizumab binds to VEGF-A and its isoforms in particular, but only to a limited extent to other VEGFR ligands like VEGF-B and VEGF-C. Recently developed antiangiogenic drugs, on the other hand, bind to VEGFR-2- [ramucirumab (IMC-1121B, ImClone), Clinical Trials.gov NCT00703326] and prevent any ligands from interacting with this target (Kumar and Aggarwal 2016). Inhibiting VEGFR in this manner might result in a more thorough target inhibition and more efficient angiogenesis suppression. Inhibitors of the PARP [Poly

**Table 11.1** Conventional treatment of TNBC[a]

| Conventional Treatment | Drugs | Scheme/Dose | Mechanism | Ref |
|---|---|---|---|---|
| Adjuvant agents | Taxanes + Anthracyclines | Doxorubicin 20 mg/m$^2$ + 75 mg/m$^2$ docetaxel +600 mg/m$^2$ for six cycles cyclophosphamide q3 weeks. | Cytotoxicity | Barkat et al. (2020) |
| Neoadjuvant therapy (gold standard) but metastatic and advanced | Taxanes + AnthracyclinesorIxabepilone and Taxane Capecitabine monotherapy or Ixabepilone + Capecitabine | Cyclophospamide 600 mg/m$^2$ along with doxorubicin 20 mg/m$^2$ plus 28 days followed by paclitaxel 80 mg/m$^2$ for 84 daysfor 14 days, 1250 mg/m$^2$ Capecitabine + Ixabepilone 40 mg/m$^2$ and docetaxel 75 mg/m$^2$ per 21 days | Cytotoxicity stabilization microtubules | Sikov et al. (2015) |
| New neoadjuvant agents (BRCA mutations) | Bevacizumab, Platinums (carboplatin) nab-paclitaxel. | The typical strategy for adding up 125 mg/m$^2$ Abraxane, AUC carboplatin, 10 mg/kg bevacizumab | Cytotoxicity and VEGF immunotherapy | CALGB 40,603 trial Martín et al. (2013) |
| Surgery: Surgical treatment is breast preservation. A radiation treatment after a lumpectomy may be a choice (National Comprehensive Cancer Network guidelines) | | | | |
| Radiotherapy: Chemotherapy and radiation therapy (RT) are often used in cancer treatment. After surgery, RT may prove beneficial. Most likely, BRCA mutations reap the benefits of this treatment. | | | | |

[a]The standard treatment for TNBC that is presently used in hospitals. It is assessed by TNM clinical-stage, imaging (ultrasound, mammography, PET, CT-Scan), blood tests, and treatment tolerability, which is frequently accompanied by corticosteroids such as dexamethasone and symptom-controlling medicines such as ondansetron to decrease side effects

(ADP-ribose) polymerase] enzyme, such as iniparib and olaparib, are also being explored as potential TNBC treatments. While preserving cells with normal BRCA function, PARP agents produce synthetic lethality in homozygous BRCA-deficient individuals. PARP-1 overexpression and BRCA-mediated DNA repair impairment in TNBC provide an attractive biological platform for PARP inhibition. IGFR (insulin-like growth factor receptor) inhibitors such as BMS-754807 (Litzenburger et al. 2009) are also potential targets as elevated insulin-like growth factor-1 receptor (IGF-IR) levels are observed in TNBC owing to the alterations in genes such as BRCA1 and p53 that inhibit IGF-IR promoter expression (Litzenburger et al. 2011). EGFR overexpression is seen in approximately 60% of TNBC tumors, and its suppression may indeed serve as a promising therapeutic target in TNBC (Cheang et al. 2008). Cetuximab (Erbitux), a chimeric monoclonal antibody in combination with carboplatin, demonstrated therapeutic effectiveness in phase II clinical trials (Carey et al. 2008). FGFR inhibitors such as lucitanib (a potent inhibitor of FGFR1/2/3, VEGFR1/2/3, and PDGFR) are being investigated because FGF receptor overexpression is enhanced in TNBC; TNBCs with augmentation of FGFR1 and FGFR2 are seen in around 9% and 2–4% of cases, respectively. (Lehmann and Pietenpol 2014).

Survival, proliferation, and growth are the most important biological implications of AKT activation in cancer cells. AKT may also possibly play a role in the beginning and course of BC. MK-2206, Ipatasertib, GSK2141795, and AZD5363 are the top four AKT inhibitors under research as possible candidates for the treatment of TNBCs (Tripathy et al. 2015). Another class of drugs, called Epothilones, are microtubule-targeting agents in which the mechanism of action hinders cell division by interfering with tubulin. Ixabepilone improved pCR rates in ER/PR-negative cancers in a neoadjuvant trial when compared to tumors with hormone receptors (Baselga et al. 2009). Platinum-based compounds have recently been studied as a possible treatment for TNBC. As a result of platinum coupling, they are thought to cause DNA damage via double-strand cross-links and impairments in BRCA-associated DNA repair (Weisenthal 2009). Small molecule inhibitors of the receptor tyrosine kinases (TKIs), such as geftinib, are under investigation as well in patients with TNBC (Baselga et al. 2005). The Src tyrosine kinase, also known as Rous sarcoma virus, is upregulated in TNBC and has been linked to the progression of metastatic cancer (Verbeek et al. 1996). Dasatinib is a Src inhibitor that is taken orally and has shown modest but intriguing single-agent action in patients with advanced triple-negative breast tumors, with a clinical benefit rate of 9.3% (4/43 patients) (Finn et al. 2009). For the best use of these agents in clinical practice, further research is warranted. The PI3K, i.e., phosphoinositide 3-kinase signaling pathway, is mediated by the tumor suppressor PTEN and AKT, and the mammalian target of rapamycin (mTOR) is an effector of PI3K. PI3K pathway protein mutations are often observed in BC, and loss of PTEN is a typical finding in TNBC, which results in greater mTOR activation in the condition. (Saal et al. 2005). As a result, mTOR inhibitors are currently being evaluated in patients with TNBC. Many clinical trials (Phase I and phase II) employing everolimus alone or in conjunction with other drugs such as lapatinib are enrolling patients with

TNBC (ClinicalTrials.gov NCT01272141). In metastatic TNBC, the mTOR inhibitor temsirolimus is being studied in a phase I/II clinical study in conjunction with neratinib (ClinicalTrials.gov NCT01111825). In addition, cellular and molecular immunotherapies like anti-PD-1 and anti-PD-L1 appear to be particularly advantageous in the treatment of TNBC. Several investigations have shown that TNBC outcome is linked to tumor immune infiltration, which supports the hypothesis. Aurora kinase, which is elevated in TNBC, has sparked interest, leading to the development of Aurora kinase inhibitors such as AS703569 and ENMD-2076 for TNBC treatment. The summary of the potential agents under development for the treatment of TNBC is shown in Fig. 11.1.

Since there are so many genetic anomalies seen in TNBCs, researchers have shifted their emphasis to various treatment approaches, several of which are now being evaluated in clinical trials. These include kinase inhibitors, such as tGF-β, TRAIL receptor, androgen receptor, and high-dose chemotherapy, vaccines, and others. The enormous complexity of TNBC's resistance to single-agent treatment has prompted researchers all over the world to propose various novel targeted signaling pathways in order to make advances in TNBC management. As we go forward to the next decade, cytotoxic chemotherapy may continue to be beneficial in the treatment of TNBC. Several challenges with chemotherapy, such as multidrug resistance, rapid in vivo clearance, endosome degradation in the cytosol, and severe systemic toxicity, have led to novel approaches to its management, such as Trojan horse-like carriers that mimic eukaryotic cell components to avoid immune recognition. TNBC management with Trojan horse-like carriers has been studied in conjunction with previously recognized therapeutic targets, such as EGFR inhibitors, VEGF inhibitors, mTOR inhibitors, AR receptor antagonists, protein 90 inhibitors, PARP inhibitors, heat shock, and so on. A greater understanding of TNBC carcinogenesis and progression, as well as the causes of phenotypic heterogeneity, may aid in the development of novel, tailored treatments for this disease. However, before these treatments can be implemented in clinical practice, accurate predictive biomarkers must be discovered. Biomarker-selected medicines may soon be a reality attributable to robust biomarkers and randomized studies that are promising with well-designed prospective. It is possible that newer technology for unraveling the disease's geno-molecular roots may also lead to new therapy options.

## 11.3 Nanomedicine-Based Strategies for Targeting Triple-Negative Breast Cancer

The aberrant tumor microenvironment and vasculature impact the precision of nanotechnology-based treatment. In the last decade, this has garnered attention in the field of targeted drug delivery and cancer therapy. The most important component is size; the targeted size of nanoparticles (NPs) (1 – 200 nm) and the particle's travel dynamics are dictated by its shape and conformation, which is vital in the development of nanoformulation. Additionally, the encapsulation and surface charge capabilities of the NPs are crucial for precisely site-specific drug delivery

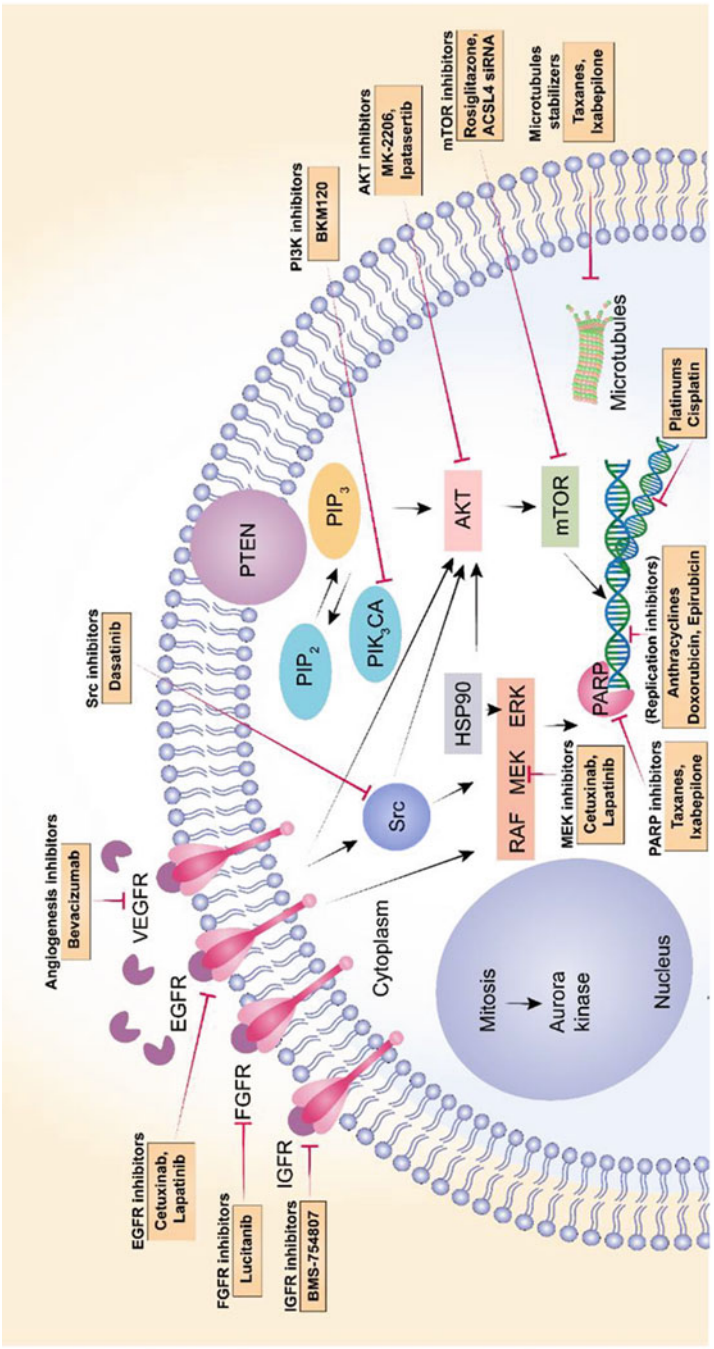



**Fig. 11.1** Current and future potential therapeutic targets in TNBC

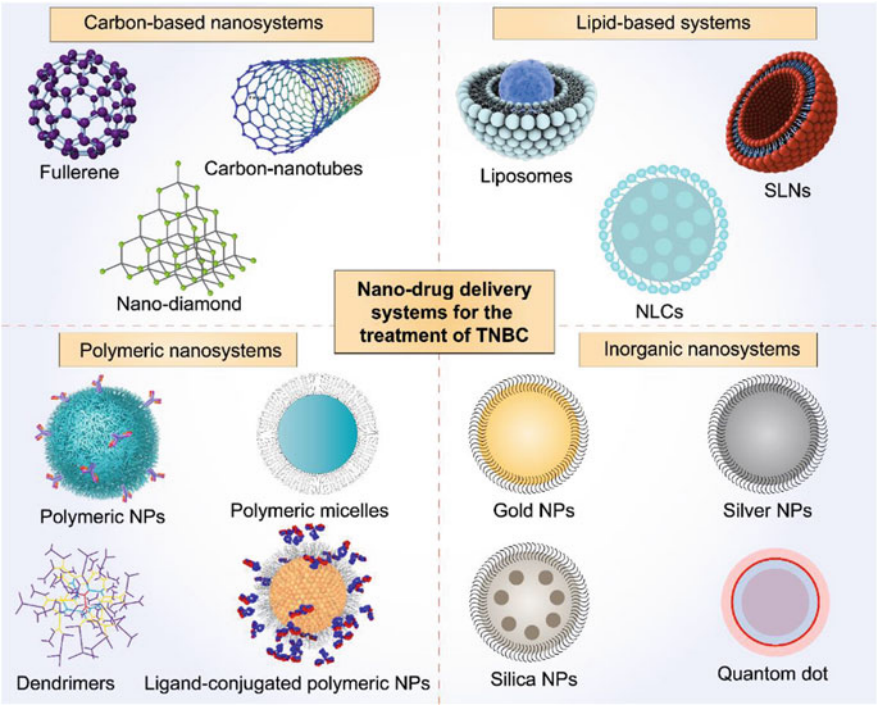



**Fig. 11.2** Emerging nanotechnologies for the treatment of triple-negative breast cancer

employing a particular conjugated ligand against the cancer cell's target receptor. Long circulation half-life with minimal systemic toxicity, high drug loading efficiency, high tumor adhesion, selective localization, augmented internalization into the tumor facilitated by endocytosis, controlled or sustained release of cytotoxic drugs and imaging agents over the appropriate time period, and safe bioelimination from the body are all main attributes of nano-based drug delivery systems. In addition, technical practicality (high recuperation with regulated drug loading and release) and economic stability for large-scale manufacturing are also significant considerations in cancer nanomedicine research. Nanomedicines, such as drug-loaded NPs, micelles, liposomes, polymeric micelles, polymeric drug conjugates, inorganic NPs, etc., have unique properties that allow them to pass through biological membranes and deliver the entrapped drug to cells (Fig. 11.2.). Hence, nano-based drug delivery systems can serve as a novel therapeutic strategy to overcome current challenges in TNBC treatment. Recently, investigated nanotherapeutics for TNBC therapy are represented in Table 11.2.

Nanoparticles (NPs) are a class of nanocarriers that are ultra-dispersed solid supramolecular structures having a size range of 10-1000 nm. A recent surge in interest in the field of nanopharmaceuticals has resulted in a slew of new developments. Drugs can be encapsulated, entrapped, dissolved, or linked to an

**Table 11.2** Recent research on nanotechnologies for the treatment of triple-negative breast cancer

| Type of nanosystem | Composition | Active ingredient | Research findings | Ref |
|---|---|---|---|---|
| Nanoparticles | Indocyanine green (ICG) | Paclitaxel | The combination of nanoparticles and αPD-L1-targeted immune checkpoint blockade therapy for TNBC resulted in tumor shrinkage, metastasis suppression, and recurrence avoidance. | Feng et al. (2020) |
| Nanoparticles | Hyaluronic acid-d-α-tocopherol succinate-(4-carboxybutyl) triphenyl phosphonium bromide | Lapatinib | After intravenous injection, the developed formulation had a stronger tumor growth inhibition profile than the other groups. MDA-MB-231 cells also showed improved antiproliferation, apoptotic effectiveness, and mitochondrial destabilizing activity. | Lee and Cho (2019) |
| SLNs | Trilaurin, cholesterol, chitosan, lecithin-phosphatydilcoline, sodium taurocholate, PEG-60 hydrogenated castor oil | Curcumin | CURC-loaded SLNs were shown to be 5–10 times more effective than free CURC at boosting intracellular retention and doxorubicin cytotoxic effects in patients with TNBC who expressed Pgp. Moreover, it was effective in restoring doxorubicin sensitivity in drug-resistant TNBC tumors while providing no systemic side effects. | Fathy Abd-Ellatef et al. (2020) |
| Polymeric nanostructures | Poly(N-isopropylacrylamide) | Doxorubicin | When compared to free doxorubicin, an increase in the IC50 of doxorubicin was found. Furthermore, great selectivity as well as quick absorption into the MDA-MB-231 cell line were found to be beneficial. | Bobrin et al. (2020) |
| Polymeric nanoparticles | Chitosan, polylactide | Tamoxifen | Increased drug-loaded nanoparticle concentration resulted in TNBC cell death and cell cycle arrest, resulting in increased efficacy for treating TNBC. | |

| | | | | |
|---|---|---|---|---|
| Dendrimer | Poly(amidoamine), EGFR-binding peptide 1, rans-activating transcriptional activator peptide | Doxorubicin | The developed dendrimer greatly increased drug accumulation at the tumor site, resulting in far greater tumor growth suppression effectiveness and prolonged survival. | Liu et al. (2019) |
| Gold (au) nanoparticles | Hexadecyltrimethylammonium chloride, 1, 6-Bis (N,N-hexadecyl dimethylammonium) adipate (16-6-16), *Azadirachta indica* | NA | The fabricated AuNPs exhibited outstanding cytotoxicity against TNBC cell lines while causing no damage to normal cells. After 24 hours at dose-dependent concentrations, increased apoptotic cell death was seen as a result of the cellular apoptosis in conjunction with mitochondrial membrane potential loss, which was reported. | Mohammed Siddiq et al. (2019) |
| Mesoporous silica nanoparticles | Cetyltrimethylammonium bromide, tetraethyl orthosilicate, aminopropyltriethoxysilane | Doxorubicin | The in vivo studies showed that the developed NPs had a better antitumor effect than DOX alone on xenograft tumor-bearing mice. | Xu et al. (2020) |
| Nanodiamond | Protamine sulphate, indocyanine green | Apoptozole, doxorubicin | The developed formulation boosted MDA-MB-231 cell sensitivity by reducing thermal/drug resistance through autophagy suppression, resulting in an enhanced combination treatment that is effective both in vitro and in vivo. | Cui et al. (2021) |

NP matrix that acts as a drug reservoir, which could have an impact on cancer treatment. For the synthesis of NPs, polyethylene glycol (PEG), poly (lactic-co-glycolic acid) (PLGA), and modified PLGA are common polymers. To increase treatment effectiveness against perlecan (maintains endothelial barrier function), PEG-maleimide factionalized with PTX loading PLGA NPs were coupled with an antibody in TNBC. As these carriers demonstrate better cellular absorption, increased cytotoxicity, and decreased tumor growth, this study revealed that NPs can enhance the delivery of TNBC drugs. (Khanna et al. 2019). With numerous intriguing evidences blooming in recent years where novel drug delivery and imaging technologies are developed, there is an optimistic vision for NPs as drug delivery systems and their clinical translation might be achieved in the near future to revolutionize cancer therapy.

### 11.3.1 Polymeric Nanoparticles and Polymeric Micelles

Polymeric NPs are another type of NPs which have specialized structural arrangements for drug delivery that are generated by various monomers. The polymeric core can be surface-adsorbed with active ingredients, or the components can be encapsulated inside the core and may range in size from 1 to 1000 nm. Biocompatibility is a particular benefit of polymeric NPs. Chen et al. (Chen et al. 2021) verified the overexpression of PD-L1 and CD155 in TNBC cell lines and developed CD155 siRNA-containing mPEG-PLGA-PLL NPs coated with antibodies that block PD-L1 to asynchronously inhibit CD155 and PD-L1 in a spatiotemporal way. In a syngeneic 4 T1 orthotopic TNBC tumor model, the developed NPs showed good TNBC targeting and produced CD8+ TILs-dominant intratumor antitumor immunity to effectively limit TNBC development and metastasis with great safety. A combination of the efforts put forth in the development of novel polymeric materials that may meet the specific requirements of a specific delivery system, the greater understanding scientists have of disease mechanisms, and the collaborative research work carried out across all scientific disciplines will improve the current state of polymeric NPs use in the medical field, resulting in improved and safer therapeutic interventions.

Polymeric micelles are nanoscopic core/shell structures composed of amphiphilic block copolymers. Because of their semisolid hydrophobic core made of a biodegradable polymer such as PLA, PCL, and PLGA, the polymer micelles have a diameter of 10 to 100 nm and are suitable for supporting a variety of water-insoluble chemotherapy drugs. (Das et al. 2020c). NBC-specific folate receptor-targeted micellar NPs carrying orlistat were developed and evaluated by Paulmurugan et al. (Paulmurugan et al. 2016). The developed NPs showed higher cytotoxicity than the free drug. Moreover, the developed micellar NPs demonstrated significantly higher tumor volume reduction as compared to the control group. The ever-increasing discovery of poorly soluble compounds has proven to be a big concern for pharmaceutical industry formulators, and polymeric micelles may pave the way to

successfully develop a universal formulation with decreased risks of additive toxicities and formulation instability.

### 11.3.2 Metal and Inorganic Nanoparticles

Inorganic NPs have a larger surface area to volume ratio than organic NPs. They have a versatile surface conjugation chemistry and are simple to prepare, albeit this generally comes at the expense of poor biodegradability and biocompatibility. Carbon nanotubes (CNTs), magnetic NPs (MNPs), gold NPs (AuNPs), quantum dots, and silica NPs (SNPs) are among the inorganic NPs that have been investigated. Surapaneni et al. (Surapaneni et al. 2018) developed gold NPs. The authors reported that administration with AuNPs increases the sensitivity of MDA-MB-231 cells to 5-FU by lowering the levels of thymidylate synthetase, resulting in cytotoxicity in TNBC cells. In another study, Snyder et al. synthesized silver nanoparticles (AgNPs) and evaluated their capacity to destroy mesenchymal TNBCs in a targeted manner. Researchers discovered that the synthesized AgNPs eliminated mesenchymal TNBCs by a process involving protein and lipid oxidation without causing similar damage to normal breast cells, resulting in improved TNBC therapeutic outcomes.

To circumvent the limitations of other delivery carriers, mesoporous silica nanoparticles (MSNs) are being employed as emerging nanocarriers. Because of the enormous internal pore capacity, they can encapsulate a significant number of anticancer medications, and the supramolecular components function as a cap, allowing active ingredients to be captured and released. Ahir et al. (Ahir et al. 2020) developed mesoporous silica NPs (MSNs) for the simultaneous delivery of antisense-miR-10b and miR-34a-mimic which were then coated with a PEG-PLGA polymer with a hyaluronic acid attachment for more precise targeting. TNBC tumor targeting was found to be highly specific in both in vitro and in vivo studies, resulting in effective tumor growth reduction and metastasis retardation, supporting the system's therapeutic application potential. In another study, Yang et al. (Yang et al. 2018) developed graphene quantum dots (GQDs) which were further integrated into the cavity of hollow MSNs to form GQDs@hMSN-PEG NPs. The developed MSNs exhibited a long retention duration in the tumor and strong drug loading effectiveness, indicating that it might be a suitable option for combinational TNBC treatment with photodynamic therapy and chemotherapy modules combined into one system. Inorganic NPs with intriguing inherent features for cancer treatment have been developed at a rapid pace during the last three decades. Many of these synthetic technologies have advanced to full-scale commercial production after demonstrating proof-of-concept efficiency. However, ensuring biological applicability requires optimization of suitable size scales and batch-to-batch repeatable synthetic techniques of NPs with unique magnetic or optical characteristics.

### 11.3.3 Lipid-Based Nanosystems

Lipid-based nanomedicines have been proposed as a possible solution to improve delivery of therapeutic interventions into tumor site while lowering side effects and countering resistance to a number of drugs. Lipidic colloids may be synthesized from a range of materials and are used in cancer research for a variety of therapeutic and diagnostic purposes. They offer benefits such as biocompatibility, biodegradability, and site-specific delivery when developed as SLNs, NLCs, or self-micro/nano emulsified formulations (SMEDDS/SNEDDS), SLNs, and NLCs.

Slow tumor development and metastasis were seen in SMEDDS containing doxorubicin hydrochloride and LyP-1. LyP-1 is a peptide that targets the p32 receptor and is found in abundance in malignant BC cells. The 4 T1, p32-expressing BC cells, and MDA-MB 231 (TNBC) cell lines were used in in vitro cytotoxicity tests, which resulted in substantial cell death (Timur et al. 2019). Pindiprolu et al. (Pindiprolu et al. 2019) developed niclosamide-containing SLNs for the effective treatment of TNBC. In an in vitro anticancer investigation, niclosamide-containing SLNs outperformed niclosamide drug alone in terms of cytotoxicity, which is due to improved cell absorption of the SLN formulation. In another study, Chand et al. (Chand et al. 2021) prepared NLCs containing cabazitaxel (CBT), a taxane analog and microtubule inhibiting drug. In vitro cell culture experiments revealed that CBT-containing NLCs showed increased cytotoxicity by a factor of 6 to 2.5 against MDA-MB-468 cell lines, respectively, than the pure drug. In MDA-MB-468 cell lines, cellular uptake of NLC was also found to be 2.5 and 2.1 times greater than CBT alone. Moreover, CBT-loaded NLCs dramatically increased apoptosis and hindered MDA-MB-468 and MCF-7 cell mobility. In another study, DSPE-PEG2000-tLyp-1 peptide-modified liposomal formulation encapsulated with a new miRNA sense strand of 25 nucleotides in length was developed by suppressing the Slug gene in order to cure TNBC. TNBC cancer-bearing mice were treated with functional miRNA liposomes, which had a greater chemotherapeutic impact than functional vinorelbine liposomes, and coupling the 2 treatments resulted in almost total prevention of tumor development in the mice. Furthermore, the functioning liposomes boosted the drug's half-life in the blood of cancer-bearing nude mice, as well as its accumulation in breast cancer tissues (Yan et al. 2019). Liposomes, owing to their biocompatibility and adaptability, are the most extensively utilized; however, SLNs and NLCs have lately gained a lot of interest. Some of these lipid-based systems have even progressed to clinical trials, and some, such as Abraxane®, Doxil®, Myocet®, and nal-IRI, have been authorized in clinic for the treatment of cancer. As a result, lipid-based systems appear to be very promising for TNBC treatment in the future.

### 11.3.4 Carbon-Based Nanomedicines

Carbon-based nanocarriers like carbon nanotubes (CNTs), diamonds, graphene, fullerenes, etc. have piqued the interest of scientists owing to their varied chemical

functionalization, biocompatibility, effective drug delivery approach, and stable physicochemical properties. Recent research suggests that these carbon-based nanocarriers can be utilized for regulated drug administration of medications as well as contrast agents for imaging, localizing therapies within malignancies, and diagnosing.

For combined thermal-photo-chemo treatment of TNBC, very recently Gadeval et al. (Gadeval et al. 2020) developed green graphene oxide (GO) nanoplates reduced and stabilized by quercetin and driven by folate receptors. MDA-MB-231 cells treated with the near-infrared laser at 808 nm demonstrated very high absorption of the quercetin-loaded GO nanoparticles-FA and cytotoxic effects (p 0.001), suggesting that this treatment could be beneficial for the treatment of TNBC. In another study, Liao et al. (Liao et al. 2019) developed a nanocomposite by employing nanodiamond conjugated with cetuximab and paclitaxel (ND-PTX-Cet) with the purpose of concentrating treatment on TNBC cells that express the EGFR gene. The developed formulation decreased cell viability and triggered mitotic catastrophe in MDA-MB-231 cell lines; in contrast, the nanodiamond alone did not cause apoptosis. Additionally, it was able to bind to EGFR selectively, enhancing anticancer effects such as mitotic catastrophe, drug absorption, and cell death in EGFR-expressing MDA-MB-231 cells. The research findings reported that ND-PTX-Cet can serve as a viable treatment option for TNBC. Carbon-based NPs, being one of the most commonly employed nanomaterials, have been the subject of much research over the last two decades. Carbon-based NPs have been widely employed in a variety of applications due to their inherent mechanical, optical, electrochemical, and electrical capabilities. Because of the presence of both inorganic and organic π–π stacking features, carbon-based NPs are emerging as potential new nanocarriers. Chemical modification techniques have been introduced and effectively applied in bio-applications such as cancer therapy to reduce their detrimental effects on the biological system. However, since carbon-based NPs still exhibit toxicity, more comprehensive research is needed to evaluate their toxicity and pharmacokinetics.

## 11.4 Future Perspectives and Conclusions

Given the aforementioned limitations, the therapeutic strategy for TNBCs should focus on cellular targeting to eliminate tumor mass, related blood vessels, and key subpopulations. The treatment objective should be founded on the notion of customized medicine, with each patient or cell subpopulation having unique needs. The ability to use a basic grasp of cellular biology to investigate the role of cell-to-cell communication in tumor resistance might open up new possibilities for TNBCs therapy. However, while immunotherapy has a high success rate, a hyperthermia-based strategy also contributes significantly to the current treatments' overall success. Antioxidants derived from plants have a critical role in the treatment of cancer as well. Comprehensive screening and comprehending the notion of conventional treatment methods that aid the development of excellent immunity in

the human body are still needed to further study natural compounds' potential. Employing a reliable multivariate data analysis on a well-programmed repository of multidimensional, multimodal sets of data provided by the simultaneous application of various 'omics' at preclinical and clinical levels and analyzing the outcomes within a suitable Systems Biology approach, a breakthrough in the field may be achievable. A constructive collaborative effort between a molecular biologist, a phytochemist, a formulation scientist, and a physician might serve as a stepping stone for therapeutic interventions in clinical that addresses the specific needs of a cancer patient.

A plethora of nano-sized formulations, including herbal nanotherapeutics and targeted therapeutic systems, have been designed for the effective management and treatment of TNBCs (Bharadwaj et al. 2021; Das et al. 2020a; Barkat et al. 2021). Next-generation TNBC therapeutics include NPs loaded with pharmacological agents with molecular specificity and imaging functionality. Various new pharmacological compounds have proven to be effective TNBC inhibitors; however, there is no evidence in the literature that these medicines are being investigated for use as nanomedicine. With greater knowledge of biological processes and nanotechnology advancements, future nanomedicine treatments for TNBC will be more compatible. Nevertheless, breakdown of the barriers between material research, preclinical testing, and clinical reality, a more rational design of nanomedicines for the treatment of TNBCs, as well as therapeutically appropriate preclinical treatment settings in which nanomedicines are evaluated, are necessary.

## References

Ahir M, Upadhyay P, Ghosh A, Sarker S, Bhattacharya S, Gupta P, Ghosh S, Chattopadhyay S, Adhikary A (2020) Delivery of dual miRNA through CD44-targeted mesoporous silica nanoparticles for enhanced and effective triple-negative breast cancer therapy. Biomater Sci 8(10):2939–2954. https://doi.org/10.1039/D0BM00015A

Anders C, Carey LA (2008) Understanding and treating triple-negative breast cancer. Oncology 22(11):1233–1243. https://pubmed.ncbi.nlm.nih.gov/18980022

Barkat HA, Barkat MA, Taleuzzaman M, Das SS, Rizwanullah M, Hadi HA (2021) Receptor-based combinatorial Nanomedicines: a new Hope for cancer management. In: Kumar S, Rizvi MA, Verma S (eds) *Handbook of research on advancements in cancer therapeutics* (pp. 339–355). IGI Global. https://doi.org/10.4018/978-1-7998-6530-8.ch011

Barkat HA, Das SS, Barkat MA, Beg S, Hadi HA (2020) Selective targeting of cancer signaling pathways with nanomedicines: challenges and progress. Future Oncol 16(35):2959–2979. https://doi.org/10.2217/fon-2020-0198

Baselga J, Albanell J, Ruiz A, Lluch A, Gascón P, Guillém V, González S, Sauleda S, Marimón I, Tabernero JM, Koehler MT, Rojo F (2005) Phase II and tumor Pharmacodynamic study of Gefitinib in patients with advanced breast cancer. J Clin Oncol 23(23):5323–5333. https://doi.org/10.1200/JCO.2005.08.326

Baselga J, Zambetti M, Llombart-Cussac A, Manikhas G, Kubista E, Steger GG, Makhson A, Tjulandin S, Ludwig H, Verrill M (2009) Phase II genomics study of ixabepilone as neoadjuvant treatment for breast cancer. J Clin Oncol 27(4):526–534

Bertucci F, Finetti P, Cervera N, Charafe-Jauffret E, Mamessier E, Adélaïde J, Debono S, Houvenaeghel G, Maraninchi D, Viens P, Charpin C, Jacquemier J, Birnbaum D (2006) Gene

expression profiling shows medullary breast cancer is a subgroup of basal breast cancers. Cancer Res 66(9):4636–4644. https://doi.org/10.1158/0008-5472.CAN-06-0031

Bharadwaj P, Das SS, Beg S, Rahman M (2021) Formulation and biological stability of nanomedicines in cancer treatment. In: Beg S, Choudhry H, Ahmad FJ, Rahman M, Souto EB (eds) Nanoformulation strategies for cancer treatment-micro and Nano Technologies. Elsevier, pp 277–289. https://doi.org/10.1016/C2019-0-01815-8

Bobrin VA, Lin Y, He J, Qi Y, Gu W, Monteiro MJ (2020) Therapeutic delivery of polymeric tadpole nanostructures with high selectivity to triple negative breast cancer cells. Biomacromolecules 21(11):4457–4468. https://doi.org/10.1021/acs.biomac.0c00302

Carey LA, Rugo HS, Marcom PK, Irvin W, Ferraro M, Burrows E, He X, Perou CM, Winer EP (2008) TBCRC 001: EGFR inhibition with cetuximab added to carboplatin in metastatic triple-negative (basal-like) breast cancer. J Clin Oncol 26(15_suppl):1009. https://doi.org/10.1200/jco.2008.26.15_suppl.1009

Chand P, Kumar H, Badduri N, Gupta NV, Bettada VG, Madhunapantula SV, Kesharwani SS, Dey S, Jain V (2021) Design and evaluation of cabazitaxel loaded NLCs against breast cancer cell lines. Colloids Surf B Biointerfaces 199:111535. https://doi.org/10.1016/j.colsurfb.2020.111535

Cheang MCU, Voduc D, Bajdik C, Leung S, McKinney S, Chia SK, Perou CM, Nielsen TO (2008) Basal-like breast cancer defined by five biomarkers has superior prognostic value than triple-negative phenotype. Clin Cancer Res 14(5):1368–1376. https://doi.org/10.1158/1078-0432.CCR-07-1658

Chen C, Guo Q, Fu H, Yu J, Wang L, Sun Y, Zhang J, Duan Y (2021) Asynchronous blockade of PD-L1 and CD155 by polymeric nanoparticles inhibits triple-negative breast cancer progression and metastasis. Biomaterials 275:120988. https://doi.org/10.1016/j.biomaterials.2021.120988

Cui X, Liang Z, Lu J, Wang X, Jia F, Hu Q, Xiao X, Deng X, Wu Y, Sheng W (2021) A multifunctional nanodiamond-based nanoplatform for the enhanced mild-temperature photothermal/chemo combination therapy of triple negative breast cancer via an autophagy regulation strategy. Nanoscale 13(31):13375–13389. https://doi.org/10.1039/D1NR03161A

Curtis C, Shah SP, Chin S-F, Turashvili G, Rueda OM, Dunning MJ (2012) The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. Nature 486(7403):346–352. https://doi.org/10.1038/nature10983

Das SS, Alkahtani S, Bharadwaj P, Ansari MT, Kahtani AL, Pang Z, Hasnain MS, Nayak AK, Aminabhavi TM (2020b) Molecular insights and novel approaches for targeting tumor metastasis. Int J Pharm 585:119556. https://doi.org/10.1016/j.ijpharm.2020.119556

Das SS, Bharadwaj P, Bilal M, Barani M, Rahdar A, Taboada P, Bungau S, Kyzas GZ (2020c) Stimuli-responsive polymeric Nanocarriers for drug delivery, imaging, and Theragnosis. Polymers 12(6):1397. https://doi.org/10.3390/polym12061397

Das SS, Verma PRP, Kar S, Singh SK (2020a) Quercetin-loaded Nanomedicine as oncotherapy. In: Rahman M, Beg S, Kumar V, Ahmad FJ (eds) Nanomedicine for bioactives: healthcare applications, 1st edn. Springer, Singapore, pp 155–183. https://doi.org/10.1007/978-981-15-1664-1_5

DeSantis CE, Ma J, Gaudet MM, Newman LA, Miller KD, Goding Sauer A, Jemal A, Siegel RL (2019) Breast cancer statistics. CA Cancer J Clin 69(6):438–451. https://doi.org/10.3322/caac.21583

Fathy Abd-Ellatef G-E, Gazzano E, Chirio D, Ragab Hamed A, Belisario DC, Zuddas C, Peira E, Rolando B, Kopecka J, Assem Said Marie M, Sapino S, Ramadan Fahmy S, Gallarate M, Zaki Abdel-Hamid A-H, Riganti C (2020) Curcumin-loaded solid lipid nanoparticles bypass P-glycoprotein mediated doxorubicin resistance in triple negative breast cancer cells. Pharmaceutics 12(2):96. https://www.mdpi.com/1999-4923/12/2/96

Feng B, Niu Z, Hou B, Zhou L, Li Y, Yu H (2020) Enhancing triple negative breast cancer immunotherapy by ICG-Templated self-assembly of paclitaxel nanoparticles. Adv Funct Mater 30(6):1906605. https://doi.org/10.1002/adfm.201906605

Finn R, Bengala C, Ibrahim N, Strauss L, Fairchild J, Sy O, Roche H, Sparano J, Goldstein L (2009) Phase II trial of dasatinib in triple-negative breast cancer: results of study. CA180059 69-(2 Supplement):3118. https://doi.org/10.1158/0008-5472.SABCS-3118

Gadeval A, Maheshwari R, Raval N, Kalyane D, Kalia K, Tekade RK (2020) Green graphene nanoplates for combined photo-chemo-thermal therapy of triple-negative breast cancer. Nanomedicine 15(6):581–601. https://doi.org/10.2217/nnm-2019-0380

Gluz O, Liedtke C, Gottschalk N, Pusztai L, Nitz U, Harbeck N (2009) Triple-negative breast cancer–current status and future directions. Ann Oncol 20(12):1913–1927. https://doi.org/10.1093/annonc/mdp492

Huober J, Gelber S, Goldhirsch A, Coates AS, Viale G, Öhlschlegel C, Price KN, Gelber RD, Regan MM, Thürlimann B (2012) Prognosis of medullary breast cancer: analysis of 13 international breast cancer study group (IBCSG) trials. Ann Oncol 23(11):2843–2851. https://doi.org/10.1093/annonc/mds105

Khanna V, Kalscheuer S, Kirtane A, Zhang W, Panyam J (2019) Perlecan-targeted nanoparticles for drug delivery to triple-negative breast cancer. Future Drug Discov 1(1):FDD8. https://doi.org/10.4155/fdd-2019-0005

Kumar P, Aggarwal R (2016) An overview of triple-negative breast cancer. Arch Gynecol Obstet 293(2):247–269. https://doi.org/10.1007/s00404-015-3859-y

Lee SY, Cho H-J (2019) Mitochondria targeting and destabilizing hyaluronic acid derivative-based nanoparticles for the delivery of Lapatinib to triple-negative breast cancer. Biomacromolecules 20(2):835–845. https://doi.org/10.1021/acs.biomac.8b01449

Lehmann BD, Bauer JA, Chen X, Sanders ME, Chakravarthy AB, Shyr Y, Pietenpol JA (2011) Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. J Clin Investig 121(7):2750–2767. https://doi.org/10.1172/JCI45014

Lehmann BD, Pietenpol JA (2014) Identification and use of biomarkers in treatment strategies for triple-negative breast cancer subtypes. J Pathol 232(2):142–150. https://doi.org/10.1002/path.4280

Liao W-S, Ho Y, Lin Y-W, Naveen Raj E, Liu K-K, Chen C, Zhou X-Z, Lu K-P, Chao J-I (2019) Targeting EGFR of triple-negative breast cancer enhances the therapeutic efficacy of paclitaxel- and cetuximab-conjugated nanodiamond nanocomposite. Acta Biomater 86:395–405. https://doi.org/10.1016/j.actbio.2019.01.025

Linderholm BK, Hellborg H, Johansson U, Elmberger G, Skoog L, Lehtiö J, Lewensohn R (2009) Significantly higher levels of vascular endothelial growth factor (VEGF) and shorter survival times for patients with primary operable triple-negative breast cancer. Ann Oncol 20(10):1639–1646. https://doi.org/10.1093/annonc/mdp062

Litzenburger BC, Creighton CJ, Tsimelzon A, Chan BT, Hilsenbeck SG, Wang T, Carboni JM, Gottardis MM, Huang F, Chang JC, Lewis MT, Rimawi MF, Lee AV (2011) High IGF-IR activity in triple-negative breast cancer cell lines and Tumorgrafts correlates with sensitivity to anti–IGF-IR therapy. Clin Cancer Res 17(8):2314–2327. https://doi.org/10.1158/1078-0432.CCR-10-1903

Litzenburger B, Tsimelzon A, Hilsenbeck S, Creighton C, Carboni J, Gottardis M, Wicha M, Chang J, Lewis M, Lee A (2009) High IGF-IR activity in triple-negative breast cancer cell lines correlates with sensitivity to IGF-IR inhibitor BMS-754807 in this subtype of human breast cancer. Cancer Res 69(24 Supplement):1132. https://doi.org/10.1158/0008-5472.SABCS-09-1132

Liu C, Gao H, Zhao Z, Rostami I, Wang C, Zhu L, Yang Y (2019) Improved tumor targeting and penetration by a dual-functional poly(amidoamine) dendrimer for the therapy of triple-negative breast cancer. J Mater Chem B 7(23):3724–3736. https://doi.org/10.1039/C9TB00433E

Martín M, Ruiz A, Borrego MR, Barnadas A, González S, Calvo L, Vila MM, Antón A, Rodríguez-Lescure A, Seguí-Palmer MA, Muñoz-Mateu M, Ribugent JD, López-Vega JM, Jara C, Espinosa E, Fernández CM, Andrés R, Ribelles N, Plazaola A, Sánchez-Rovira P, Bofill JS, Crespo C, Carabantes FJ, Servitja S, Chacón JI, Rodríguez CA, Hernando B, Álvarez I,

Carrasco E, Lluch A (2013) Fluorouracil, doxorubicin, and cyclophosphamide (FAC) versus FAC followed by weekly paclitaxel as adjuvant therapy for high-risk, node-negative breast cancer: results from the GEICAM/2003-02 study. J Clin Oncol 31(20):2593–2599. https://doi.org/10.1200/jco.2012.46.9841

Miles DW, Diéras V, Cortés J, Duenne AA, Yi J, O'Shaughnessy J (2013) First-line bevacizumab in combination with chemotherapy for HER2-negative metastatic breast cancer: pooled and subgroup analyses of data from 2447 patients. Ann Oncol 24(11):2773–2780. https://doi.org/10.1093/annonc/mdt276

Mohammed Siddiq A, Thangam R, Madhan B, Alam MS (2019) Green (gemini) surfactant mediated gold nanoparticles green synthesis: effect on triple negative breast cancer cells. Nano-Struct Nano-Objects 19:100373. https://doi.org/10.1016/j.nanoso.2019.100373

Paulmurugan R, Bhethanabotla R, Mishra K, Devulapally R, Foygel K, Sekar TV, Ananta JS, Massoud TF, Joy A (2016) Folate receptor–targeted polymeric micellar Nanocarriers for delivery of Orlistat as a repurposed drug against triple-negative breast cancer. Mol Cancer Ther 15(2):221–231. https://doi.org/10.1158/1535-7163.MCT-15-0579

Pindiprolu SKSS, Chintamaneni PK, Krishnamurthy PT, Ratna Sree Ganapathineedi K (2019) Formulation-optimization of solid lipid nanocarrier system of STAT3 inhibitor to improve its activity in triple negative breast cancer cells. Drug Dev Ind Pharm 45(2):304–313. https://doi.org/10.1080/03639045.2018.1539496

Saal LH, Holm K, Maurer M, Memeo L, Su T, Wang X, Yu JS, Malmström P-O, Mansukhani M, Enoksson J, Hibshoosh H, Borg Å, Parsons R (2005) PIK3CA mutations correlate with hormone receptors, node metastasis, and ERBB2, and are mutually exclusive with PTEN loss in human breast carcinoma. J Cancer Res 65(7):2554–2559. https://doi.org/10.1158/0008-5472-CAN-04-3913

Shimoi T, Nagai SE, Yoshinami T, Takahashi M, Arioka H, Ishihara M, Kikawa Y, Koizumi K, Kondo N, Sagara Y, Takada M, Takano T, Tsurutani J, Naito Y, Nakamura R, Hattori M, Hara F, Hayashi N, Mizuno T, Miyashita M, Yamashita N, Yamanaka T, Saji S, Iwata H, Toyama T (2020) The Japanese breast cancer society clinical practice guidelines for systemic treatment of breast cancer., 2018 edition. Breast Cancer 27(3):322–331. https://doi.org/10.1007/s12282-020-01085-0

Sikov WM, Berry DA, Perou CM, Singh B, Cirrincione CT, Tolaney SM, Kuzma CS, Pluard TJ, Somlo G, Port ER, Golshan M, Bellon JR, Collyar D, Hahn OM, Carey LA, Hudis CA, Winer EP (2015) Impact of the addition of carboplatin and/or bevacizumab to neoadjuvant once-per-week paclitaxel followed by dose-dense doxorubicin and cyclophosphamide on pathologic complete response rates in stage II to III triple-negative breast cancer: CALGB 40603 (Alliance). Am J Clin Oncol 33(1):13–21. https://doi.org/10.1200/JCO.2014.57.0572

Surapaneni SK, Bashir S, Tikoo K (2018) Gold nanoparticles-induced cytotoxicity in triple negative breast cancer involves different epigenetic alterations depending upon the surface charge. Sci Rep 8(1):12295. https://doi.org/10.1038/s41598-018-30541-3

Timur SS, Yöyen-Ermiş D, Esendağlı G, Yonat S, Horzum U, Esendağlı G, Gürsoy RN (2019) Efficacy of a novel LyP-1-containing self-microemulsifying drug delivery system (SMEDDS) for active targeting to breast cancer. Eur J Pharm Biopharm 136:138–146. https://doi.org/10.1016/j.ejpb.2019.01.017

Tripathy D, Chien AJ, Hylton N, Buxton MB, Ewing CA, Wallace AM, Forero A, Kaplan HG, Nanda R, Albain KS, Moulder SL, Haley BB, DeMichele A, Symmans WF, van't Veer L, Paoloni M, Esserman L, Berry DA, Yee D (2015) Adaptively randomized trial of neoadjuvant chemotherapy with or without the Akt inhibitor MK-2206: graduation results from the I-SPY 2 trial. J Clin Oncol 33(15_suppl):524. https://doi.org/10.1200/jco.2015.33.15_suppl.524

Verbeek BS, Vroom TM, Adriaansen-Slot SS, Ottenhoff-Kalff AE, Geertzema JGN, Hennipman A, Rijksen G (1996) C-Src protein expression is increased in human breast cancer. An immunohistochemical and biochemical analysis. J Pathol 180(4):383–388. https://doi.org/10.1002/(SICI)1096-9896(199612)180:4<383::AID-PATH686>3.0.CO;2-N

Weigelt B, Reis-Filho JS (2009) Histological and molecular types of breast cancer: is there a unifying taxonomy? Nat Rev Clin Oncol 6(12):718–730. https://doi.org/10.1038/nrclinonc.2009.166

Weisenthal L (2009) Activity of cisplatin in triple-negative breast cancer in comparison to other cancer types in fresh tumor cell culture assay using a cell death endpoint. In 2009 breast cancer symposium. Dent Abstr 61:8–10

Wetterskog D, Lopez-Garcia MA, Lambros MB, A'Hern R, Geyer FC, Milanezi F, Cabral MC, Natrajan R, Gauthier A, Shiu K-K, Orr N, Shousha S, Gatalica Z, Mackay A, Palacios J, Reis-Filho JS, Weigelt B (2012) Adenoid cystic carcinomas constitute a genomically distinct subgroup of triple-negative and basal-like breast cancers. J Pathol 226(1):84–96. https://doi.org/10.1002/path.2974

Xu P, Yao J, Li Z, Wang M, Zhou L, Zhong G, Zheng Y, Li N, Zhai Z, Yang S (2020) Therapeutic effect of doxorubicin-chlorin E6-loaded mesoporous silica nanoparticles combined with ultrasound on triple-negative breast cancer. Int J Nanomedicine 15:2659

Yagata H, Kajiura Y, Yamauchi H (2011) Current strategy for triple-negative breast cancer: appropriate combination of surgery, radiation, and chemotherapy. Breast Cancer 18(3):165–173. https://doi.org/10.1007/s12282-011-0254-9

Yan Y, Li X-Q, Duan J-L, Bao C-J, Cui Y-N, Su Z-B, Xu J-R, Luo Q, Chen M, Xie Y, Lu W-L (2019) Nanosized functional miRNA liposomes and application in the treatment of TNBC by silencing slug gene. Int J Nanomedicine 14:3645–3667. https://doi.org/10.2147/IJN.S207837

Yang D, Yao X, Dong J, Wang N, Du Y, Sun S, Gao L, Zhong Y, Qian C, Hong H (2018) Design and investigation of Core/Shell GQDs/hMSN nanoparticles as an enhanced drug delivery platform in triple-negative breast cancer. Bioconjug Chem 29(8):2776–2785. https://doi.org/10.1021/acs.bioconjchem.8b00399

# Effect of Thymoquinone and its Delivery through Using of Nanomedicine in Benign Prostatic Hyperplasia

12

Swati Pandey, Prateek Pathak, Chiagoziem A. Otuechere, Mahfoozur Rahman, and Amita Verma

**Abstract**

Thymoquinone (TQ) is a water-insoluble phytochemical compound (black caraway) isolated from Nigella sativa, which is highly recommended as a carcinogen. The reason for this appearance is the polymeric nanoscale formula, a strong lipid nanoparticle, for TQ to enhance the chitosan nanoparticle to release the demanding conditions of its delivery. Poly-lactide-co-glycolide (PLGA) formulations of TQ-encapsulated nanoparticles (NPs) containing chitosan, pectin, and alginate, which participate in the improved transport across mucous membranes and increase drug shipping. In most cancer cells, TQ is encapsulated in nanoparticles by PEG nanoprecipitation. Every other chemical compound used is polymeric nanoparticle poly-A-caprolactone (PCL) which is a perishable polyester. Formulations are distinguished in terms of their cell length, drug loading performance, and drug launch. Formula Cultured is trying to confirm its antiproliferative effect on human maxillary cancer cellular scars as a potential anticancer nanomedicine. Polymeric NPs may be successfully established a

S. Pandey · A. Verma (✉)
Bioorganic and Medicinal Chemistry Research Laboratory, Department of Pharmaceutical Sciences, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, India
e-mail: amita.verma@shiats.edu.in

P. Pathak
Laboratory of Computational Modeling of Drugs, Higher Medical and Biological School, South Ural State University, Chelyabinsk, Russia

C. A. Otuechere
Department of Biochemistry, Redeemer's University, Ede, Nigeria

M. Rahman
Department of Pharmaceutical Sciences, Faculty of Health Sciences, SHUATS, Allahabad, Uttar Pradesh, India



M. Rahman et al. (eds.), *Hormone Related Cancer Mechanistic and Nanomedicines*,
https://doi.org/10.1007/978-981-19-5558-7_12

nanoscale transport stage layout for TQ which could manipulate its biopharmaceutical limitations boundaries.

**Keywords**

Thymoquinone · Polymeric nanoparticles · PLGA · PCL · Encapsulated nanoparticles · Antiproliferative

## 12.1 Introduction

Benign prostate hyperplasia (BPH) is one of the foremost common diseases in older men which might cause lower tract symptoms (LUTS). Glandular cancer typically grows terribly slowly, and finding and treating it before symptoms occur might not enhance convenience health or facilitate them live longer. Prostate dysplasia grows and varies from man to man as they age, and a prostate enlargement might constrict and compress the duct and cause issues with micturition and urine outcome is decreased (Lerner et al. 2021). BPH might cause tract infections, bladder or excretory organ harm, bladder stones, or incontinence. BPH is that the most typical disorder of the endocrine, and therefore, the most typical identification by urologists for males is between the ages of 50 and 75. More than half of men in their sixties and as several as 90% in their seventies and eighties have some symptoms of BPH.

In the control of inauspicious LUTS, it is critical that tending dealers cognize the multifaceted bladder dynamics, prostate, bladder neck, and canal. Similarly, signs and symptoms might result from the interaction of these organs likewise to the primary device or extraordinary trendy diseases (e.g., metabolic syndrome and symptom heart failure) (Lim 2017).

Testosterone does not act in competition to BHP. The mechanism through manner of that androgenic hormone exerts several of its functional effects on the endocrine through dihydrotestosterone (DHT), an androgenic hormone within the prostate to be able to inspire the growth of cell. Androgen, which incorporates, androgenic hormone, is the rectangular degree made via using the adrenal glands and Leydig cells. When manufacturing, androgenic hormone is circulated through the blood to the endocrine, then center into the cellular via clean diffusion.

Once intra cytoplasmic, the androgenic hormone is regenerated to its activated remember DHT via the accelerator of five alpha enzymes. DHT elaborates with androgenic hormone receptors which can be then conveyed to the nucleus. At intervals within the nucleus, its results to transcription of deoxyribonucleic acid. Those required for improvement in conventional treatment of the endocrine carcinoma, e.g., Benign prostatic hyperplasia (BPH) (BPH 2021).

Thymoquinone may be a natural drug. Its seasoner medicines employed in medical specialty properties against many illnesses several disease TQ effectively transforms the signals of cancer progression. It does not solely improve the antineoplastic activity of therapy medicine however additionally attenuates their facet effects. It reduces the adverse consequences of respiratory disorders, inflammation,

arthritis, gastro, and liver issues. TQ has several attributed clinical specialty moves, as well as an inhibitor, anti-inflammatory effects. It additionally exhibits hepatoprotective, gastroprotective, neuroprotective, and nephroprotective activities (Prostate Cancer–Patient Version 2021).

## 12.2 Benign Prostatic Hyperplasia

### 12.2.1 Cause

- The prostate is found simply at a lower place than the bladder. The prostate is regarding the scale of hazel and adjacent is the canal that passes through the middle of the prostate (the tube that empties pee from the bladder) (Auanet.org 2021).
- Once the prostate is enlarged because of cause benign ductless gland, dysplasia begins to dam pee flow. Most men have continued prostate growth in older age of fifty, up to five-hundredths of men might have histologically prominent BPH with reported generality increasing to ninetieth by age ninety (Jokisch et al. 2017) (Fig. 12.1).
- Prostatic dysplasia is usually caused by an associate degree underlying condition that affects your alteration in sex steroid metabolism.
- Family history is additionally to blame for BHP. Cognate, like a father or a brother, with a prostate downside, means that additional seemingly to transfer that downside.
- In polygenic disorder and cardiopathy use, beta-blockers would possibly increase the danger of BPH.
- Lack of exercise and blubber will increase the danger of BPH.

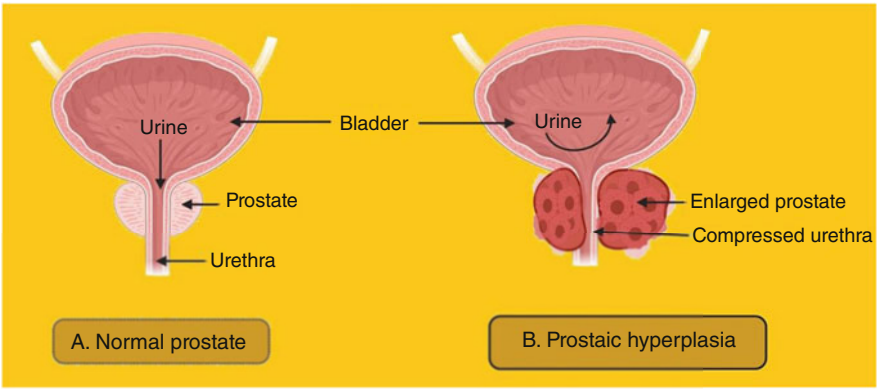



**Fig. 12.1** (**a**) Normal prostate. (**b**) Prostatic hyperplasia

### 12.2.2 Mechanisms of Cause of Benign Prostatic Hyperplasia

- The main mechanism of BPH is increase of ductless gland tissue that takes place in older age, and the endocrine becomes enlarged. Dihydrotestosterone (DHT) is a male secretion that plays a crucial position in prostate growth and improvement. Inhibits or decreases in blood androgenic hormone (testosterone) tiers in older men still turn out and acquire excessive ranges of DHT within the prostate. This accumulation of DHT might encourage prostate cells to nonetheless grow to cause benign ductless gland dysplasia and obstruction of (pee) urine flow (Gravas et al. 2018).
- In men, bladder outlet obstruction has been ascribed to static obstacle, it is because of the enlargement of the prostate incursive upon the ductless gland canal and bladder outlet, whereas the dynamic impediment is said to the stress of prostate sleek muscle.
- Testosterone androgenic hormone directly stimulates androgen-dependent processes. Testosterone (androgenic hormone) converts to DHT by the action of the 5-alpha reductase enzyme (alpha-adrenergic receptors settled at intervals the graceful muscle tone, deteriorating symptoms of obstruction, and retention of urine and declining the urinary flow rate) at these target tissues. DHT is the most potent hormone among the androgens. Testosterone androgenic hormone and DHT both bind to the similar receptor. However, DHT will be additional expeditiously. DHT is transported to focus on cells (postal cell and animal tissue cell). Binding of DHT to androgenic hormone receptor with sequent modulate of sequence. There is an unbalanced proliferation of cell and programmed cell death, because of this reason, abnormal cell growth causes prostate enlargement referred to as benign ductless gland dysplasia [BHP] also known as benign prostate hyperplasia (Symptoms and Overview of BPH 2021) (Fig. 12.2).

### 12.2.3 Symptoms of Benign Hyperplasia

- *Frequency of urination*—A large prostate can place pressure on urinary system, in this condition, not much urine can be held in bladder and cause frequent urination. Urination takes place eight or more times a day.
- *Urinary urgency*—An overactive bladder puts pressure on the urethra to release urine even when bladder is not full.
- *Dribbling*—Urinary incontinence is leaking of urine that is the inability to delay urination. Loss of bladder control that is ordinarily seen in older age. The accidental loss of urine.
- *Kidney damage*—Renal failure is a lower urinary tract indications (LUTS), which occur due to benign prostatic hyperplasia (BPH) in people over the age of 50. Injury to the excretory organs (kidneys) due to obstructive disease occurs sporadically.

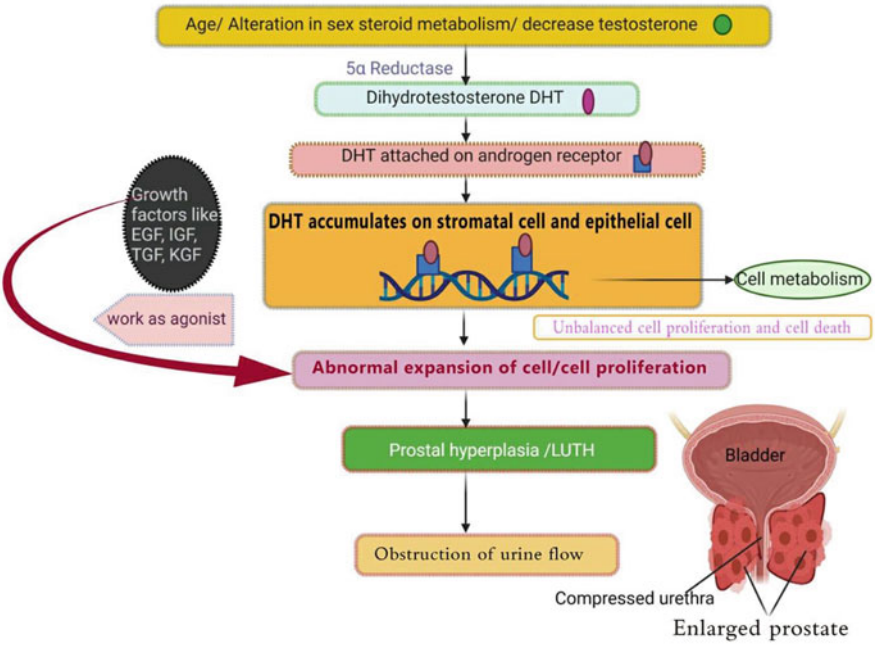



**Fig. 12.2** Mechanism of cause of benign prostatic hyperplasia

- *Bladder stone*—Bladders basically collect urine from kidney until you need to urine ejaculation. Therefore, bladder should be empty due to frequent urination. In some cases, due to the lack of fluids, some of the elements in the urine start to stick together and form the crystals followed by bladder stone.
- *Painful orgasms*—Inflammation is associated with BHP, that is why pain occurs after ejaculation or during urination.
- *UTI*—In benign prostatic hyperplasia, obstruction in urine may inhibit the complete emptying of the bladder, bacterial infection leading to urinary tract infection. Urine that has an unusual color or smell.
- *Blood in the urine*—Due to infection in bladder and kidney in benign prostatic hyperplasia causes hematuria in men.
- *Urinary hesitancy*—Trouble starting a urine stream. Urinary hesitancy is most common in older man due to enlarged prostate.
- *Urinary retention*—An obstruction because of enlarged prostate or bladder stone, a blockage occur, and urine cannot flow unimpeded through urinary tract.
- *Nocturia*—Frequent urination during sleep time. Nocturia is a commonly cause by urinary tract infection or enlargement of prostate or bladder infection or overactive bladder (Gravas et al. 2018; Symptoms and Overview of BPH 2021) (Fig. 12.3).

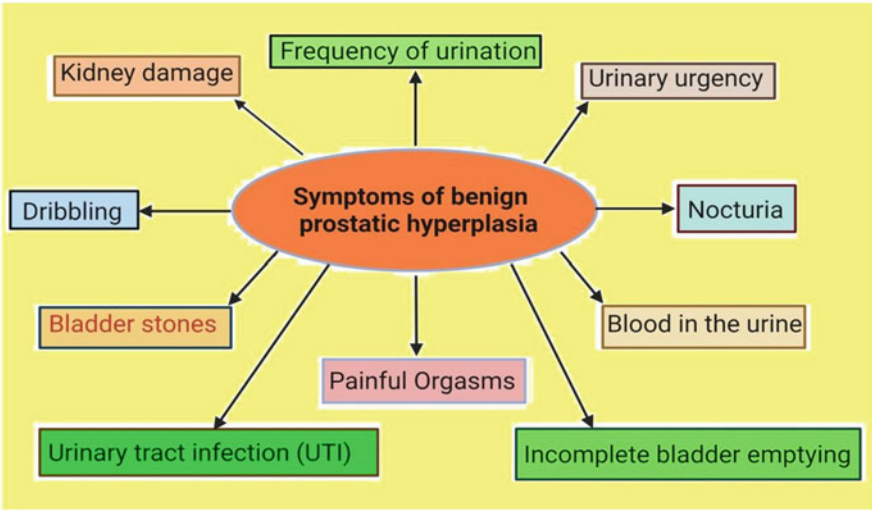
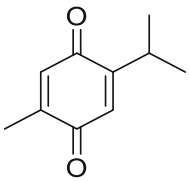



**Fig. 12.3** Symptoms of benign prostatic hyperplasia

## 12.3 Thymoquinone

Bioactive compound derived from the seeds of Nigella sativa is thymoquinone (TQ), commonly known as black cumin. Evidence indicates that the medicinal properties of TQ have been recognized for more than thousands of years. TQ has been shown to exhibit anticancer and anti-inflammatory activities. TQ in nanoparticles is more powerful than TQ in suppressing and inhibiting the proliferation of prostate cancer, colon cancer, breast cancer, etc. (Gupta et al. 2016).

### 12.3.1 Properties

| Structure | Properties |
|---|---|
|  Thymoquinone | Chemical formula: $C_{10}H_{12}O_2$, Molecular weight: 164.20 IUPAC name: 2-methyl-5-isopropyl-1,4- benzoquinone Boiling point: 232.0°C Melting point: 45.5°C (10) |

### 12.3.2 Pharmacology and Mechanism of TQ

Prostatic adenocarcinoma is the most dangerous urinary and genital tumor in men (Gomathinayagam et al. 2020). TQ suppresses the development of prostate adenocarcinoma by decreasing the expression of epithelial-mesenchymal cells (epithelial cell loss) in DU145, PC3, and LNCaP prostatic adenocarcinoma cell lines. Transforming beta growth (TGF-β) that plays a necessary role within the control of animate signal material (Siegel et al. 2019). TQ may be an operative story for the active sites of hemoprotein P450 enzymes, which are thought to be a significant target in the medical care of prostatic adenocarcinoma (Othman et al. 2018; Dirican et al. 2015). TGF-β itself exhibits a complex 0receptor made up of two completely different proteins, a TFT-β type II receptor and a TFT receptor called activin receptor-like 5-alpha reductase (kinase enzyme five), ALK-5, each expressed in a cell type. TQ suppressed carcinogenesis of prostate cancer at forty-five units of weight by downregulating IL-6 expression, phosphorylation of STAT3, AKT, and protein-regulated enzyme (ERK) proteins in PC3 prostatic adenocarcinoma (Ma et al. 2017; Das et al. 2012).

As a naturally occurring chemical, TQ allows a non-enzymatic chemical plagiarism reaction (reduction followed by oxygen oxidation) to be accelerated, resulting in the antioxidant-like release of antioxidants (Bolton et al. 2000; Mansour et al. 2002). TQ performs its antitumor function by modifying the profile-like expression of key communication skeletons involved in the growth and development of neoplastic cells. TQ has antitumor function. Thus, its regulation of inflammatory pathways participated in cell proliferation, survival, death, invasive metastasis, and inflammatory pathways via multidrug/epigenetic regulation. LNCaP glandular carcinoma cells treated with TQ show a decrease in E2F-1 secretion of controlled proteins and cyclin A with a G1/S component of cell cycle binding (Kaseb et al. 2007). In metastatic tumor cells, T2 cell cycle binding caused by TQ is associated with the excretion of an increasing number of nuclear cells, cyclin B1, and CDK1 alongside expanded expression of p53 and p21 (Paramasivam et al. 2015).

### 12.3.3 Other Combination of Drug

Combination drug therapy is most widely used in all over the world. Combination drugs have many advantages like increased compliance, synergy, enhanced activity, and increased efficacy. Some combination drugs and disease treated are shown in Table 12.1.

## 12.4 Nanomedicines of TQ

“Nanomedicine is that the branch of drugs. There’s no nanomedicine, there’s applied science in medication. Although the demonstration or expression “nanomedicine” has been widely used for some of the years, it’s additional correct to talk to

**Table 12.1** Combination of other drugs with TQ

| S. No. | Combination of drug | Disease treated | References |
|---|---|---|---|
| 1 | TQ + CYS (cyclophosphamide) | Rheumatoid arthritis, Breast cancer | Pal et al. (2021) |
| 2 | TQ + IM | Colorectal cancer | Imran et al. (2018) |
| 3 | TQ + 5FU (fluorouracil) | Colorectal, gastric, Nasopharyngeal cancer | Imran et al. (2018); El-Shemi et al. (2016) |
| 4 | TQ+ DTX (docetaxel) | Prostate and breast cancer | Singh et al. (2019); Alkhatib et al. (2020) |
| 5 | TQ+ ZA (Zoledronic acid) | Prostate cancer | Dirican et al. (2014) |
| 6 | TQ+ TMZ (Temozolomide) | Glioblastoma | Pazhouhi et al. (2018) |
| 7 | TQ+ CDDP (cisplatin) | Esophageal, gastric, lung, ovarian, oral, squamous cancer | Jafri et al. (2010); Wang et al. (2019) |
| 8 | TO+PAC (paclitaxel) | Breast cancer | Şakalar et al. (2016) |
| 9 | TQ + BTZ (Bortezomib) | Myeloma | Şakalar et al. (2016) |
| 10 | TQ+ TP (Topotecan) | Leukemia colon cancer | Fatfat et al. (2021); Mostofa et al. (2017) |
| 11 | TQ+ TAM (doxorubicin) | Breast cancer | Khalife et al. (2016) |
| 12 | TQ+ DOX (doxorubicin) | Leukemia, breast, liver cancer | Rajput et al. (2013); Effenberger-Neidnicht and Schobert (2011); Jehan et al. (2020) |
| 13 | TQ + AS (arsenic trioxide) | Leukemia | Houssein et al. (2020) |

"nanotechnology-enabled medication" in numerous medicines like medical specialty, treatment, or observation" (Vinogradov and Wei 2012).

Nanomedicine could be a moderately new field of science and technology "nanotechnology-enabled medication" in various drugs like medical science, treatment, or observation." It is often vague and the exact definition of the word may vary, especially between Europe and the U.S. By interacting with living molecules, thus in nanoscale, applied science expands the broad field of analysis and application. The interaction between cells produced by cells or nanodevices and biomolecules is often understood individually within body fluids and the protoplasm of human cells. The application of nanoscale allows the escape of visible structures that are completely different from those expressed on a small scale such as surface quantitative relation. Tested diagnostic applications are often considered in vitro additionally as in vivo diagnostics.

The second area that shows strong training is "nanomedicines," where nanoparticles are engineered to deliver targeted drugs. The use of such carriers facilitates drug delivery, directing active molecules to diseased tissues while

preserving healthy tissues. A third application area of regenerative medicine, where nanotechnology enables the creation of biocompatible compounds that promote cell proliferation, is used in cell therapy. The use of nanotechnology in medicine poses new problems because of the new applications they allow, if possible: Can the medical field harness the power of this new experiment? Does this mean treating a patient with no clinical symptoms? Nanomedicine can provide the loading of individual drugs for diagnosis, imaging, and drug delivery for the management of disease (Shah et al. 2011).

The most commonly used components of nanomedicine are as follows:

- Delivery of drug combinations.
- In vitro and in vivo diagnostic equipment, including imaging.
- Rejuvenating (regenerative) medicine.
- Implant medical equipment.

### 12.4.1 Type of TQ Nanoparticles

#### 12.4.1.1 Polymeric Nanoparticle of TQ PLGA and β-Cyclodextrin Nanoparticles

The nanoprecipitation method used to immobilize the structure of macromolecular nanoparticles has several advantages, which make this suitable for the optimal drug delivery. Nanoparticles are lesser in size than cells, steady in blood stream, and non-toxic in nature, which also promote decomposition and regulate drug delivery. Poly-lactide-coglycolide (PLGA) nanoparticles are the most abundant polymer blends (Saghir et al. 2021) used in the field of nanomedicine. Addition of PLGA with pectin, chitosan, and alginate can improve mobility across all mucosal barriers and improve drug delivery. Polyethylene glycol (PEG) is a water-insoluble and non-toxic polymeric nanoparticle, which is used in a combination and protect certain drugs, such as doxorubicin (Nallamuthu et al. 2013). TQ is incorporated into PEG nanoparticles by nanoprecipitation in cancer cells [43)]. The formulation made by PEG also acts as a safe transporter of various hydrophobic drugs, such as TQ, which have nontoxic effects on rat hippocampal neurons with a drug loading capacity of 97.5%. Therefore, it may be useful for the interpretation of clinical TQ (Gali-Muhtasib et al. 2006). The nanoprecipitation method was developed for the preparation of mixed states of nanoparticles and has many advantages, suitable for drug delivery. The localized nanoparticle unit is cellular, blood-stable, non-toxic, promotes biodegradability, and regulates drug delivery (Sunoqrot et al. 2020). PLGA nanoparticle is an amazing synthetic claim used in the field of nanomedicine. The glycol synthetic resin (Notch) is a localized unit of nanoparticle, which is a clear and non-toxic compound (Saghir et al. 2021). Notch is used to regenerate and protect certain drugs, such as antibiotics (Bhattacharya et al. 2015). TQ was reconstituted in Notch nanoparticles by nanoprecipitation, in cancer cells (Gali-Muhtasib et al. 2006). Another nanoparticle used is polyεcaprolactone (PCL), which is a perishable

polyester. What else, cyclodextrin (CD) is used for two-fold encapsulation, which is an external hydrophilic unit and an internal hydrophobic (Nallamuthu et al. 2013). The CD is low in toxicity, prevents conjugated physic degradation, and is supplied in several sizes. The natural integration style is used to combine TQ with CD, where TQ mixed with bCD with direct volume correlation (4 TQ1 CD) developed a TQ lot that inhibits proliferation in a time-dependent manner; inhibitory concentration (IC50) is estimated to be five times greater compared to free TQ (Abu-Dahab et al. 2013; Cardoso et al. 2012; Omtvedt et al. 2021).

#### 12.4.1.2 Solid Lipid Nanoparticle of TQ

Solid lipid nanoparticles are lipid nanoparticles with specific potential for application in drug delivery, which, based on the rapidly evolving field of nanotechnology and its size-dependent properties, offer an opportunity for development of new treatment modalities (Mukherjee et al. 2009).

Different types of solid lipid-based nanocarriers, such as solid lipid-based nanoparticles, nanostructured carriers, and drug conjugates, are deliberated along with their structural variances. SLNs offer distinctive features such as lesser size, bulky surface area, maximum drug loading and step-by-step interactions at interfaces, and the ability to increase the activity of pharmaceuticals, nutraceuticals, and other materials (Ramachandran and Thangarajan 2016).

Liposomes, first prototype lipid-based carriers, labeled as non-toxic, synthetic, supple, and fully degradable. (Mukherjee et al. 2009) They mainly consist of phospholipid bilayers, phosphatidylcholine, and phosphatidylethanolamine. Liposomes also consist other bilayer components such as cholesterol and lipophobic polymers surround each lipid vesicle.

The cytotoxicity of 1,2-dipalmitoylsnglycerol3phosphocholine phospholipids against T47D and MCF7 cancers was induced using a 90% wrapping efficiency using a conventional thin-layer hydration technique (Fig. TQLP). The cell line has little effect on normal cells (Scioli Montoto et al. 2020).

They have promising characteristics such as biodegradability, biocompatibility, and non-toxic nature. TQ assay in overexpressed active MCF7 cells, tamoxifen resistant MCF7 (TAM) cells. Furthermore, TQ and Akt siRNA (siRNANioAuTQ) with a TQ loading efficiency of 82% and exerted higher in vivo effect in the BALB/ xenograft murine model of MCF7/TAM (Sarangi et al. 2019). In all experiments, TQ nanoparticles showed better efficacy than free TQ in reducing cellular ACT levels and significantly reducing tumor mass and volume. Research by vivo showed that TQSLN increased five times in rat plasma compared with free TQ after administration. On the other hand, during in vitro studies, the TQSLN formulation exhibits controlled drug release with a 70% maximum release as compared to 47% for free TQ. In addition, TQ concentrations were increased with SLN administration in organs such as the heart, lungs, prostate, spleen, brain, liver, and kidneys (Mukherjee et al. 2009; García-Pinel et al. 2019). However, inorganics such as gold and silica based NP-TQs have also been investigated for enhanced antitumor activity.

#### 12.4.1.3 Chitosan-Based TQNP

Chitosan consists higher degradation compatibility. Therefore, it is one of the most widely used natural biofilm forming agents in the pharmaceuticals. These rights have led to them being used in compiling nanoparticles for medicine over the past 20 years. Incorporating compounds with completely diverse phases (hydrophilic and hydrophobic compounds) in a chitosan carrier can be difficult due to the hydrophobic nature of chitosan (de Sousa et al. 2020). The use of L-ascorbic acid (LAA) and thymoquinone (TQ) effective as antioxidant compounds with poor absorption and limited bioavailability. Nanoparticles (NPs) have solved this problem by acting as a charge carrier for them because they need higher levels (Naskar et al. 2019).

#### 12.4.1.4 Thymoquinone Loaded Gold Nanoparticle

Gold nanoparticles (AuNPs) may be used as nanocarriers (nanomaterials being employed as a transport medicament) for corrective to inhibit its toxic properties and enhance the healing property. Thymoquinone (TQ) has anti-tumor properties and thymoquinone with AuNPs will increase and enhances its conditioning against anti-cancer or tumors. The combined treatment of thymoquinone-containing AuNPs increases tumor-regulating and immunomodulatory potential. AuNPs will accumulate in cancer cells and exhibit ocular diffusion. Therefore, these nanoparticles will play a vital role as collaborators in the study of carcinoma cells. These nanoparticles are used to identify cancers. In a general sense, the identification of specific cells, organs, and tissues has become a central question and a challenge. Drug delivery systems are a promising substitute to orthodox chemotherapy that can provide truly targeted delivery and modulate a range of biochemical barriers in the body such as the blood barrier and brain. Large amounts of AuNPs are used to treat cancer to speed up blood capillaries. The small size of AuNPs should be avoided due to trappy macrophages within the reticuloendothelial system (RES). The prepared or complete size of the AuNPs used in the treatment of cancer is recommended to be approximately 50–100 nm to gather in the tumor tissue to increase the efficiency of "improved accessibility and retention (EPR)" (Song et al. 2013; Gomaa et al. 2018).

## 12.5 Conclusion

TQ is the main bioactive constituent in *N. Sativa* that has been intensively shown to own many therapeutic properties, together with malignant neoplasm (anticancer) activity. Its effectiveness on nanoparticles of TQ enhances higher survival rates, reduced tumor volume, reduced pre-cancerous molecules, and elevated anti-tumorigenesis in vitro studies; TQ has shown the power to inhibit cancer staging like migration, proliferation, and invasion or cell death induction by inhibiting the activation of significant pathways, like JAK/STAT and PI3K/AKT/mTOR. However, the TQ impact mechanism on cancers continues to be not absolutely understood. It is significant to mention that the introduction of dangerous carcinogens (tyrosine kinase inhibitors (TKIs)) represents a revolution in anticancer medication. TQ has been incorporated into lipid polymeric nanoparticles (LPNPs) composed of

phosphatidylcholine (PC) and PLGA to enhance oral TQ delivery and malignancy. AuNPs suppress the progression of ductless gland dysplasia in a size-dependent manner. AuNPs suppress the progression of ductless gland dysplasia in a size-dependent manner. Although 20 nm AuNPs ameliorate non-ductal benign glandular hyperplasia with the effect of reducing prostate cell proliferation, inflammation, and maturation, 50 nm AuNPs can certainly exacerbate the incidence of prostate cancer, hyperplasia, mainly by enhancing the inflammatory process. It can be concluded that NPs supported the perishable polymer mPEG-PCL can be a nanoscale delivery system for the TQ. The optimum TQ NP relies on the mPEG-PCL polymer with the larger PCL MW. However, the nanoparticles formulation enhances the antitumor efficiency compared to the free drug of TQ.

## References

Abu-Dahab R, Odeh F, Ismail SI, Azzam H, Al BA (2013) Preparation, characterization and antiproliferative activity of thymoquinone-beta-cyclodextrin self assembling nanoparticles. Pharmazie 68(12):939–944

Alkhatib MH, Bawadud RS, Gashlan HM (2020) Incorporation of docetaxel and thymoquinone in borage nanoemulsion potentiates their antineoplastic activity in breast cancer cells. Sci Rep 10(1):18124

Auanet.org. [cited 2021 Oct 17]. Available from: https://www.auanet.org/documents/education/clinical-guidance/Benign-Prostatic-Hyperplasia.pdf

Benign Prostatic Hyperplasia (BPH) Guideline–American Urological Association [Internet]. Auanet.org. [cited 2021 Oct 17]. Available from: https://www.auanet.org/guidelines/guidelines/benign-prostatic-hyperplasia-(bph)-guideline

Bhattacharya S, Ahir M, Patra P, Mukherjee S, Ghosh S, Mazumdar M et al (2015) PEGylated-thymoquinone-nanoparticle mediated retardation of breast cancer cell migration by deregulation of cytoskeletal actin polymerization through miR-34a. Biomaterials 51:91–107

Bolton JL, Trush MA, Penning TM, Dryhurst G, Monks TJ (2000) Role of quinones in toxicology. Chem Res Toxicol 13(3):135–160

Cardoso T, Galhano CIC, Ferreira Marques MF, Moreira da Silva A (2012) Thymoquinone-β-Cyclodextrin nanoparticles system: a preliminary study. Spectrosc Int J 27:329–336

Das S, Dey KK, Dey G, Pal I, Majumder A, MaitiChoudhury S et al (2012) Antineoplastic and apoptotic potential of traditional medicines thymoquinone and diosgenin in squamous cell carcinoma. PLoS One 7(10):e46641

de Sousa VR, da Cunha M, Santos A, Viana de Sousa B, de Araújo NG, de Lima N, Santana L, Rodrigues MR (2020) A review on chitosan's uses as biomaterial: tissue engineering, drug delivery systems and cancer treatment. Materials (Basel) 13(21):4995

Dirican A, Atmaca H, Bozkurt E, Erten C, Karaca B, Uslu R (2015) Novel combination of docetaxel and thymoquinone induces synergistic cytotoxicity and apoptosis in DU-145 human prostate cancer cells by modulating PI3K-AKT pathway. Clin Transl Oncol 17(2): 145–151

Dirican A, Erten C, Atmaca H, Bozkurt E, Kucukzeybek Y, Varol U et al (2014) Enhanced cytotoxicity and apoptosis by thymoquinone in combination with zoledronic acid in hormone- and drug-resistant prostate cancer cell lines. J BUON 19(4):1055–1061

Effenberger-Neidnicht K, Schobert R (2011) Combinatorial effects of thymoquinone on the anti-cancer activity of doxorubicin. Cancer Chemother Pharmacol 67(4):867–874

El-Shemi A, Kensara O, Mohamed A, Refaat B, Idris S, Ahmad J (2016) Thymoquinone subdues tumor growth and potentiates the chemopreventive effect of 5-fluorouracil on the early stages of colorectal carcinogenesis in rats. Drug Des Devel Ther 10:2239–2253

Fatfat Z, Fatfat M, Gali-Muhtasib H (2021) Therapeutic potential of thymoquinone in combination therapy against cancer and cancer stem cells. World J Clin Oncol 12(7):522–543
Gali-Muhtasib H, Roessner A, Schneider-Stock R (2006) Thymoquinone: a promising anti-cancer drug from natural sources. Int J Biochem Cell Biol 38(8):1249–1253
García-Pinel B, Porras-Alcalá C, Ortega-Rodríguez A, Sarabia F, Prados J, Melguizo C, López-Romero JM (2019) Lipid-based nanoparticles: application and recent advances in cancer treatment. Nano 9(4):638
Gomaa S, Aboshafey A, Aladawy A (2018) Combination treatment of thymoquinone-loaded gold nanoparticles and cisplatin potentiates anti-tumor activity and immunomodulatory effects in breast cancer model. Egypt J Exp Biol Zool 14(1):21
Gomathinayagam R, Ha JH, Jayaraman M, Song YS, Isidoro C, Dhanasekaran DN (2020) Chemopreventive and anticancer effects of thymoquinone: cellular and molecular targets. J Cancer Prev 25(3):136–151
Gravas S, Cornu JN, Drake MJ, Gacci M, Gratzke C, Herrmann TRW (2018) Management of non-neurogenic male lower urinary tract symptoms LUTS. Limited update
Gupta B, Ghosh KK, Gupta RC (2016) Thymoquinone. In: Nutraceuticals. Elsevier, pp 541–550
Houssein M, Fatfat M, Habli Z, Ghazal N, Moodad S, Khalife H et al (2020) Thymoquinone synergizes with arsenic and interferon alpha to target human T-cell leukemia/lymphoma. Life Sci 251(117639):117639
Imran M, Rauf A, Khan IA, Shahbaz M, Qaisrani TB, Fatmawati S et al (2018) Thymoquinone: a novel strategy to combat cancer: a review. Biomed Pharmacother 106:390–402
Jafri SH, Glass J, Shi R, Zhang S, Prince M, Kleiner-Hancock H (2010) Thymoquinone and cisplatin as a therapeutic combination in lung cancer: in vitro and in vivo. J Exp Clin Cancer Res 29(1):87
Jehan S, Zhong C, Li G, Zulqarnain Bakhtiar S, Li D, Sui G (2020) Thymoquinone selectively induces hepatocellular carcinoma cell apoptosis in synergism with clinical therapeutics and dependence of p53 status. Front Pharmacol 11:555283
Jokisch J-F, Herlemann A, Weinhold P, Magistro G, Stief CG, Gratzke C (2017) Management of benign prostatic hyperplasia (BPH). MMW Fortschr Med 159(9):58–64
Kaseb AO, Chinnakannu K, Chen D, Sivanandam A, Tejwani S, Menon M et al (2007) Androgen receptor and E2F-1 targeted thymoquinone therapy for hormone-refractory prostate cancer. Cancer Res 67(16):7782–7788
Khalife R, Hodroj M, Fakhoury R, Rizk S (2016) Thymoquinone from Nigella sativa seeds promotes the antitumor activity of noncytotoxic doses of Topotecan in human colorectal cancer cells in vitro. Planta Med 82(04):312–321
Lerner LB, McVary KT, Barry MJ, Bixler BR, Dahm P, Das AK et al (2021) Management of lower urinary tract symptoms attributed to benign prostatic hyperplasia: AUA GUIDELINE PART I-initial work-up and medical management. J Urol 206(4):806–817
Lim KB (2017) Epidemiology of clinical benign prostatic hyperplasia. Asian J Urol 4(3):148–151
Ma J, Hu X, Li J, Wu D, Lan Q, Wang Q et al (2017) Enhancing conventional chemotherapy drug cisplatin-induced anti-tumor effects on human gastric cancer cells both in vitro and in vivo by Thymoquinone targeting PTEN gene. Oncotarget 8(49):85926–85939
Mansour MA, Nagi MN, El-Khatib AS, Al-Bekairi AM (2002) Effects of thymoquinone on antioxidant enzyme activities, lipid peroxidation and DT-diaphorase in different tissues of mice: a possible mechanism of action. Cell Biochem Funct 20(2):143–151
Mostofa AGM, Hossain MK, Basak D, Bin Sayeed MS (2017) Thymoquinone as a potential adjuvant therapy for cancer treatment: evidence from preclinical studies. Front Pharmacol 8:295
Mukherjee S, Ray S, Thakur RS (2009) Solid lipid nanoparticles: a modern formulation approach in drug delivery system. Indian J Pharm Sci 71(4):349–358
Nallamuthu I, Parthasarathi A, Khanum F (2013) Thymoquinone-loaded PLGA nanoparticles: antioxidant and anti-microbial properties. Int Curr Pharm J 2(12):202–207
Naskar S, Koutsu K, Sharma S (2019) Chitosan-based nanoparticles as drug delivery systems: a review on two decades of research. J Drug Target 27(4):379–393

Omtvedt LA, Kristiansen KA, Strand WI, Aachmann FL, Strand BL, Zaytseva-Zotova DS (2021) Alginate hydrogels functionalized with β-cyclodextrin as a local paclitaxel delivery system. J Biomed Mater Res A 109(12):2625–2639

Othman N, Masarudin M, Kuen C, Dasuan N, Abdullah L, Md. Jamil S. (2018) Synthesis and optimization of chitosan nanoparticles loaded with L-ascorbic acid and thymoquinone. Nanomaterials (Basel) 8(11):920

Pal RR, Rajpal V, Singh P, Saraf SA (2021) Recent findings on thymoquinone and its applications as a nanocarrier for the treatment of cancer and rheumatoid arthritis. Pharmaceutics 13(6):775

Paramasivam A, Raghunandhakumar S, Priyadharsini JV, Jayaraman G (2015) In vitro anti-neuroblastoma activity of thymoquinone against neuro-2a cells via cell-cycle arrest. Asian Pac J Cancer Prev 16(18):8313–8319

Pazhouhi M, Sariri R, Khazaei MR, Moradi MT, Khazaei M (2018) Synergistic effect of temozolomide and thymoquinone on human glioblastoma multiforme cell line (U87MG). J Cancer Res Ther 14(5):1023–1028

Prostate Cancer–Patient Version [Internet]. Cancer.gov. [cited 2021 Oct 17]. Available from: https://www.cancer.gov/types/prostate

Rajput S, Kumar BNP, Sarkar S, Das S, Azab B, Santhekadur PK et al (2013) Targeted apoptotic effects of thymoquinone and tamoxifen on XIAP mediated Akt regulation in breast cancer. PLoS One 8(4):e61342

Ramachandran S, Thangarajan S (2016) A novel therapeutic application of solid lipid nanoparticles encapsulated thymoquinone (TQ-SLNs) on 3-nitroproponic acid induced Huntington's disease-like symptoms in wistar rats. Chem Biol Interact 256:25–36

Saghir SAM, Al-Gabri NA, Ali AA, Al-Attar A-SR, Al-Sobarry M, Al-Shargi OYA et al (2021) Ameliorative effect of thymoquinone-loaded PLGA nanoparticles on chronic lung injury induced by repetitive intratracheal instillation of lipopolysaccharide in rats. Oxidative Med Cell Longev 2021:5511523

Şakalar Ç, İzgi K, İskender B, Sezen S, Aksu H, Çakır M et al (2016) The combination of thymoquinone and paclitaxel shows anti-tumor activity through the interplay with apoptosis network in triple-negative breast cancer. Tumour Biol 37(4):4467–4477

Sarangi B, Jana U, Palei NN, Mohanta GP (2019) Solid lipid nanoparticles: a potential approach for drug delivery system. Nanoscience Nanotechnol Asia 9(ue 2)

Scioli Montoto S, Muraca G, Ruiz ME (2020) Solid lipid nanoparticles for drug delivery: pharmacological and biopharmaceutical aspects. Front Mol Biosci 7:587997

Shah M, Choi MH, Ullah N, Kim MO, Yoon SC (2011) Synthesis and characterization of PHV-block-mPEG diblock copolymer and its formation of amphiphilic nanoparticles for drug delivery. J Nanosci Nanotechnol 11(7):5702–5710

Siegel RL, Miller KD, Jemal A (2019) Cancer statistics, 2019: cancer statistics, 2019. CA Cancer J Clin 69(1):7–34

Singh SK, Apata T, Gordetsky JB, Singh R (2019) Docetaxel combined with thymoquinone induces apoptosis in prostate cancer cells via inhibition of the PI3K/AKT signaling pathway. Cancers (Basel) 11(9):1390

Song K, Xu P, Meng Y, Geng F, Li J, Li Z et al (2013) Smart gold nanoparticles enhance killing effect on cancer cells. Int J Oncol 42(2):597–608

Sunoqrot S, Alfaraj M, Hammad AM, Kasabri V, Shalabi D, Deeb AA et al (2020) Development of a thymoquinone polymeric anticancer nanomedicine through optimization of polymer molecular weight and nanoparticle architecture. Pharmaceutics. 12(9):811

Symptoms & Overview of BPH [Internet]. Enidurologyassociates.com. [cited 2021 Oct 17]. Available from: https://www.enidurologyassociates.com/bph-benign-prostatic-hyperplasia/what-is-bph

Vinogradov S, Wei X (2012) Cancer stem cells and drug resistance: the potential of nanomedicine. Nanomedicine 7(4):597–615

Wang X, Li H, Shi J (2019) LncRNA HOXA11-AS promotes proliferation and cisplatin resistance of oral squamous cell carcinoma by suppression of miR-214-3p expression. Biomed Res Int 2019:8645153

# Concept of Nanomedicine in Endocrine Hormone Cancer Treatment

13

Ashish Dhiman, Aman Pareek, and Rahul Shukla

**Abstract**

Cancer is the deadliest disease that led to the death of millions of people around the world. Endocrine cancer is one of the most common types of cancer and badly affects the endocrine system of the patient. Currently, due to the alarming increase in the endocrine cancer patients, there is a need for nanotechnology like therapeutic options to treat the endocrine cancer. This chapter describes about the steroid-based hormone receptors in the cancer biology and remedies like hormone-based therapy to target the different forms of cancers. However, the nanomedicines could be the ideal strategy to treat the endocrine cancer using different forms of nanoproducts. The chapter also briefs about different types of drugs approved for cure of different types of cancer along with their recent status in the market. There is numerous research available in the field of hormone-based treatment for cancer through use of nanotechnology but still no milestone is established.

**Keywords**

Cancer · Nanomedicines · Hormone · Endocrine

A. Dhiman · A. Pareek · R. Shukla (✉)
Department of Pharmaceutics, National Institute of Pharmaceutical Education and Research-Raebareli, Lucknow, India
e-mail: rahul.shukla@niperraebareli.edu.in



M. Rahman et al. (eds.), *Hormone Related Cancer Mechanistic and Nanomedicines*,
https://doi.org/10.1007/978-981-19-5558-7_13

## 13.1 Introduction

Cancer is one of the humanity's most challenging diseases, and it is increasingly becoming a worldwide health issue. Localized malignancies respond well to surgery and radiation therapy, but only chemotherapy can stop the disease from spreading throughout the body. Nanomedicine has progressed in recent years to become a game-changing tool for the treatment and diagnosis of several types of cancers (Feinberg 2004). Scientists are trying to find ligand-based targeted therapeutic techniques with the aid of the conjugation of different drug therapies with a variety of MAbs (monoclonal antibodies) or the ligands that are selectively bound to antigens and receptors on the surface of the tumour cells (Coulson et al. 2014). Despite the fact that this approach outperforms chemotherapy in preclinical and clinical trials, for instance, problems like limited targeted selectivity and potency must need to be considered.

According to statistics, approximately eight million people across the globe and 5,00,000 people in the United States die each year because of the deadly and dreadful disease called "cancer" (Lozano et al. 2012). The chances of cancer occurrence increase with age, and studies prove that it will affect almost 22 million people by the year 2030. Cancer is a disease produced by the spontaneous and regular fragmentation of aberrant cells inside the body. Tumours can be solid or filled with liquid, and in some situations, they can progress to malignancy. It is crucial to understand that not every tumour is cancerous. In general, abnormal tissue growth defines one of the three conditions: malignant, pre-malignant, or benign. According to biopsies, a benign tumour is not a carcinogenic type, and it will not be able to penetrate neighbouring tissues. If removed, it will not relapse, and it will remain confined to its original location without migrating to nearby tissues (Hakim et al. 2015). Haemangiomas, a benign tumour known as strawberry markings, showed an increase in blood. The premalignant type of tumour requires constant monitoring since it has the capability to mature into proper cancer. The malignant form of tumours develops and spreads quickly, attacking neighbouring cells and tissues by making entry into the bloodstream (Lin and Pollard 2007). This is known as the metastatic cancer and proved to be a devastating disease that puts patients' lives in danger. The basic difference between the tumour and cancer is that the tumour is the uncontrolled growth of cells and cancer is the irregular development in the growth of the cells that can be migrated to other parts of the body and damage the surrounding tissues (Loree et al. 2018). The movement of the cells from the specific place of the body to distant regions of organs is known as metastasis. Tobacco use, radiation, loss of daily physical activities, several infections, and obesities are all known to raise the risk of cancer. Nanomedicines are helpful in cancer due to their superior physicochemical and pharmacokinetic features. Nanoparticles, unlike traditional cancer treatments, have several characteristics that put them on a superior list for oncological applications.

The endocrine system is a type of messenger system in which the internal glands secrete hormones in an organism directly into the blood system of circulation. These hormones provide feedback loops that help in the regulation of the targeted organs

residing at distant sites (Lauretta et al. 2019). The adrenal glands and thyroid gland are the principal glands of the human endocrine system. The hypothalamus is the brain activity control center for all endocrine systems. Endocrine glands produce hormones directly into interstitial spaces, where they are absorbed into the bloodstream, irrespective of the duct so called as ductless glands. The pancreas, adrenal glands, pituitary gland, ovaries, parathyroid gland, testes, hypothalamus, pineal gland, and thyroid gland are the primary glands of the endocrine system (Hiller-Sturmhöfel and Bartke 1998). Neuroendocrine organs include the hypothalamus and pituitary gland. Mineralocorticoids, glucocorticoids, and androgens are the three types of steroid hormones produced by the adrenal cortex. Mineralocorticoids (like aldosterone) produced in the zona glomerulosa assist regulate blood pressure and electrolyte balance. In the zona fasciculata, the glucocorticoids cortisol and cortisone are generated, which aid in metabolic regulation and immune system inhibition (Rosol et al. 2001). Three hormones, namely, calcitonin (peptide hormone), T4 (thyroxine), and T3 (triiodothyronine) are released by the thyroid gland. These three hormones of the thyroid gland control the growth and development in children and plays a role in the synthesis of proteins and basal metabolic rate. Homeostasis of the calcium ions is aided with the help of the calcitonin hormone. Thyroid cancer is a very prevalent form of endocrine cancer type and exists in many forms (Cabanillas et al. 2016). Nanomedicine solves biopharmaceutical issues associated with traditional therapies, such as lack of selectivity, drug water solubility, and multidrug resistance (MDR), which results from the repeated administration of the same drug.

Nanotechnology deals with the deliberate creation of substances for practical use in all sectors of sciences by the cutting edge shape and size of the substances based upon the nano-size range of 1–100 nm (Ehdaie 2007). Multi-modular treatment can be obtained by modifying the surface of these nanoparticles (NPs) with diverse moieties such as ligands. Furthermore, the form of NPs has a significant impact on medication uptake and viability in cells. Because of these unique properties, NPs have attracted huge studies in the field of biomedical sciences as a means of transporting cancer therapies (Misra et al. 2010). Nanotechnology-dependent drug delivery systems have improved pharmacokinetic properties, for example, high volume of distribution, good bioavailability to the cancerous cells, and increased clearance value as a consequence of raised enhanced permeation and retention (EPR) effect (Greish 2010). Furthermore, nanomedicine-based techniques would assess molecular alterations and serve as a vital tool for transferring treatments across the biological domains. Nanoparticles having 5.5 nm hydrodynamic diameter can clear the renal track without causing negative effects (Choi et al. 2010). Nanocarriers are appealing because of the safe administration of medications to prevent non-targeted cells and tissues from major unwanted symptoms. The use of nanoparticles as effective carriers is their ease of modification and regarded as distinguished property of NPs. To reduce unwanted side effect and optimize proper response opposite to malignant cells nanocarriers are effective for such aid. However, precise targets, as well as nanocarriers, must be wisely selected. The cure can deposit the medicine in malignant cells by the leaky vasculature with the aid of increased EPR effect (Greish 2010). Furthermore, it becomes crucial to determine the ability of the nanoparticles

as well as the qualities of medicines to predict their capability to cross the biological membrane, their release, and biocapability to deliver the desired medicine to a specific spot.

## 13.2 Steroid-Based Hormone Receptors in Cancer Biology

Several types of human cancers are affected by steroid hormones throughout their formation and proliferation. As a result, altering the endocrine milieu is an effective palliative treatment for hormone-dependent cancer patients. Many essential cell regulators are mediated by steroid hormone receptors (SHRs), which are intracellular transcription factors that are dependent on the ligand. SHRs are ligand stimulated transcription factors that include receptors for GR (glucocorticoids), ER (oestrogens), PR (progesterone), AR (androgens), as well as mineralocorticoids (MR) (Ahmad and Kumar 2011). They are distinguished by the similarity of certain modular domains, with the DNA binding domain (DBD) providing the most direct link. Certain portions of the SHR proteins fulfil specific tasks, and these areas might potentially be swapped in themselves to generate molecules that function as a hybrid, allowing SHR to function independently. SHRs appear to have a minimum of four key functional regions: C-terminal, NTD (N-terminal domain), DBD (DNA binding domain), and LBD (ligand-binding domain) (Lavery and McEwan 2005). SHRs have tissue and cell-specific actions, as well as developmental-specific regulation, which cause malignancies that include prostate cancer, breast cancer, lung cancer, ovarian cancer, lymphoma, and leukaemia (Miller and Langdon 1997; Huang et al. 2009). The invention of phenomena of the target site selectivity for the modification of the responses of antagonist and agonist. This must focus on the total pharmacological effects of the signal transduction through SHRs with concurrent competition from internal hormones, which is the central issue for SHR pharmacology research (Ahmad and Kumar 2011). The discovery of the underlying mechanisms that control this activity could lead to the creation of tissue-specific modulators SHR. The therapy by the use of the hormones plays a vital role in the cure of numerous malignancies, resulting in a lower death rate and raised lifespan. The main problem of concern is the time period and levels of therapy by hormones in the development of new steroid-based medicines, which could lead to resistance for the steroid over time. The problem has become a serious therapeutic issue, necessitating the production of new drugs to overcome developed resistance in various types of populations of cancer patients.

### 13.2.1 Leukaemia and Glucocorticoid

Glucocorticoids have been used to treat inflammatory illnesses for decades, and their therapeutic benefits are mediated by changing the transcriptional process of the glucocorticoid receptors through binding to the glucocorticoid receptors (GR) (Schleimer 1993). For many lymphomas, glucocorticoids are the basic parts

of therapy and leukaemia because of their ability to limit malignant cell development and trigger apoptosis (Ploner et al. 2005). The key component contributing to glucocorticoids' therapeutic effectiveness is their ability to decrease inflammation and cause apoptosis. Apoptosis introduced by the dexamethasone in cells of lymphoma occurs via the intracellular route as per the recent understanding of GR-dependent apoptosis in leukaemia (Rambal et al. 2009). Dexamethasone, a glucocorticoid, induces apoptosis in some cell types while having ineffective or anti-apoptotic in others. Various types of mechanisms, include GR isoform expression patterns and post-translational changes, and hypothesized to describe the specificity for cellular apoptotic effect of glucocorticoid. It has been proven that in malignant lymphoid cells, enhanced levels of stimulated intracellular glucocorticoid receptors and an interval of regular exposure to steroids lead to the participation of a complicated signalling process transmission and invoking a network of regulatory mechanisms (Reichardt and Schütz 1998). These GR-mediated lymphoid cell loss mechanisms are influenced significantly by signalling systems cells which have an active role at the level of the process of post-translation. Death of lymphoid cells occurs due to the different kinase mechanisms when working in sequence with the GR (Distelhorst 2002).

A mutation in the GR gene causes glucocorticoid resistance through altering, GR-cofactor interactions, nuclear translocation, ligand-binding affinity, or GR stability. The increased activity of FKBP51 has been linked with glucocorticoid resistance (Scammell et al. 2001). The kinase functional role of p38 MAPKs, ERK, and JNK has also been associated with glucocorticoid resistance (Pace et al. 2007). Combining glucocorticoids with kinase/phosphatase inhibitors has the potential to modify the activity of GR in a gene-specific, cell-specific, and/or time-dependent behaviour (Ismaili and Garabedian 2004).

Incorporating the kinase inhibitors and glucocorticoids and various other types of therapies that treat the response of inflammation is not simple as it seems due to the phosphatases or kinases with signalling glucocorticoid receptors (Beck et al. 2009). As a consequence, extensive studies done for investigating the role of phosphatases and kinases in signalling controlled by the glucocorticoid might lead to novel treatment targets for glucocorticoid-controlled deleterious effects and the lowering of glucocorticoid resistance. Some investigations imply that mineralocorticoids, at least in some leukaemic cells, are accountable for excessive cell growth, which may have therapeutic implications, specifically in resistance related to the glucocorticoid.

### 13.2.2 Breast Cancer and Oestrogen

The most common malignancy found in women is breast cancer, due to which several types of threat and defensive factors have been established in the last two decades or more (Al-Hajj et al. 2003). The most crucial hormone for the normal activity of the female reproductive system is estrogens and is required for the differentiation and growth of the epithelium of the healthy and normal breast (Bonney et al. 1983). This ovarian hormone impacts cell division rates and hence

affects breast cancer risk by promoting breast epithelial cell proliferation, which is prone to genetic mistakes during the replication of DNA (Subramani et al. 2017). These types of genetic mistakes can eventually result in a cancerous phenotype. Early menarche and late menopause, both of which result in increased oestrogen build-up in the breast epithelium, are well-known threats for breast cancer. Two forms of genes, BRCA2 and BRCA1, frequent in most of the cancer patients, and discovered as genes linked to breast cancer susceptibility (Ford et al. 1998). Breast cancer risk has also been linked to ER gene polymorphisms, that can hinder the linkage of ER receptors to the action factor DNA or any other proteins which coregulates and directly affects the further ER target gene transcription. According to the American Cancer Society, about 60% of premenopausal breast cancers and 80% of those detected after menopause are ER-positive, demonstrating the expression of ER as a potent indicator of responsiveness to endocrine treatment in patients of breast cancer. Estradiol increases proliferation in MCF-7 cells of breast cancer in human by upregulating c-myc, a gene that controls multiple cell cycle-related signalling pathways (Lee et al. 2015). However, disrupting the ER-a signalling system with a pure anti-estrogen completely limits estrogen's apoptotic effect. The ER-a signalling route has been proved to be important for estrogen-induced tumour regression and death. Raloxifene and tamoxifen which are SERMs (selective eestrogen receptor modulators) have proved to show the reduction in invasive breast cancer in the current recent trials in women (Lee et al. 2008). The use of raloxifene plus tamoxifen for the high risk associated with breast cancer in women has been licensed by the US FDA (United States Food and Drug Administration) (Vogel et al. 2010). Following oestrogen deprivation using anti-estrogens, cell death of apoptotic tumour can be induced by the very little number of estrogens. The advancement of new genomic technologies has resulted in the progression of sophisticated tools for identifying genes which are differently expressed between cancers.

### 13.2.3 Prostate Cancer and Androgen

In males, the most common form of non-skin type of cancer is prostate cancer, and this cancer is prevailing as the second most reason for the death in the United States of America (Scardino 2003). Cancer related to the prostate gland is uncommon before the age of 40, but the disease's risk rises dramatically with age, and exceeds that of any other cancers (Muir et al. 1991). Prostate cancer is 50–70% more common in African/Americans, i.e., Caucasians. Japanese and Chinese males, as well as probably other Asian groups, have lesser chances of being diagnosed with the prostate cancer across the globe (Paris et al. 1999). Androgen, particularly dihydrotestosterone, is required for normal prostate growth (DHTb) (Traish and Morgentaler 2009). AR regulates all the biological activities of androgens. In prostate cancer, AR and its modulators continue to be relevant. Through signal transduction pathways that involve changed expression/binding of AR coregulators, dysregulation of AR function results in prostate cancer progression (Gioeli 2005). During the beginning of prostate cancer, AR expression is profound and can be

observed in the hormone-refractory tumours and hormone-sensitive tumours throughout their course. The most common endocrine-based treatment for prostate cancer is ablation therapy and controlling the circulating testicular androgens (Sriprasad et al. 2009; Labrie et al. 1983). However, deficiency of androgen causes prostate cancer cells to die, these may evolve tolerance to cell death triggered by androgen ablation. A protein generated specifically in the tissue of the prostate called a PSA (prostate-specific antigen) is a widely and normally used biomarker for diagnosis of prostate cancer (Hernández and Thompson 2004). In normal prostate, the PSA levels are more when compared to the serum and serum PSA levels have been found to rise considerably as prostate cancer progresses. PolyQ (polyglutamine) chain repeated sequence encoded by polymeric CAG repeated sequence in the AF1 region of AR gene. The polyQ chain length is inversely proportional to the AR's transcriptional activity (Giovannucci et al. 1997). Because androgens are required for prostate carcinogenesis, and shorter polyQ repeat lengths are linked to increased AR transcriptional activity, it is hypothesized that there are more chances of threat for prostate cancer in males with shorter repeat lengths. The androgen receptor is found on the X chromosome, and it is constant with an X-linked hereditary component for prostate cancer that a brother's history of the disease is riskier than a father (Nelson and Witte 2002). These surprising findings prompted researchers to investigate whether the polyQ chain length in the AR is linked to the development of prostate cancer.

### 13.2.4 Ovarian Cancer and Progesterone

Ovarian malignancy is the fifth most commonly found cancer in females, accounting for approximately one out of five of all gynaecologic cancers. When ovarian cancer is diagnosed, it has likely progressed to a metastatic stage in most women, resulting in a high fatality rate (Ho 2003). The worldwide use of reproductive medicines that trigger the release of follicles from the ovaries is one of the primary reasons for raised chances of ovarian cancer (Sueblinvong and Carney 2009). Levels of progesterone are quite excess during pregnancy, and certain studies indicate that continual ovulation in females might prone to ovarian cancer (Scully 1995). Some data show that progesterone suppresses the proliferation of ovarian cancer cells in women. It is indicated that the doses equivalent to the progesterone during pregnancy are very effective similarly to the inducers of apoptosis in cells of ovarian cancer and where this mechanism is blocked by estrogens (Dor et al. 2002). In hospital-based investigation, the chance of ovaries-related malignancy for ever use of HRT was 0.9, and in population-dependent survey was 1.1, with no uniform duration—risk relationship. Longer-term usage of HRT has been linked to an augmented risk of ovarian malignancy (Gambacciani et al. 2003). The amount of PR-A isoform action is drastically lowered in tissues of ovaries with specimens of malignant cancer, whereas PR-B is unchanged, showing that PR-mediated transcriptional regulation of the target genes (Akahira et al. 2002). Progesterone has also been shown to limit the proliferation of ovarian cancer cells, perhaps via changing genes such as p53,

c-myc, bcl-2, and others which are apoptotic in nature (Syed et al. 2002; Bu et al. 1997; Yu et al. 2001). Multiple SHR coregulator expression levels must have a critical function in ovarian cancer tissue sensitivity and response to progesterone.

### 13.2.5 Multiple Endocrine Neoplasia

Multiple endocrine neoplasia (MEN) is categorized into two types: type 2 and type 1. MEN2-A and MEN2-B are further classified in the MEN type-2 (Marx 2005). There are some new classified types of MEN; but somehow, they are placed under these two types: MEN4 is currently thought to be a variant of MEN1, while another variant of MEN2 is familial medullary thyroid cancer (FMTC). Anterior pituitary tumours, parathyroid tumours, and pancreatic islet cell tumours are all symptoms of MEN1 (Trump et al. 1996). The autosomal dominant mode of inheritance is common for MEN1. When the MEN1 linked primary hyperparathyroidism is compared with the non-MEN1 primary hyperparathyroidism, it is seen that MEN1 linked primary hyperparathyroidism is most prone to develop in younger aged individuals and may affect numerous glands (Thakker et al. 2012). Carcinoid tumours of the lungs, gastrointestinal system, pancreas, and thymus have all been reported in MEN1 patients. One of the most common manifestations of MEN1 is cutaneous symptoms. One-third of MEN1 patients have been diagnosed with subcutaneous lipomas. The MEN1 gene which codes for Menin protein is found on the 11th chromosome and has a vital function in genome stability and transcription regulation (Nord et al. 2010). The MEN1 gene has been connected to the loss of heterozygosity in the genomic area, indicating that it can act as a blocker for tumour growth.

Multiple endocrine neoplasia (MEN2)—type 2 is an autosomal dominant disease which affects roughly one in every 30,000 people (Moline and Eng 2011). There are two types of MEN2: MEN2A and MEN2B. The traditional MEN2A, MEN2A with cutaneous lichen amyloidosis (CLA), MEN2A with familial medullary thyroid tumour, and Hirschsprung's disease (HD) are four clinical variations of MEN2A. MEN2 is defined by the coexistence of a number of malignant and benign endocrine neoplasia with non-endocrine disorders such as pheochromocytoma, parathyroid gland adenomatosis. The RET gene, which is responsible for MEN2, has been localized at 10q11.2. There are 21 exons in total. It is a proto-oncogene for tyrosine kinase receptors. Stimulation of the germline point mutations in the RET gene which encodes for receptor tyrosine kinase caused the MEN2A, MEN2B, and FMTC (Santoro et al. 2002). Both MEN2A and MEN2B have a high penetrance and follow an autosomal dominant inheritance pattern. MEN2 can be commonly treated either by visiting to a clinical geneticist or endocrinologist. Individuals with MTC require molecular genetics and biochemical testing for diagnosis and monitoring of their illness state.

## 13.3 Rationale of Nanomedicine in Hormone-Based Cancer Therapy

Drug delivery has the advantage of eliminating some of the challenges associated with traditional DDS, such as drug absorption while passing through the membrane of cell, insignificant biodistribution, and low bioavailability, all of which impede optimal therapeutic results (Aftab et al. 2018). Targeted DDS in form of nanomedicines is the ideal strategy in this regard. Since it can maintain the needed tissue drug levels as well as plasma concentration inside the body, all types of hazardous effects on the tissues which are fit and healthy in nature are prevented. These DDS reduce healthcare costs by increasing product longevity, product differentiation, and patient compliance. Targeted medication distribution is one of the most significant characteristics since it reduces undesirable toxicity and side effects in the tissues (Dharap et al. 2005). Small capillaries have a diameter of between 5 and 6 microns. Because the diameter of cancer medications is bigger than that of capillaries, they cannot penetrate effectively unless their particle size is reduced. Because nanoparticles are smaller than microparticles, they can easily pass through capillaries and deliver drugs for successful treatment. Furthermore, when drugs are transported by nanoparticles, their efficiency increases because they are efficiently digested by cells. Greater bioavailability, prolonged circulation, improved tumour disposal, appropriate half-life, and transporting the high concentrations of drug at tumour site are all important aspects of nanomedicines (Saha et al. 2010).

## 13.4 Recent Development in Nanomedicine-Based Hormone Cancer Therapy

FDA is actively creating regulations for items including nanomedicines by forming a "Nano Task Force" that gives sufficient information on the FDA's process for accepting nanomedicines. So far, we've looked into genetic changes that affect tumour prognosis, as well as several targetable biomarkers and imaging techniques that can aid in efficient cancer therapy. Polymer chemistry has progressed because of recent advancements in protein engineering to create complex nano formulations that meet basic criteria (increased pharmacokinetic (PK), safety and controlled release) while also optimizing the delivery system for improved efficacy (via active targeting) and concurrent surveillance of adverse reactions and toxic effects. During the last five decades, endocrine treatment for metastatic prostate cancer has been the primary line of treatment. However, newer kinds of hormonal therapy have broadened the therapeutic options in recent times. Newer options to estrogens and orchiectomy include inhibitors of androgen production, also referred as "antiandrogens," and LHRH (luteinizing hormone-releasing hormone) analogues, which can be used alone or in combination (Labrie et al. 2005). Many people consider orchiectomy to be the "gold standard" form of endocrine therapy since it causes a rapid drop in serum testosterone, although there are concerns regarding the surgery's psychological effects. The recently available antiandrogen flutamide,

when paired with LHRH analogues or orchiectomy, can prevent the "flare," and may enhance survival time, especially when the illness is less severe (Hellerstedt and Pienta 2002). Because LHRH analogues may elicit an initial increase or "flare" in blood testosterone before it declines to castrate levels, they should not be administered alone to individuals with neurologic issues or substantial discomfort. Tamoxifen inhibits the expression of oestrogen receptors in breast cancer cells. It prevents oestrogen from binding to cancerous cells and instructs them to proliferate and develop. Tamoxifen functions as an oestrogen in other types of tissues, such as the uterus and bones, while having an anti-estrogen effect in breast cells. As a result, it is known as a selective oestrogen receptor modulator (SERM). It should be employed to treat both menopausal and non-menopausal women with breast cancer. Hormone-based treatment of cancer has a range of side effects that vary depending on the kind of hormone therapy and how your body reacts to it. Vaginal dryness, hot flashes, lack of interest in sex, changes in your cycles (if you haven't entered menopause yet), weariness, nausea, and mood swings are all possible symptoms. So, recent studies are in pipeline to develop a formulation with lesser side effects and toxicity.

## 13.5 Clinical Status

Doxil, the first licensed nanomedicine by the FDA, was introduced in 1995. It's liposomal doxorubicin that is been PEGylated. It was licensed for the therapy of Kaposi's sarcoma, refractory breast cancer, and ovarian cancer, and at the beginning. A list of drugs developed under nanotechnology with their advantages is discussed in Table 13.1.

## 13.6 Conclusion

The efficacy of nanomedicine-based therapy depends on early cancer identification. Nanomedicines are advantageous because they are highly exposed and specific in imaging cellular tumour, making them excellent candidates as one of the best carriers for giving treatments against cancer with minimum damage to normal tissues. The conventional method of drug delivery has a limitation that a huge release of the drug(s) occurs during the circulation of the blood. Traditional systems are affected by the rapid drug(s) release during circulation of blood, so advancement in the availability of nanomedicines provides more beneficial health results. Nanomedicines which act to little modifications in the biological activities may be able to replace current prescription formulations, despite a variety of limitations and flaws. The synthesis flexibility, biocompatibility, optimum size, and long-term release give incredible applications. Hormone-based cancer therapy is the treatment of choice for many cancers like breast cancer, prostate cancer, endometrial cancer, ovarian cancer, and others. But it offers certain side effects along with some toxicities in the patient body, so these should be noticed while considering cancer

**Table 13.1** List of drugs developed using nanoengineered approaches in clinicals

| S.No. | Name | Constitution | Type of cancer | Approval status |
|---|---|---|---|---|
| 1 | *Immunoevasion* | | | |
| | Doxil<br>Onivyde | Liposomal doxorubicin (pegylated)<br>Liposomal irinotecan (pegylated) | HV-related Kaposi sarcoma ovarian cancer, and multiple myeloma<br>Advanced pancreatic cancer | Approved in the US (1995)<br>Approved in the US (2015) |
| 2 | *Hemodynamics* | | | |
| | Nanoparticle generator | Porous silicon micropartticle with polymeric doxorubicin | Breast cancer lung metastasis | planning of phase I |
| 3. | *Protection from degradation* | | | |
| | Atu027<br>ALN-VSP02<br>DCR-MYC<br>MRX34 | Liposomal small interfering RNA (siRNA)<br>Liposomal siRNA<br>Lipid nanoparticle with Dicer-substrate siRNA<br>Liposomal micro-RNA (miRNA) | Advanced or metastatic pancreatic cancer<br>Solid tumours with liver involvement<br>Advanced solid tumours<br>Advanced cancers | Phase I/II completed (2016)<br>Phase I completed (2011)<br>Phase I/II terminated (2016)<br>Phase I terminated (2016) |
| 4. | *Unique electromagnetic properties* | | | |
| | Nano Therm<br>AuroShell | Iron oxied NP<br>Gold nanoshells | Brain tumours<br>Prostate cancer | Approved in Europe (2011)<br>Phase I ongoing |
| 5. | *Combination therapy* | | | |
| | Vyxeos | Liposomal cytarabine and daunorubicin | High-risk acute myeloid leukaemia | Approved in the US (2017) |
| 6. | *Unique transport properties (EPR) effect Enhanecd permeability and retention* | | | |
| | SMANCS<br>DaunoXome<br>Myocet | Polymer-neocarzinostatin conjugate<br>Liposomal daunorubicin | Liver and renal cancer<br>HIV-associated Kaposi's sarcoma | Approved in Japan (1993)<br>Approved in the US (1996) |

treatment. Nanotherapeutics have shifted the clinical therapeutic paradigm of cancer from nonspecific chemotherapy (give the appropriate medicine to the correct disease) to site-specific therapy (deliver the right drug to the right spot at the right time).

**Conflict of Interest** The authors declare no conflict of interest among themselves.

**Acknowledgments** The authors acknowledge Department of Pharmaceuticals, Ministry of Chemical and Fertilizers, Govt. of India, for their support. The NIPER-R communication number is NIPER-R/Communication/277.

**Disclosures** There is no conflict of interest and disclosures associated with the manuscript.

## References

Aftab S, Shah A, Nadhman A, Kurbanoglu S, Aysıl Ozkan S, Dionysiou DD et al (2018) Nanomedicine: an effective tool in cancer therapy. Int J Pharm 540. https://doi.org/10.1016/j.ijpharm.2018.02.007

Ahmad N, Kumar R (2011) Steroid hormone receptors in cancer development: a target for cancer therapeutics. Cancer Lett 300:1–9. https://doi.org/10.1016/j.canlet.2010.09.008

Akahira JI, Suzuki T, Ito K, Kaneko C, Darnel AD, Moriya T et al (2002) Differential expression of progesterone receptor isoforms a and B in the normal ovary, and in benign, borderline, and malignant ovarian tumors. Jpn J Cancer Res 93. https://doi.org/10.1111/j.1349-7006.2002.tb01323.x

Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF (2003) Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci U S A 100:3983–3988. https://doi.org/10.1073/pnas.0530291100

Beck IME, Vanden BW, Vermeulen L, Yamamoto KR, Haegeman G, de Bosscher K (2009) Crosstalk in inflammation: The interplay of glucocorticoid receptor-based mechanisms and kinases and phosphatases. Endocr Rev 30:830–882. https://doi.org/10.1210/er.2009-0013

Bonney RC, Reed MJ, Davidson K, Beranek PA, James VHT (1983) The relationship between 17β-Hydroxysteroid dehydrogenase activity and Oestrogen concentrations in human breast Tumours and in normal breast tissue. Clin Endocrinol 19:727–739. https://doi.org/10.1111/j.1365-2265.1983.tb00051.x

Bu SZ, Yin DL, Ren XH, Jiang LZ, Wu ZJ, Gao QR et al (1997) Progesterone induces apoptosis and up-regulation of p53 expression in human ovarian carcinoma cell lines. Cancer 79. https://doi.org/10.1002/(SICI)1097-0142(19970515)79:10<1944::AID-CNCR15>3.0.CO;2-V

Cabanillas ME, McFadden DG, Durante C (2016) Thyroid cancer. Lancet 388:278395. https://doi.org/10.1016/S01406736(16)30172-6

Choi HS, Liu W, Liu F, Nasr K, Misra P, Bawendi MG et al (2010) Design considerations for tumour-targeted nanoparticles. Nat Nanotechnol 5:42–47. https://doi.org/10.1038/nnano.2009.314

Coulson A, Levy A, Gossell-Williams M (2014) Monoclonal antibodies in cancer therapy: mechanisms, successes, and limitations. West Indian Med J 63:650–654. https://doi.org/10.7727/wimj.2013.241

Dharap SS, Wang Y, Chandna P, Khandare JJ, Qiu B, Gunaseelan S et al (2005) Tumor-specific targeting of an anticancer drug delivery system by LHRH peptide. Proc Natl Acad Sci U S A 102. https://doi.org/10.1073/pnas.0504274102

Distelhorst CW (2002) Recent insights into the mechanism of glucocorticosteroid-induced apoptosis. Cell Death Differ 9:6–19. https://doi.org/10.1038/sj.cdd.4400969

Dor J, Lerner-Geva L, Rabinovici J, Chetrit A, Levran D, Lunenfeld B et al (2002) Cancer incidence in a cohort of infertile women who underwent in vitro fertilization. Fertil Steril:77. https://doi.org/10.1016/S0015-0282(01)02986-7

Ehdaie B (2007) Application of nanotechnology in cancer research: review of progress in the National Cancer Institute's alliance for nanotechnology. Int J Biol Sci 3:doi:10.7150/ijbs.3.108

Feinberg AP (2004) The epigenetics of cancer etiology. Semin Cancer Biol 14:427–432. https://doi.org/10.1016/j.semcancer.2004.06.005

Ford D, Easton DF, Stratton M, Narod S, Goldgar D, Devilee P et al (1998) Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. Am J Hum Genet 62:676–689. https://doi.org/10.1086/301749

Gambacciani M, Monteleone P, Sacco A, Genazzani AR (2003) Hormone replacement therapy and endometrial, ovarian and colorectal cancer. Best Practice and Research: Clinical Endocrinology and Metabolism 17. https://doi.org/10.1016/S1521-690X(02)00086-6

Gioeli D (2005) Signal transduction in prostate cancer progression. Clin Sci 108. https://doi.org/10.1042/CS20040329

Giovannucci E, Stampfer MJ, Krithivas K, Brown M, Brufsky A, Talcott J et al (1997) The CAG repeat within the androgen receptor gene and its relationship to prostate cancer. Proc Natl Acad Sci U S A 94. https://doi.org/10.1073/pnas.94.7.3320

Greish K (2010) Enhanced permeability and retention (EPR) effect for anticancer nanomedicine drug targeting. Methods Mol Biol. (Clifton, NJ) 624:25–37. https://doi.org/10.1007/978-1-60761-609-2_3

Hakim DN, Pelly T, Kulendran M, Caris JA (2015) Benign tumours of the bone: a review. J Bone Oncol 4:37–41. https://doi.org/10.1016/j.jbo.2015.02.001

Hellerstedt BA, Pienta KJ (2002) The current state of hormonal therapy for prostate cancer. CA Cancer J Clin 52:doi:10.3322/canjclin.52.3.154

Hernández J, Thompson IM (2004) Prostate-specific antigen: a review of the validation of the most commonly used cancer biomarker. Cancer 101. https://doi.org/10.1002/cncr.20480

Hiller-Sturmhöfel S, Bartke A (1998) The endocrine system–an overview. Alcohol Res Health 22:153–164

Ho SM (2003) Estrogen, progesterone and epithelial ovarian cancer. Reprod Biol Endocrinol 1. https://doi.org/10.1186/1477-7827-1-73

Huang P, Chandra V, Rastinejad F (2009) Structural overview of the nuclear receptor superfamily: insights into physiology and therapeutics. Annu Rev Physiol 72:247–272. https://doi.org/10.1146/annurev-physiol-021909-135917

Ismaili N, Garabedian MJ (2004) Modulation of glucocorticoid receptor function via phosphorylation. Ann N Y Acad Sci 1024:86–101. https://doi.org/10.1196/annals.1321.007

Labrie F, Bélanger A, Luu-The V, Labrie C, Simard J, Cusan L et al (2005) Gonadotropin-releasing hormone agonists in the treatment of prostate cancer. Endocr Rev 26. https://doi.org/10.1210/er.2004-0017

Labrie F, Dupont A, Belanger A, Lacoursiere Y, Raynaud JP, Husson JM et al (1983) New approach in the treatment of prostate cancer: complete instead of partial withdrawal of androgens. Prostate 4. https://doi.org/10.1002/pros.2990040605

Lauretta R, Sansone A, Sansone M, Romanelli F, Appetecchia M (2019) Endocrine disrupting chemicals: effects on endocrine glands. Front Endocrinol 10:178. https://doi.org/10.3389/fendo.2019.00178

Lavery DN, McEwan IJ (2005) Structure and function of steroid receptor AF1 transactivation domains: induction of active conformations. Biochem J 391:449–464. https://doi.org/10.1042/BJ20050872

Lee WL, Cheng MH, Chao HT, Wang PH (2008) The role of selective estrogen receptor modulators on breast cancer: from tamoxifen to raloxifene. Taiwanese J Obstetric Gynecol 47:24–31. https://doi.org/10.1016/S1028-4559(08)60051-0

Lee A, Oesterreich S, Davidson NE (2015) MCF-7 cells–changing the course of breast cancer research and care for 45 years. JNCI: J Nat Cancer Institute 107:djv073. https://doi.org/10.1093/jnci/djv073

Lin EY, Pollard JW (2007) Tumor-associated macrophages press the Angiogenic switch in breast cancer. Cancer Res 67:5064. https://doi.org/10.1158/0008-5472.CAN-07-0912

Loree JM, Pereira AAL, Lam M, Willauer AN, Raghav K, Dasari A et al (2018) Classifying colorectal cancer by tumor location rather than sidedness highlights a continuum in mutation

profiles and consensus molecular subtypes. Clin Cancer Res 24:1062. https://doi.org/10.1158/1078-0432.CCR-17-2484

Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V et al (2012) Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the global burden of disease study 2010. Lancet 380:2095–2128. https://doi.org/10.1016/S0140-6736(12)61728-0

Marx SJ (2005) Molecular genetics of multiple endocrine neoplasia types 1 and 2. Nat Rev Cancer 5. https://doi.org/10.1038/nrc1610

Miller WR, Langdon SP (1997) Steroid hormones and cancer: (III) observations from human subjects. Eur J Surg Oncol 23:163–177. https://doi.org/10.1016/s0748-7983(97)80014-5

Misra R, Acharya S, Sahoo SK (2010) Cancer nanotechnology: application of nanotechnology in cancer therapy. Drug Discov Today 15:842–850. https://doi.org/10.1016/j.drudis.2010.08.006

Moline J, Eng C (2011) Multiple endocrine neoplasia type 2: an overview. Genet Med 13. https://doi.org/10.1097/GIM.0b013e318216cc6d

Muir CS, Nectoux J, Staszewski J (1991) The epidemiology of prostatic cancer: geographical distribution and time-trends. Acta Oncol 30. https://doi.org/10.3109/02841869109092336

Nelson KA, Witte JS (2002) Androgen receptor CAG repeats and prostate cancer. Am J Epidemiol 155:883–890. https://doi.org/10.1093/aje/155.10.883

Nord KH, Magnusson L, Isaksson M, Nilsson J, Lilljebjörn H, Domanski HA et al (2010) Concomitant deletions of tumor suppressor genes MEN1 and AIP are essential for the pathogenesis of the brown fat tumor hibernoma. Proc Natl Acad Sci U S A 107. https://doi.org/10.1073/pnas.1013512107

Pace TWW, Hu F, Miller AH (2007) Cytokine-effects on glucocorticoid receptor function: relevance to glucocorticoid resistance and the pathophysiology and treatment of major depression. Brain Behav Immun 21:9–19. https://doi.org/10.1016/j.bbi.2006.08.009

Paris PL, Kupelian PA, Hall JM, Williams TL, Levin H, Klein EA et al (1999) Association between a CYP3A4 genetic variant and clinical presentation in African-American prostate cancer patients. Cancer Epidemiology Biomarkers and Prevention 8

Ploner C, Schmidt S, Presul E, Renner K, Schröcksnadel K, Rainer J et al (2005) Glucocorticoid-induced apoptosis and glucocorticoid resistance in acute lymphoblastic leukemia. J Steroid Biochem Mol Biol 93:153–160. https://doi.org/10.1016/j.jsbmb.2004.12.017

Rambal AA, Panaguiton ZLG, Kramer L, Grant S, Harada H (2009) MEK inhibitors potentiate dexamethasone lethality in acute lymphoblastic leukemia cells through the pro-apoptotic molecule BIM. Leukemia 23:1744–1754. https://doi.org/10.1038/leu.2009.80

Reichardt HM, Schütz G (1998) Glucocorticoid signalling–multiple variations of a common theme. Mol Cell Endocrinol 146:1–6. https://doi.org/10.1016/S0303-7207(98)00208-1

Rosol TJ, Yarrington JT, Latendresse J, Capen CC (2001) Adrenal gland: structure, function, and mechanisms of toxicity. Toxicol Pathol 29:41–48. https://doi.org/10.1080/019262301301418847

Saha RN, Vasanthakumar S, Bende G, Snehalatha M (2010) Nanoparticulate drug delivery systems for cancer chemotherapy. Mol Membr Biol 27. https://doi.org/10.3109/09687688.2010.510804

Santoro M, Melillo RM, Carlomagno F, Fusco A, Vecchio G (2002) Molecular mechanisms of RET activation in human cancer, vol 963. Anna New York Acad Sci. https://doi.org/10.1111/j.1749-6632.2002.tb04102.x

Scammell JG, Denny WB, Valentine DL, Smiths DF (2001) Overexpression of the FK506-binding immunophilin FKBP51 is the common cause of glucocorticoid resistance in three New World primates. Gen Comp Endocrinol 124:152–165. https://doi.org/10.1006/gcen.2001.7696

Scardino PT (2003) The prevention of prostate cancer–The dilemma continues. N Engl J Med 349. https://doi.org/10.1056/nejme038109

Schleimer RP (1993) An overview of glucocorticoid anti-inflammatory actions. Eur J Clin Pharmacol 45:S3–S7. https://doi.org/10.1007/BF01844196

Scully RE (1995) Pathology of ovarian cancer precursors. J Cell Biochem 59. https://doi.org/10.1002/jcb.240590928

Sriprasad S, Feneley MR, Thompson PM (2009) History of prostate cancer treatment. Surg Oncol 18. https://doi.org/10.1016/j.suronc.2009.07.001

Subramani R, Nandy SB, Pedroza DA, Lakshmanaswamy R (2017) Role of growth hormone in breast cancer. Endocrinology 158:1543–1555. https://doi.org/10.1210/en.2016-1928

Sueblinvong T, Carney ME (2009) Current understanding of risk factors for ovarian cancer. Curr Treat Options in Oncol 10. https://doi.org/10.1007/s11864-009-0108-2

Syed V, Ulinski G, Mok SC, Ho S-M (2002) Reproductive hormone-induced, STAT3-mediated interleukin 6 action in Normal and malignant human ovarian surface epithelial cells. JNCI: J Nat Cancer Institute 94:617–629. https://doi.org/10.1093/jnci/94.8.617

Thakker RV, Newey PJ, Walls GV, Bilezikian J, Dralle H, Ebeling PR et al (2012) Clinical practice guidelines for multiple endocrine neoplasia type 1 (MEN1). J Clin Endocrinol Metab 97. https://doi.org/10.1210/jc.2012-1230

Traish AM, Morgentaler A (2009) Epidermal growth factor receptor expression escapes androgen regulation in prostate cancer: a potential molecular switch for tumour growth. Br J Cancer 101. https://doi.org/10.1038/sj.bjc.6605376

Trump D, Farren B, Wooding C, Pang JT, Besser GM, Buchanan KD et al (1996) Clinical studies of multiple endocrine neoplasia type 1 (MEN1). QJM-Monthly Journal of the Association of Physicians 89. https://doi.org/10.1093/qjmed/89.9.653

Vogel VG, Costantino JP, Wickerham DL, Cronin WM, Cecchini RS, Atkins JN et al (2010) Update of the national surgical adjuvant breast and bowel project study of tamoxifen and Raloxifene (STAR) P-2 trial: preventing breast cancer. Cancer Prev Res 3:696–706. https://doi.org/10.1158/1940-6207.CAPR-10-0076

Yu S, Lee M, Shin S, Park JS (2001) Apoptosis induced by progesterone in human ovarian cancer cell line SNU-840. J Cell Biochem 82. https://doi.org/10.1002/jcb.1171

# Neurocognitive Underpinning of Neurological Disorders: Role of Default Mode Network

14

Karamjeet Kaur Sarna, Azizuddin Khan, Waheeda Khan, and Sumant Saini

**Abstract**

Over the past few decades, the brain's default mode network (DMN), consisting of a constellation of brain regions active during rest, has been associated with many neurological disorders. However, this network has recently shown activation during high-order social cognitive tasks attributed to self-referential processing and theory of mind. Furthermore, in several disorders with deficits in social cognition, or disrupted physiology including autism spectrum disorder (ASD), Alzheimer's, Parkinson's, hormonal cancer, and strikingly aberrant patterns of brain activity have been observed in certain cortical regions overlapping the DMN. This suggests that DNM overlaps the social brain network extensively involved in social cognitive processes and is often compromised in brain disorders. Here, we explore how an altered DMN may be plausibly extended as an indicator of neurological disorders.

K. K. Sarna
Amity Institute of Neuropsychology and Neurosciences (AINN), Amity University, Noida, India

Psychophysiology Laboratory, Department of Humanities and Social Sciences, Indian Institute of Technology Bombay, Mumbai, India

Faculty of Behavioural Sciences, SGT University, Gurugram, India

A. Khan (✉)
Psychophysiology Laboratory, Department of Humanities and Social Sciences, Indian Institute of Technology Bombay, Mumbai, India
e-mail: aziz@hss.iitb.ac.in; khanaziz@iitb.ac.in

W. Khan
Faculty of Behavioural Sciences, SGT University, Gurugram, India

S. Saini
University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh, India



M. Rahman et al. (eds.), *Hormone Related Cancer Mechanistic and Nanomedicines*,
https://doi.org/10.1007/978-981-19-5558-7_14

**Keywords**

Brain's default mode network · Hormonal neuronal tumours · Therapeutics models

## 14.1 Introduction

As the saying goes, "*An idle mind never rests,*" neuroscientists set out afoot to navigate the regions in the human cerebral cortex which are active during rest. In 1997, Shulman was the first to report reduced activity in some regions of the cerebral cortex while performing non-referential and goal-directed tasks (Shulman et al. 1997). In 2001, Marcus Raichle introduced positron emission tomography (PET) measurements and observed distinct and bilaterally symmetrical cortical areas showing suppression during active tasks, collectively referred to as the brain's default mode network (DMN) (Raichle et al. 2001). It has been highlighted that even when an individual is at rest and mind is left undirected, their thoughts tend to gravitate towards self or other people related to them, which implies that the idle brain is indeed constantly active and at work. Researchers from neuroimaging studies have encountered some brain areas showing activation during self-referential tasks (Mars et al. 2012).

Multiple approaches have been adopted to characterize the DMN components, from which core regions identified are the medial prefrontal cortex, posterior cingulate cortex, inferior parietal lobule, and medial temporal lobe, which show enhanced responses at cognitive rest (Xu et al. 2016; Alves et al. 2019). In addition, some subcortical regions like basal forebrain, cholinergic nuclei, anterior, and mediodorsal thalamic nuclei are structurally and functionally connected to other regions of the DMN (Alves et al. 2019). Functionally, the default network is active when an individual is engaged in social cognitive and mental processes, including autobiographical memory retrieval, envisioning the future, self-reference, and theory of mind (ToM) (Buckner et al. 2008). Apart from that, it is involved in mind wandering and spontaneous thought processes. However, the exact mechanisms of the contribution of DMN to this social cognitive behaviour are still subject to further research (Fig. 14.1).

To understand the function of the DMN, we need to understand the functional role of each component of the DMN individually. The essential nodes in the DMN attributing to the psychology of self and others are the medial posterior cortex, specifically posterior cingulate cortex (PCC) and often the precuneus, the medial frontal cortex, and bilateral inferior parietal and posterior temporal areas around the temporoparietal junction area (TPJ) (Mars et al. 2012). Interestingly, different regions of DMN serve diverse cognitive functions such as PCC is involved in autobiographical episodic memory and self-referential processes, the medial prefrontal cortex is linked to social processing, and medial temporal lobe is associated with episodic memory. The inferior parietal cortex is implicated in attention and semantic processing (Mevel et al. 2011) (Fig. 14.2).

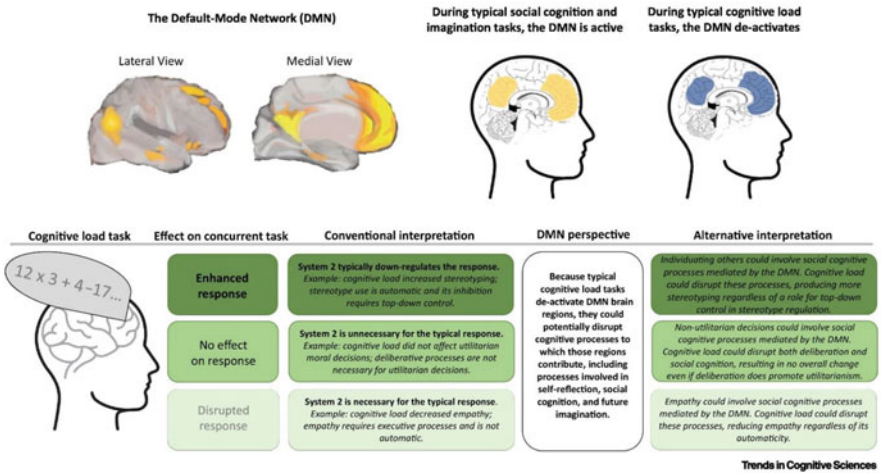



**Fig. 14.1** An overview of the DMN perspective with regard to cognitive load tasks, reproduced with permission

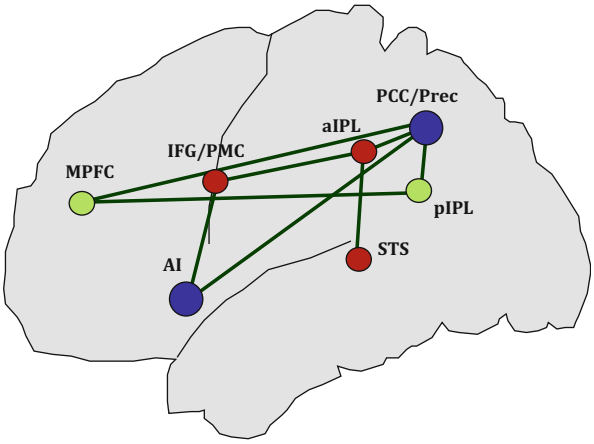



**Fig. 14.2** Functional connections underlying interactions between the DMN and MNS. The DMN, a system for psychological self-relevant processing and mentalizing, and the MNS, a system for physical self-recognition and embodied simulation, may interact through densely connected "hubs" such as the AI and PCC/Prec. Green, DMN nodes; red, MNS nodes; blue, interaction nodes; MPFC, medial prefrontal cortex; pIPL, posterior inferior parietal lobule; PCC/Prec, posterior cingulate cortex/precuneus; IFG/PMC, inferior frontal gyrus/premotor cortex; aIPL, anterior inferior parietal lobule; STS, superior temporal sulcus; AI, anterior insula; and gray lines indicate possible functional connections

Moreover, PCC is involved in working memory tasks, whose attenuated activity during rest in Alzheimer's disease is thought to be susceptible to atrophy (Greicius et al. 2004). While comparing activity in DMN during baseline state and visual

processing tasks, the functional connectivity maps between posterior cingulate cortex (PCC) and anterior cingulate cortex (ACC) were identical, regions implicated in DMN suggesting that the DMN is minimally disrupted by sensory processing tasks with low cognitive load (Greicius et al. 2003). The ventral and dorsal regions of mPFC are thought to mediate emotional processing and cognitive functions, respectively (Gusnard et al. 2001; Raichle 2015). Both PCC and mPFC mediate introspective processes such as self-referential and emotional processing, which show reduced activity when attention is directed externally (Gusnard et al. 2001). Together how these interconnected regions contribute to social cognition is still unclear. The integrity of spatially distinct but functionally connected regions of DMN plays a crucial role in the health of the mind; however, DMN disruptions are noted in Alzheimer's disease (Jones et al. 2011; Cha et al. 2013) and Parkinson's disease [in PD Sect. 14.3].

## 14.2 Default Mode Network Dysfunctions in Neurological and Neuropsychiatric Disorders

### 14.2.1 Alzheimer's Disease

Alzheimer's disease (AD) is a neurocognitive disorder associated with structural and functional changes in the brain networks that gets exacerbated on its progression. One of the earliest clinical manifestations of AD is episodic memory problems leading to cognitive decline. Memory consolidation is thought to be supported by the DMN during resting state (Yildirim and Büyükişcan 2019). Hence, alterations in the activity of the DMN have been associated with working memory impairments in Alzheimer's disease (Greicius et al. 2004). Another reason why it is relevant to study DMN in patients with AD is that the brain regions that are DMN structures are vulnerable to amyloid plaque deposition, atrophy and show a reduced glucose metabolism. For instance, prominent atrophy and metabolic abnormalities were observed in the posterior cortical and medial temporal regions of the DMN (Buckner et al. 2005). Studies have investigated reduced functional connectivity between the hippocampus and mPFC, PCC, precuneus and ventral ACC in AD patients with mild memory impairments (Wang et al. 2006). Few studies have shown a greater decrease in functional connectivity between the frontal cortex and the hippocampus in moderate AD patients than patients with mild AD, showing an association between DMN connectivity and disease severity (Allen et al. 2007; Zhang et al. 2010).

On the contrary, some studies have found evidence for increased resting-state connectivity between the left hippocampus and right lateral PFC (Wang et al. 2006) and between PCC and mPFC (Zhang et al. 2010). One possible explanation for this inconsistency could be the compensatory-recruitment hypothesis, which assumes that AD patients use additional neural resources to compensate for the loss of cognitive function (Grady et al. 2003). The functional connectivity can also be used to trace alterations in the DMN of AD patients after treatment (Dennis and Thompson 2014). Also, a decreased task-induced deactivation was observed in

medial and lateral parietal regions during associative memory paradigm in AD patients, together suggesting disrupted neural responses in the DMN due to amyloid pathology (Celone et al. 2006; Hafkemeijer et al. 2012).

The first pathological mechanism of AD is the deposition of amyloid-β (Aβ) fibrils decades before disease manifestation (Jack Jr et al. 2013). A study has reported an initial accumulation of Aβ predominantly in the precuneus, medial orbitofrontal, and posterior cingulate cortices, regions mainly located within the DMN. This early Aβ burden further associates hypoconnectivity within the DMN, which contributes to cognitive changes (Palmqvist et al. 2017). Age-related effect on DMN activity was observed in APOE-$\varepsilon$4 carriers, where young $\varepsilon$4 carriers demonstrated high activity in the retrospinal, medial temporal, and medial-prefrontal cortices compared to young non-carriers, and older APOE-$\varepsilon$4 carriers were characterized by reduced DMN activity in anterior and posterior cingulate, and cerebellum compared to old non-carriers (Filippini et al. 2009; Mevel et al. 2011). The strength of DMN's functional connectivity partly depends on white matter tracts connectivity. APOE-$\varepsilon$4 carriers have shown disruptions in the white matter tracts, primarily in the cingulum, which interconnects PCC and hippocampus (Heise et al. 2011). These white matter disruptions are reported in individuals at high AD risk before cognitive decline (Gold et al. 2012). APOE-$\varepsilon$4 carriers have reduced functional connectivity between PCC and hippocampus, suggesting early hippocampal structural alterations. This hippocampal atrophy is thought to induce structural and functional perturbations along with memory impairments through cingulum disruptions (Chételat et al. 2003; Villain et al. 2008). A study reported grey matter atrophy in the PCC following cingulum disconnection, leading to amnestic mild cognitive impairment (aMCI) to AD. In a study employing sensory-motor processing tasks, reduced activity in the DMN was observed in patients with Alzheimer's disease, particularly in the PCC and medial temporal lobe (Grady et al. 2003). This study revealed a significant role of the hippocampus in DMN activity. As the disease progresses, these disruptions spread within the cortex, suggesting that the disruptions are not restricted to hippocampus only (Gili et al. 2011).

Some studies show disruptions of functional connectivity within DMN nodes that may lead to cognitive decline. Furthermore, healthy ageing and AD have significant and distinct effects on DMN activity or connectivity; thus, early disruptions in DMN can be considered the potential biomarkers of AD. Therefore, DMN seems to be an essential network that can help us answer many vital questions at the forefront of this debilitating disease. A few literature instances are summarized in Table 14.1.

### 14.2.2 Parkinson's Disease

Parkinson's disease (PD) is a neurodegenerative condition accompanied by cognitive, emotional, and motor symptoms, which are often associated with dysfunction in the DMN (Tepper et al. 2017; Weil et al. 2019). It has been noted that the functional connectivity between the anterior and posterior hubs of the DMN affect cognitive

**Table 14.1** Literature instances of exploration of DMN functionality in Alzheimer's disease

| Technique | Inference | Reference |
|---|---|---|
| fMRI and FDG-PET | AD patients showed reduced input into the medial parietal system and absent input from hippocampus into medial prefrontal cortex. | Scherr et al. (2021) |
| fMRI | Prominent coactivation detected in the hippocampus suggests the involvement of default-mode network in episodic memory processing. In AD group, reduced resting-state activity in the posterior cingulate and hippocampus suggest that disrupted connectivity between these two regions accounts for the posterior cingulate hypometabolism commonly detected in positron emission tomography studies of early AD.<br>As a result, a goodness-of-fit analysis applied at the individual subject level demonstrates that activity in the default-mode network may be a sensitive and specific biomarker for incipient AD. | Greicius et al. (2004) |
| Resting-state functional magnetic resonance imaging | A study indicated significant variability of dALFF in the cerebellar posterior and middle temporal lobe among the AD, MCI, and HC groups. In AD patients, these brain regions had high dALFF variability. The AD group showed lower dfALFF in the left calcarine cortex and white matter than the MCI group. | Li et al. (2021) |
| MRI | While all RSNs represent an increase in connectivity within networks by enhancing inner cohesive ability, 7 out of 10 RSNs were characterized by a decrease in connectivity between networks, indicating a weakened connector among networks from the early stage to dementia.<br>This abnormal network connectivity is significantly correlated with the levels of molecular biomarkers of AD. | Wang et al. (2020) |
| fMRI | This study suggests that cognitive decline in Alzheimer's disease is a reciprocal of a decreased signal complexity in DMN nodes, which might further lead to disrupted DMN functional connectivity. | Grieder et al. (2018) |
| fMRI | The functional connectivity of the default-mode network (DMN) in Alzheimer's disease (AD) patients is weaker than that in healthy participants. However, upon considering physiological noise, such as breathing and heart beating, DMN difference between AD patients and healthy controls became insignificant, owing to importance of controlling physiological noise in the resting-state fMRI analysis to obtain clinically related characterizations in AD. | Li et al. (2020) |

functions; for instance, visual hallucinations in PD is thought to result from disrupted connectivity in the resting state network (Shine et al. 2014). Several fMRI studies have reported impaired deactivation of the DMN during executive task performances along with disturbed connectivity between medial prefrontal cortex and rostral ventromedial caudate nucleus in patients with PD. One of the major hallmarks of PD is the loss of dopaminergic neurons projecting to the striatum, leading to functional disconnection in the striato-thalamo-frontal loop resulting in cognitive deficits. Hence, it has been suggested that the dopamine depletion might lead to functional disconnection in the DMN, inappropriately modulating DMN activity and ultimately failing at executive tasks (van Eimeren et al. 2009).

The network underlying the disease is suggested to influence functional connectivity between the DMN and the Central Executive Network (CEN), which is important in cognitive functions such as attention, reasoning, working memory, and inhibition. These two networks show anticorrelation in a healthy brain. In contrast to PD patients who show an atypical pattern of network interaction, positive coupling between the right CEN and DMN is shown (Fox et al. 2005; Sridharan et al. 2008). A study investigated the three neurocognitive networks supporting efficient cognition, salience network (SN), CEN, and DMN in PD patients and reported increased connectivity within the SN and DMN but decreased connectivity with CEN (Tessitore et al. 2017). Another study examining resting-state networks (RSNs) in PD showed a significant decrease in connectivity within at least seven RSNs as compared to healthy controls (Ghasemi et al. 2021). Aberrant intra- and inter-functional network connectivity in left frontoparietal, basal ganglia network, SA, and DMN were demonstrated in patients diagnosed with PD with depression (DPD) (Zhang et al. 2021). Enhanced functional coactivation was reported within the DMN of PD patients with visual hallucinations; however, low functional coactivation was found within the DMN of PD patients with visual hallucinations and without compared to the control group (Yao et al. 2014).

Network-based analysis in PD patients shows a decreased morphological connection in the DMN and sensorimotor network while increased connections in the salience and frontoparietal networks (Hafkemeijer et al. 2012). Healthy coupling between SN and DMN is essential for efficient cognition, which seems to be dysfunctional in PD (Mevel et al. 2011). The degree centrality (DC) mapping approach found functional connectivity alterations in patients with PD and PD with mild cognitive impairment (PD-MCI) compared to healthy controls. Comparing PD patients with no cognitive impairment (PD-NCI), the PD-MCI group showed abnormal DC in the left fusiform gyrus, precuneus, cerebellum VI, cuneus, and hippocampus. The severity of cognitive impairment in PD-MCI patients is correlated to altered DC in the left fusiform gyrus and left precuneus (Filippini et al. 2009). These alterations were mainly exhibited in the DMN core hubs, for instance, precuneus, which regulates cognitive function. Also, studies showed precuneus dysfunctions association with cognitive impairment in patients with PD (Heise et al. 2011). Increased metabolic and functional connectivity along frontoparietal connections was identified in PD-MCI patients compared to controls and unimpaired patients (Gold et al. 2012). A study also demonstrated that the DMN dysfunction

**Table 14.2** Literature instances of exploration of DMN functionality in Parkinson's disease

| Technique | Inference | Reference |
|---|---|---|
| fMRI | The RSN scores showed a significant decrease in connectivity particularly striking on the lateral and medial posterior occipital cortices in 7 ICs patients with PD compared to HCs. The results show altered connectivity in cluster of the default mode network in PD patients. | Ghasemi et al. (2021) |
| fMRI | Glucose metabolism was significantly reduced in all DMN hubs in both PD patient groups. However, an increased metabolic and functional connectivity was identified along fronto-parietal connections in PD-MCI patients compared to controls and unimpaired patients. Functional connectivity is reported to negatively correlate with cognitive functioning. Thus, the study provides the importance of measuring brain network activity to highlight cognitive symptoms in PD, which seems to be a potential network approaches for identifying prospective biomarkers. | Ruppert et al. (2021) |

improved upon treating the patients with levodopa, typically regulating activity in two midline components of DMN, MPFC, and PCC, which suggests rewiring in the DMN improves symptoms of PD patients (Villain et al. 2008). A few literature instances are summarized in Table 14.2.

### 14.2.3 Schizophrenia

Schizophrenia is a psychotic disorder identified by disturbed functional integrity across frontotemporal regions, and its symptoms have been attributed to disrupted connectivity among cortical regions (Fletcher et al. 1999; Friston 1999). Schizophrenia is associated with abnormal temporal and spatial patterns of activity in the DMN, primarily in the frontal, anterior cingulate, and parahippocampal gyri. The patients also show hyperactivity within PFC and between PFC and subcortical regions such as the thalamus and caudate during cognitively demanding tasks (Garrity et al. 2007; Woodward et al. 2012; Giraldo-Chica and Woodward 2017). Reduced resting-state connectivity has been found between DMN and CEN in patients with schizophrenia which could be regarded as a trademark of schizophrenia pathology (Friston 1998; Riehemann et al. 2001; Giraldo-Chica and Woodward 2017). This reduced suppression of the DMN can be considered as a failed ability to allocate attention to tasks at hand, which results in impairment in task performance. An important question remains if this reduced suppression of the DMN causes impaired cognition in neuropsychiatric disorders. In line with the existing literature, abnormal functional connectivity is found in the DMN, where hyperconnectivity is demonstrated in the posterior cingulate and inferior parietal lobule in the ventral DMN in schizophrenic compared to first-degree relatives (Li et al. 2017).

A study reported increased connectivity in the PCC, mPFC, and inferior parietal lobule in some regions of DMN during self-reflection task in schizophrenia patients;

**Table 14.3** Literature instances of exploration of DMN functionality in schizophrenia

| Technique | Inference | Reference |
|---|---|---|
| FMRI | A study used ICA to evaluate hidden spatiotemporal structures contained within fMRI data from a large group of schizophrenia patients, healthy controls, and individuals with bipolar disorder. The default mode network regions show significant and specific differences between healthy subjects, patients with schizophrenia, and patients with bipolar disorder. | Calhoun et al. (2012) |
| Resting-state functional magnetic resonance imaging | The results indicated that patients with schizophrenia show impaired interaction among DMN subsystems, with a reduced central role for posterior cingulate cortex (PCC) and anterior medial prefrontal cortex (aMPFC) hubs as well as weaker interaction between dorsal medial prefrontal cortex (dMPFC) subsystem and medial temporal lobe (MTL) subsystem. This weak DMN integration may be associated with impaired ability in making self-other distinctions and coordinating present mental states with future envisioning. | Du et al. (2016) |

however, low white matter integrity was observed compared to the healthy controls. These changes in the DMN are associated with impaired self-insight in schizophrenia (Ćurčić-Blake et al. 2015). Functional connectivity impairments have been studied in schizophrenia; however, the specific difference varies across the literature. For example, in an eyes-closed resting condition, a global and non-specific disconnectivity is observed in schizophrenic patients. At the same time, in the same, increased functional connectivity was reported in the DMN and task-positive network (Zhou et al. 2007). A study performed social cognition tasks by patients with schizophrenia demonstrated abnormal hyperactivity in the medial PCC and reduced activation of the right VmPFC (Holt et al. 2011. In addition, it has been observed that DMN activity attenuates differently in patients depending on cognitive load during goal-directed tasks (Schneider et al. 2011). These abnormal patterns connectivity in the intrinsic networks of patients with schizophrenia may be potential endophenotypes of this disorder. Some compelling evidence suggests that compared to controls, patients with schizophrenia had low connectivity strength in the PCC whereas more significant in the precuneus and inferior parietal and that the DMN responds to antipsychotic medications; for instance, olanzapine treatment in patients with schizophrenia increased the ventromedial PFC connectivity, but no changes observed in the posterior regions of the DMN. A few literature instances are summarized in Table 14.3.

### 14.2.4 Attention Deficit Hyperactivity Disorder (ADHD)

ADHD is manifested by impaired levels of impulsivity, hyperactivity, and attention, pointing to the inability of DMN to deactivate, which interferes with the task-positive activities, consequently causing attentional lapses in people with ADHD (Weissman et al. 2006; Sonuga-Barke and Castellanos 2007). Decreased network integrity is found in the DMN, particularly between the precuneus and other regions of DMN, which draws our interest to impaired precuneus connectivity in ADHD (Uddin et al. 2008). Increased functional connectivity was observed in bilateral dorsal ACC, thalamus, cerebellum, insula, and brainstem in individuals with ADHD, reflecting impairments in autonomic control functions from these regions (Tian et al. 2006). On the other hand, reduced functional connectivity has been observed between anterior and posterior regions, showing a relationship between deficits in working memory and attentional lapses in ADHD (Castellanos et al. 2008). Dysfunctional dorsal anterior cingulate cortex (dACC) has been indicated in the pathology of ADHD, showing decreased activation and reduced volumes compared with normal controls (Bush et al. 2005; Carmona et al. 2005). A study found significantly decreased resting-state functional connectivity between PCC and dACC, suggesting an abnormal balance between attentional and self-related thoughts (Makris et al. 2007).

For response inhibition, where an affected individual has trouble suppressing an ongoing, inappropriate response, the DMN must be actively inhibited to perform a task successfully. However, there are stronger connections within the nodes of DMN than with the nodes of response inhibition network, contributing to decreased task performance in ADHD (van Rooij et al. 2015). The cognitive control network, engaged during cognitively demanding tasks, works in anticorrelation with the DMN. Attenuated negative connectivity is seen between the DMN and central control network, which may be vital in ADHD pathophysiology (Cole and Schneider 2007). Another study observed increased frontoparietal connectivity in boys with ADHD. Also, it examined the effect of methylphenidate on functional connectivity in boys with ADHD and observed drastic suppression in the frontoparietal connectivity associated with improved cognitive performance (Silberstein et al. 2016). A few literature instances are summarized in Table 14.4.

### 14.2.5 Autism

Autism is a heterogeneous condition often characterized by deficits in social cognitive processes. Impaired introspective thoughts, social and emotional processing are attributed to altered patterns of DMN deactivation in ASD patients (Kennedy et al. 2006). The neural mechanism underlying the pathogenesis of the social impairments in this disorder is poorly understood. Disrupted functional connectivity in the DMN regions between ACC and PCC has been implicated in autism, proposing an absence of self-referential thoughts in people with ASD (Cherkassky et al. 2006). A resting-state condition showed reduced functional connectivity in mPFC and angular gyrus,

**Table 14.4** Literature instances of exploration of DMN functionality in ADHD

| Technique | Inference | Reference |
|---|---|---|
| Resting-state functional magnetic resonance imaging | The developmental patterns of local and global brain activities between ADHD and typically developing (TD) individuals were analyzed. Results showed that the ADHD group had abnormal amplitude of low-frequency fluctuation, fractional amplitude of low-frequency fluctuation, and regional homogeneity in the medial orbital frontal cortex, anterior cingulate cortex, postcentral gyrus, thalamus, precuneus, and cerebellum from local brain activity. | Tang et al. (2018) |
| Resting-state functional magnetic resonance imaging | ADHD symptoms are related to lower levels of detail in ongoing thought while the participants made more difficult, memory-based decisions.<br>In addition, greater ADHD scores are linked to lower levels of connectivity between the DMN and right sensorimotor cortex, and between the FPN and right ventral visual cortex. | Vatansever et al. (2019) |

also reported no anticorrelation between DMN and task-positive network in the ASD group. These abnormalities in the DMN, specifically reduced functional connectivity in mPFC associated with social and emotional processes and deficits in the mirror neuron system, suggesting atypical self-processing with others. Studies on adolescents demonstrate global patterns of underconnectivity within and between DMN and other brain areas (Nomi and Uddin 2015).

The maturation trajectory of DMN connectivity focuses on age-related patterns of DMN connectivity, which shows ASD groups do not show a typical age-related increase in connectivity between PCC and frontal areas (Wiggins et al. 2011). On the contrary, a study demonstrated an age-associated rise in negative functional connectivity between the DMN and the task-positive CEN in the control group (Lawrence et al. 2019). DMN shows variations across factors, such as age, gender, and cognitive functions. A study exploring sex differences in DMN connectivity shows that the female controls have stronger within-DMN connectivity than the male control group; in contrast to ASD, females and males reported similar strength of connectivity within DMN, which was significantly low control counterparts (Ypma et al. 2016). This hypoconnectivity in the DMN seems to be an endophenotype that suggests aberrant DMN connectivity may contribute to autism-relevant traits. Studies show DMN is underdeveloped in infants and continues to develop in adolescence; the strength of functional connectivity tends to decrease with age and is also correlated with cognitive abilities (Mak et al. 2017).

Another critical variable relevant to DMN connectivity is intellectual functioning. The low cognitive functioning group showed significant underconnectivity within the DMN compared to the high cognitive functioning group, despite managing symptoms severity (Mash et al. 2019). Structural aberrations, including increased

cortical thickness in the PCC and VmPFC in children with ASD as well as reduced gyrification in the VmPFC, were reported in male participants of mixed age group (Padmanabhan et al. 2017). Diffusion tensor imaging studies demonstrated aberrant white matter connectivity in ASD, primarily along with the cingulum bundle that connects the mPFC to PCC (Ismail et al. 2016). Atypical neuronal migration during early development may contribute to the pathophysiology of ASD. The human post-mortem analysis shows an increased density of white matter neurons in the superficial layers of PCC (Oblak et al. 2011). An imbalance in the excitatory/inhibitory (E/I) neural circuits is thought to alter inter- and intra- signaling of DMN and contribute to atypical behaviours (Padmanabhan et al. 2017). An optogenetic study on rodents shows increased E/I imbalance in the mPFC, resulting in impaired social functions. Complex social processes are supported by intrinsic connectivity between large-scale brain networks, including salience network (SN), central executive network (CEN), and DMN. Therefore, suggesting the importance of interwork interactions in the pathophysiology of ASD. Overall, the literature suggests impairments in the DMN underlie social deficits in ASD.

In the strictest sense, social cognition is regarded as the social understanding of self and others, a vital part of social life. The self-reflective processes typically begin developing during the early years of life and continue through adulthood. The task-negative network is implicated in the self-referential processing and socially pertinent information – typically by showing suppressed activity in the cortical areas during social cognitive tasks. When individuals are not engaged in any specific task, their mind is left undirected to process thoughts relating to themselves or others. Besides, there is an overlap between core DMN areas and the social brain, attributed to understanding cognitive processes of self and others (Li et al. 2014). A set of regions including PCC, mPFC, and left IPL have been demonstrated to show everyday activity to self-referential thoughts and rest (Davey et al. 2016).

A brain network dedicated to support human sense of self has been reported from self-referential tasks studies that show activation patterns in the regions of the DMN. A self-related mental process evokes a resting state; however, this poses a question, what are the different and common components between the two states. Recently, a study has shown an overlap in the regions mPFC during the self-referential and task conditions (Bernier et al. 2013). The PCC, the core functional hub of DMN, is implicated in both self and other relevant information. A study reported reduced activity in the PCC during self-related judgement tasks.

On the other hand, EEG showed enhanced alpha-band activity in the posterior hubs of the DMN during self-referential thoughts (Knyazev et al. 2011). Together, these findings imply that the DMN is the seat for self-referential processing in the brain. In autism, atypical self-referential and introspective thoughts result from low activation of DMN in the baseline state. Reduced activity is also observed between PCC and ACC, which is thought to disturb self-referential thoughts in autism (Broyd et al. 2009). ToM refers to the ability to infer the thoughts, intentions, and mental perspective of others which is often regarded as underdeveloped in people with autism spectrum disorder that results in social cognitive deficits (Baron-Cohen and Wheelwright 2004). From previous neuroimaging studies, mPFC was recognized as

a critical area for ToM tasks; however, there were certain discrepancies where Saxe demonstrated right TJP involvement in understanding mental states of others (Saxe 2006), whereas Samson argued that the left TJP was representing the mental states (Samson et al. 2004). Also, ToM studies revealed strong functional connectivity between the parietal and frontal cortex in the DMN. On the contrary, it detected lower functional connectivity within DMN during intentional mentalizing in individuals with autism (Li et al. 2014).

Humans have an incredible tendency to process mental states of self and others, and the default mode network is seen to be processing these cognitive functions. However, the default mode network is highly correlated to resting states such as daydreaming or mind-wandering. It is also seen to be active when an individual engages in thinking about self, conceiving perspectives of others, envisioning the future, and retrieving autobiographical memory. Individuals with autism spectrum disorder have social difficulties understanding others' emotional or mental states, and self-referential processing is also atypical in autism. Studies have shown that networks for social cognition, particularly higher-order tasks of attributing to others' mental states, overlap the DMN (Mars et al. 2012), and impairments in similar social cognitive abilities are reflected in autism. This overlap between the social brain network and default mode network may provide evidence for DMN dysfunction resulting in social cognitive dysfunction in patients with autism in two core domains: self-referential processing and ToM.

Autism spectrum disorder is a cluster of social impairments that makes it difficult for individuals to reciprocate social interaction due to a lack of sense of self in context to others. Hence, it is not surprising to link DMN, a core neural network processing information relevant to self and inferring the mental states of others, to ASD phenotypes. Studies have suggested that PCC and mPFC manifest aberrant patterns of self-representation in ASD (Mars et al. 2012). Therefore, the literature points towards the idea that social impairments in ASD are linked to dysfunctional DMN. Complex social behaviour is supported by interactions between large neural networks, such as the salience network, central executive network, and default mode network. However, alterations in connectivity are likely to present atypical behaviour signifying the inter-network connectivity in the pathophysiology of ASD. A few literature instances are summarized in Table 14.5.

### 14.2.6 Multiple Sclerosis (MS)

Multiple sclerosis (MS) is a debilitating demyelinating disease of the central nervous system, characterized by a cognitive decline among other motor symptoms, hampering daily life (Inglese and Petracca 2018). It has previously been demonstrated that alterations in resting-state networks are observed mainly in the DMN related to altered cognitive processes (Faivre et al. 2012). Using fMRI, changes within DMN connectivity and attentional networks were associated with cognitive impairments (Louapre et al. 2014). Furthermore, an MEG study of patients with MS reported reduced within and cross-network DMN connectivity, complementing previous

**Table 14.5** Literature instances of exploration of DMN functionality in autism

| Technique | Inference | Reference |
|---|---|---|
| Resting-state fMRI | Individuals with ASD displayed significantly impaired connectivity in the ventral medial prefrontal cortex and posterior cingulate cortex, which are two critical hubs of the default mode network (DMN). | Guo et al. (2019) |
| fMRI | This study found a small-world regimen in the DMN; however, there were no significant differences in global network of ASD participants.<br>The ASD group exhibited reduced nodal centralities in the bilateral anterior medial prefrontal cortex and increased nodal centralities in the right lateral temporal cortex and the right retrosplenial cortex.<br>Moreover, ASD participants also displayed significantly reduced and increased FC within the DMN. Overall, showing disrupted patterns of FC metrics and nodal network metrics in the DMN, that could be a potential biomarker for ASD diagnosis. | Chen et al. (2021) |
| Resting-state fMRI | Whole-brain within-connectivity showed hyper-connectivity, especially in cerebellum and brainstem in ASD children but both hyper−/hypo-connectivity in adolescents and ASD adults and between-connectivity.<br>While in the between-connectivity, negative correlation between DMN and temporal network was examined in ASD group.<br>Significant differences between cerebellum and DMN was observed from full correlation comparison between ASD adolescents and TD individuals. | Haghighat et al. (2021) |
| Rs-fMRI | Hyper-connectivity of the posterior cingulate and retrosplenial cortices with predominately medial and anterolateral temporal. Cortex was observed in ASD children.<br>On the contrary, the precuneus demonstrated hypo-connectivity with visual cortex, basal ganglia, and locally within the posteromedial cortex in ASD children.<br>In ASD, aberrant posterior cingulate cortex hyper-connectivity was associated with severity of social impairments in ASD, whereas precuneus hypo-connectivity was not linked to social deficits. | Lynch et al. (2013) |

fMRI studies (Sjøgård et al. 2021). In a study, dynamic functional connectivity (dFC) of patients with MS was measured to explore its link with fatigue experienced by more than 80% of patients. Global increase of dFC was reported in MS patients compared to healthy controls. Besides, ganglia-DMN dFC was associated with fatigue in MS, where patients with lower ganglia-DMN dFC were estimated to have higher fatigue scores, indicating a neural correlate of fatigue in MS (Tijhuis et al. 2021).

Another symptom of MS specifically associated with functional disconnection of neurotransmitter-related nuclei is depression. A study investigated a significant reduction of raphe nuclei related functional connectivity in MS patients with

**Table 14.6** Literature instances of exploration of DMN functionality in multiple sclerosis

| Technique | Inference | Reference |
|---|---|---|
| fMRI | Impairment in the anterior parts of the DMN has been shown to be associated with cognitive impairment in patients with progressive MS. | Rocca et al. (2010a, 2010b) |
| fMRI | Functional abnormalities were present in cognitive networks, including DMN of patients with relapsing–remitting multiple sclerosis (RRMS). | Nejad-Davarani et al. (2016) |
| fMRI | In benign multiple sclerosis, patients showed reduced white matter fractional anisotropy (WM FA), gray matter (GM) atrophy, and increased resting-state functional connectivity (RS FC) in fronto-temporo-parietal regions. | Riccitelli et al. (2020) |
| Resting-state fMRI | In the relapsing–remitting multiple sclerosis (RRMS) patients, DMN connectivity was found to be significantly weaker in the anterior cingulate cortex. It was significantly weaker in the core but stronger at the periphery of the posterior cingulate cortex which demonstrates possible compensatory effect on cognitive performance. | Bonavita et al. (2011) |

depression compared to those without depression. This signalling deficit may induce changes in the DMN which in turn may be behaviourally manifested in MS patients with depression (Martino et al. 2020). Research suggested that reduced activity in the anterior component of the DMN was marked more in cognitively impaired patients with MS than cognitively preserved patients (Rocca et al. 2010a, 2010b). Functional connectivity (FC) changes occur in both the ACC and PCC of the DMN in MS patients with depression. Since there are partially overlapping FC changes in the PCC of MS patients with depression and cognitive decline, FC changes within the DMN can serve as an early marker of cognitive impairment in MS patients (Bonavita et al. 2017). A few literature instances are summarized in Table 14.6.

### 14.2.7 Glioma

Gliomas are the most frequent primary intracranial tumours, whose malignant forms exhibit a high degree of tumour cell proliferation and infiltration compared to low-grade gliomas (Ostrom et al. 2014). Patients with low-grade glioma are seen with cognitive deficits that affect the quality of life. A study conducted on low-grade glioma patients after respective surgery showed increased lower alpha band connectivity within the DMN. This increase in alpha band functional connectivity after surgery is correlated with improved cognitive performance (van Dellen et al. 2012). DMN connectivity patterns differ significantly in patients with gliomas as compared to healthy individuals. Changes in the connectivity pattern of the DMN are observed at or near tumour sites and in patients who underwent a series of radiotherapy.

Furthermore, neurocognitive impairments are mainly associated with reduced connectivity in the left temporal and parietal DMN nodes; therefore, these nodes of the DMN should be considered for personalized treatment strategies (Kocher et al.

2020). Lower DMN integrity was observed in high-grade tumour with low-grade patients. That tumour in the left parietal lobe reported more impaired DMN, suggesting tumour grade and location impact DMN connectivity (Harris et al. 2014). In an fMRI study, impaired cognitive performance in glioma patients prior to treatment was associated with reduced deactivation in the DMN (Schouwenaars et al. 2020).

Tumours affect the resting state networks, reportedly significant changes are observed in the right angular gyrus/inferior parietal lobule of the DMN (Maesawa et al. 2015). Modifications of the DMN connectivity are seen to be induced by different tumour grades. A patient study showed an increased DMN integrity in the hippocampal regions, whereas reduced connectivity in the prefrontal regions (Esposito et al. 2012). In patients with frontal gliomas, the intra-hemisphere functional connectivity was reduced between the PCC and temporal-parietal junction (TPJ), suggesting that gliomas not only affect local functions but also disrupt the global functional state (Zhang et al. 2016). Tumour leads to neuronal generation, decreasing the hemispheric connectivity and disrupting connections across the corpus callosum. It has also been observed that tumours in the left parietal lobe have more impact on the DMN integrity. In contrast, tumours within DMN have no significant impact, showing how tumour locations impact connectivity (Tordjman et al. 2021). Studies also reveal the effect of tumour on brain and memory deficits induced by it depend on the DMN integrity (Romero-Garcia et al. 2021). A few literature instances are summarized in Table 14.7.

### 14.2.8 DMN in Addiction

The DMN has been implicated in the substance use disorder (SUD), both aberrant DMN function and other disturbed large scale interacting networks have been associated with impaired cognitive functions contributing to craving and relapse Jovicich et al. 2016; Pinter et al. 2016. The core midline regions of the DMN have extensively been investigated through neuroimaging studies of addiction. Both anterior and posterior parts of DMN, including medial prefrontal cortex and PCC which presumably interact with other cortical areas, have profound effect of drugs (Gusnard and Raichle 2001). A few literature instances are summarized in Table 14.8.

### 14.2.9 Epilepsy

Epileptic activity has been found to influence abnormal functional connectivity among resting state networks, particularly in the default mode network which might be associated with loss of consciousness and cognitive deficits during epilepsy (Li et al. 2015). Cerebral blood flow hypoperfusion has been indicated in some regions of the DMN, in tonic-clonic and temporal lobe seizures, inducing physiological changes in these regions (Danielson et al. 2011). An increased functional

**Table 14.7** Literature instances of exploration of DMN functionality in glioma

| Technique | Inference | Reference |
|---|---|---|
| Functional MRI | Patients with gliomas, mainly with tumours near the posterior DMN, showed reduced posterior cingulate cortex anticorrelation in task-based fMRI than controls.<br>Moreover, patients with both left- and right-hemisphere tumours, and those with grade IV tumours, showed significantly lower posterior cingulate cortex anticorrelation than controls.<br>A study concluding impaired deactivation of the default mode network in patients with gliomas. | Kenna et al. (2013) |
| Magnetoencephalography | Increased alpha band RSN functional connectivity in MEG recordings correlates with improved cognitive outcome after resective surgery. The mechanisms resulting in functional connectivity alterations after resection remain to be elucidated. Importantly, our findings indicate that connectivity of MEG RSNs may be used for presurgical prediction of cognitive outcome in future studies. | Van Dellen et al. (2013) |
| fMRI | Compared with the HCs group, the patient group exhibited significant differences in functional connectivity among default mode network, executive control network, and salience network. In addition, the number of the significant functional connectivities between the paired seeds observed in the patients was greater than that in HCs and significantly increased functional connectivity was detected between left posterior cingulate cortex and right angular gyrus. Furthermore, altered neural activities in the RSNs of patients with frontal glioma were positively associated with certain aspects of cognitive function. | Liu et al. (2019) |
| fMRI | Brain tumours and their corresponding treatment affecting brain networks that are fundamental for memory functioning such as the DMN can have a major impact on patient's memory recovery. | Assem et al. (2021) |
| fMRI | A significantly increased and reduced integration of DMN areas was observed in hippocampal area sand prefrontal regions, respectively. | Esposito et al. (2012) |
| fMRI | Functional connectivity in each default mode network region was not significantly different between task-based and resting-state maps. Task-based fMRI showed impaired deactivation of the default mode network in patients with gliomas. The functional connectivity of the default mode network in both task-based and resting-state fMRI in patients with gliomas using the posterior cingulate cortex identified in task- | Maniar et al. (2021) |

(continued)

**Table 14.7** (continued)

| Technique | Inference | Reference |
|---|---|---|
| | based fMRI as an ROI for seed-based correlation analysis has strong overlap. | |
| RS-fMRI | Tumours in the left hemisphere had the largest effect on DMN connectivity regardless of their size and type, while this effect was not observed for right hemispheric tumours. Tumours in the cerebellum also had statistically significant effects on DMN connectivity. These results suggest that DMN connectivity in the left side of the brain may be more fragile to insults by lesions. | Ghumman et al. (2016) |
| Pseudo-resting state fMRI | Higher tumour grade along with prior surgery and/or treatment cause the largest reduction in DMN functional connectivity in patients with primary gliomas, and that tumour location has an impact on connectivity. | Harris et al. (2014) |
| Resting state fMRI | These findings suggest the possibility that functionally intact regions may persist within GBMs and that the extent to which FC is maintained may carry prognostic value and inform treatment planning. | Daniel et al. (2021) |
| Resting state fMRI | Cognitive deficits in glioma patients prior to treatment are associated with reduced responsiveness of the DMN, but not with abnormal CEN activation. These results suggest that cognitive deficits in glioma patients reflect a reduced capacity to achieve a brain state necessary for normal cognitive performance, rather than abnormal functioning of executive brain regions. Solely focusing on increases in brain activity may well be insufficient if we want to understand the underlying brain mechanism of cognitive impairments in patients, as our results indicate the importance of assessing deactivation. | Schouwenaars et al. (2021) |
| fMRI | A significantly increased and reduced integration of DMN areas was observed in the hippocampal and prefrontal regions, respectively. Modifications were closely related to tumour grading. Moreover, the DMN lateralized to the hemisphere contralateral to tumour in the low-grade, but not in the high-grade tumour patients. | Esposito et al. (2012) |
| MRI | Although the tumours were localized in the left side of the brain, changes in connectivity were observed in the contralateral side. Moreover, these changes correlated with some aspects of cognitive function indicating that patients with gliomas may undergo cognitive changes even in | Maesawa et al. (2015) |

(continued)

**Table 14.7** (continued)

| Technique | Inference | Reference |
|---|---|---|
| | the absence of or before the onset of major symptoms. Evaluation of resting state networks could be helpful in advancing our hodological understanding of brain function in glioma cases. | |
| Resting state fMRI | Altered functional connectivity is reliably found with seed-based correlation analysis and independent component analysis in the DMN, dorsal attention network, and fronto-parietal executive control network in glioma patients, possibly explained by decreased connectivity between the cerebral hemispheres across the corpus callosum due to disruption of the connections. | Tordjman et al. (2021) |

connectivity was observed between DMN and the right medial temporal region as well as anterior cingulate cortex during temporal lobe epilepsy (Hsiao et al. 2015). A decreased integration within DMN and reduced functional connectivity among temporal, parietal, and frontal was observed in patients with absence epilepsy, suggesting how impaired anatomo-functional architectural integration in DMN, result in cognitive mental impairment and unconsciousness during absence seizure (Hu et al. 2017). A few literature instances are summarized in Table 14.9.

## 14.3 Conclusion and Future Prospects

DMN on the whole can be visualized as a novel perspective to neurological diagnostics. The said tool can be used as a prognostic tool and predict the advent of disease much before its clinical manifestation which can be used to control as well as to follow up the efficacy of treatment provided to subjects. A quantum of research is still underway and needed to establish the underlying mechanisms of the same and comprehend the different strata of brain connectivity and how it can be used as well explored to detect as well as treat the diseases. A early detection is a major problem with neurological diseases like AD/PD. It has advantages over current methods used for detection viz. biochemical. Treatment can be started early to reverse the damages. An alternate way of visualizing the disease rather from a biochemical or immunological perspective may shed light with upcoming research, finding the new ways to have a better understanding of the disease as well as newer methodologies for its treatment.

**Table 14.8** Literature instances of exploration of DMN functionality in addiction cases

| Technique | Drug of abuse | Inference | Reference |
|---|---|---|---|
| fMRI | Betal quid | Decreased network connectivity observed. | Zhu et al. (2017) |
| fMRI | Heroin | These findings suggest drug addicts' abnormal functional organization of the DMN, and are discussed as addiction-related abnormally increased memory processing but diminished cognitive control related to attention and self-monitoring, Which may underlie the hypersensitivity toward drug-related cues but weakened strength of cognitive control in the state of addiction. | Ma et al. (2011) |
| fMRI | Cocaine | In individuals with cocaine-use disorder, Greater D3R-related binding in the substantia nigra was associated with improved performance and greater DMN suppression. Exploratory moderated-mediation analyses indicated that DMN suppression was associated with Stroop performance indirectly through D2R in healthy comparison And D3R in cocaine-use disorder Participants. | Worhunsky et al. (2021) |
| fMRI for BOLD signal | Internet addiction | Hyper-connectivity in Cognitive control network and default mode network as well as the hypo-connectivity In visual attention network, verifying the common mechanism in IA and substance Addiction, and the underlying association between IA, and attention deficit/hyperactivity Disorder in terms of neurobiology. | Wang et al. (2019) |
| Resting stage fMRI | Alcohol addiction | rsFC within The DMN. Functionally, this finding may be associated with impairments in memory Encoding and self-referential processes commonly observed during alcohol intoxication. Future resting- state functional magnetic resonance imaging studies might therefore Also investigate memory function and test whether DMN-related connectivity Changes are associated with alcohol-induced impairments or craving. | Fang et al. (2021) |
| fMRI | Cocaine | Cocaine addiction is associated with disrupted interactions among DMN, medial temporal lobe, and salience network, which have been implicated, respectively, in self-referential functions, emotion and memory, and coordinating Between internal and external stimuli, providing novel and important insights into the neurobiological mechanisms of cocaine addiction. | Liang et al. (2015) |

**Table 14.9** Literature instances of exploration of DMN functionality in epilepsy

| Technique | Inference | Reference |
|---|---|---|
| MRI | Altered functional connectivity, particularly in dorsal attention (DAN), salience (SN), and default mode (DMN) networks, might lead to the loss of consciousness during seizures and cognitive deficits in patients with children absence epilepsy (CAE). | Li et al. (2015) |
| EEG | The theta (4–8 Hz) and alpha (8–13 Hz) bands activity in each DMN subnetwork shows alterations during epileptiform discharges which may suggest serve as an underlying mechanism for the generation and propagation of epileptiform discharges in the brain. | Cui et al. (2018) |
| BOLD-fMRI data EEG | Compared to healthy group, epilepsy patients showed significant decreased DMN functional connectivity in right uncus, left inferior parietal lobule, left supramarginal gyrus, left uncus, left parahippocampal gyrus, and left superior temporal gyrus, suggesting how patterns in BOLD-fMRI may suggest networks responsible for partial epilepsy genesis or progression. | Hu et al. (2017) |
| Functional magnetic resonance imaging (MRI) | Results: The analyses revealed that hypervariable edges within the specific DMN subsystem were shared by all subtypes (all $P$NBS $< 0.005$), and deficits in node-wise temporal variability were prominent in TLE (all $t(243) \leq 2.51$, $P$FDR $< 0.05$) and FLE (all $t(302) \leq -2.65$, $P$FDR $< 0.05$) but relatively weak in GGE-GTCS. Moreover, dynamic states were generally less stable in patients than controls (all $P$'s $< 0.001$). | Yang et al. (2021) |

## References

Allen G, Barnard H, McColl R, Hester AL, Fields JA, Weiner MF, Ringe WK, Lipton AM, Brooker M, McDonald E (2007) Reduced hippocampal functional connectivity in Alzheimer disease. Arch Neurol 64(10):1482–1487

Alves PN, Foulon C, Karolis V, Bzdok D, Margulies DS, Volle E, de Schotten MT (2019) An improved neuroanatomical model of the default-mode network reconciles previous neuroimaging and neuropathological findings. Communications biology 2(1):1–14

Assem M, Sinha R, Woodberry E (2021) Memory recovery is related to default mode network impairment and neurite density during brain tumours treatment. J Neurosurg 136(2):358–368

Baron-Cohen S, Wheelwright S (2004) The empathy quotient: an investigation of adults with Asperger syndrome or high functioning autism, and Normal sex differences. J Autism Dev Disord 34(2):163–175

Bernier R, Aaronson B, McPartland J (2013) The role of imitation in the observed heterogeneity in EEG mu rhythm in autism and typical development. Brain Cogn 82(1):69–75

Bonavita S, Gallo A, Sacco R, Corte MD, Bisecco A, Docimo R, Lavorgna L, Corbo D, Costanzo AD, Tortora F (2011) Distributed changes in default-mode resting-state connectivity in multiple sclerosis. Mult Scler J 17(4):411–422

Bonavita S, Sacco R, Esposito S, d'Ambrosio A, Della Corte M, Corbo D, Docimo R, Gallo A, Lavorgna L, Cirillo M, Bisecco A, Esposito F, Tedeschi G (2017) Default mode network

changes in multiple sclerosis: a link between depression and cognitive impairment? Eur J Neurol 24(1):27–36
Broyd SJ, Demanuele C, Debener S, Helps SK, James CJ, Sonuga-Barke EJ (2009) Default-mode brain dysfunction in mental disorders: a systematic review. Neurosci Biobehav Rev 33(3): 279–296
Buckner RL, Andrews-Hanna JR, Schacter DL (2008) The brain's default network: anatomy, function, and relevance to disease. Ann N Y Acad Sci 1124:1–38
Buckner RL, Snyder AZ, Shannon BJ, LaRossa G, Sachs R, Fotenos AF, Sheline YI, Klunk WE, Mathis CA, Morris JC (2005) Molecular, structural, and functional characterization of Alzheimer's disease: evidence for a relationship between default activity, amyloid, and memory. J Neurosci 25(34):7709–7717
Bush G, Valera EM, Seidman LJ (2005) Functional neuroimaging of attention-deficit/hyperactivity disorder: a review and suggested future directions. Biol Psychiatry 57(11):1273–1284
Calhoun V, Sui J, Kiehl K, Turner J, Allen E, Pearlson G (2012) Exploring the psychosis functional connectome: aberrant intrinsic networks in schizophrenia and bipolar disorder. Front Psych 2:75
Carmona S, Vilarroya O, Bielsa A, Trèmols V, Soliva JC, Rovira M, Tomàs J, Raheb C, Gispert JD, Batlle S, Bulbena A (2005) Global and regional gray matter reductions in ADHD: a voxel-based morphometric study. Neurosci Lett 389(2):88–93
Castellanos FX, Margulies DS, Kelly C, Uddin LQ, Ghaffari M, Kirsch A, Shaw D, Shehzad Z, Di Martino A, Biswal B, Sonuga-Barke EJS, Rotrosen J, Adler LA, Milham MP (2008) Cingulate-Precuneus interactions: a new locus of dysfunction in adult attention-deficit/hyperactivity disorder. Biol Psychiatry 63(3):332–337
Celone KA, Calhoun VD, Dickerson BC, Atri A, Chua EF, Miller SL, DePeau K, Rentz DM, Selkoe DJ, Blacker D (2006) Alterations in memory networks in mild cognitive impairment and Alzheimer's disease: an independent component analysis. J Neurosci 26(40):10222–10231
Cha J, Jo HJ, Kim HJ, Seo SW, Kim HS, Yoon U, Park H, Na DL, Lee JM (2013) Functional alteration patterns of default mode networks: comparisons of normal aging, amnestic mild cognitive impairment and Alzheimer's disease. Eur J Neurosci 37(12):1916–1924
Chen L, Chen Y, Zheng H, Zhang B, Wang F, Fang J, Li Y, Chen Q, Zhang S (2021) Changes in the topological organization of the default mode network in autism spectrum disorder. Brain Imaging Behav 15(2):1058–1067
Cherkassky VL, Kana RK, Keller TA, Just MA (2006) Functional connectivity in a baseline resting-state network in autism. Neuroreport 17(16):1687–1690
Chételat G, Desgranges B, de la Sayette V, Viader F, Berkouk K, Landeau B, Lalevée C, Le Doze F, Dupuy B, Hannequin D (2003) Dissociating atrophy and hypometabolism impact on episodic memory in mild cognitive impairment. Brain 126(9):1955–1967
Cole MW, Schneider W (2007) The cognitive control network: integrated cortical regions with dissociable functions. NeuroImage 37(1):343–360
Cui Y, Yu S, Zhang T, Zhang Y, Xia Y, Yao D, Guo D (2018) Altered activity and information flow in the default mode network of pilocarpine-induced epilepsy rats. Brain Res 1696:71–80
Ćurčić-Blake B, van der Meer L, Pijnenborg GH, David AS, Aleman A (2015) Insight and psychosis: functional and anatomical brain connectivity and self-reflection in S chizophrenia. Hum Brain Mapp 36(12):4859–4868
Daniel AG, Park KY, Roland JL, Dierker D, Gross J, Humphries JB, Hacker CD, Snyder AZ, Shimony JS, Leuthardt EC (2021) Functional connectivity within glioblastoma impacts overall survival. Neuro-Oncology 23(3):412–421
Danielson NB, Guo JN, Blumenfeld H (2011) The default mode network and altered consciousness in epilepsy. Behav Neurol 24(1):55–65
Davey CG, Pujol J, Harrison BJ (2016) Mapping the self in the brain's default mode network. NeuroImage 132:390–397
Dennis EL, Thompson PM (2014) Functional brain connectivity using fMRI in aging and Alzheimer's disease. Neuropsychol Rev 24(1):49–62

Du Y, Pearlson GD, Yu Q, He H, Lin D, Sui J, Wu L, Calhoun VD (2016) Interaction among subsystems within default mode network diminished in schizophrenia patients: a dynamic connectivity approach. Schizophr Res 170(1):55–65

Esposito R, Mattei PA, Briganti C, Romani GL, Tartaro A, Caulo M (2012) Modifications of default-mode network connectivity in patients with cerebral glioma. PLoS One 7(7):e40231

Faivre A, Rico A, Zaaraoui W, Crespy L, Reuter F, Wybrecht D, Soulier E, Malikova I, Confort-Gouny S, Cozzone PJ, Pelletier J, Ranjeva J-P, Audoin B (2012) Assessing brain connectivity at rest is clinically relevant in early multiple sclerosis. Mult Scler J 18(9):1251–1258

Fang X, Deza-Araujo YI, Petzold J, Spreer M, Riedel P, Marxen M, O'Connor SJ, Zimmermann US, Smolka MN (2021) Effects of moderate alcohol levels on default mode network connectivity in heavy drinkers. Alcohol Clin Exp Res 45(5):1039–1050

Filippini N, MacIntosh BJ, Hough MG, Goodwin GM, Frisoni GB, Smith SM, Matthews PM, Beckmann CF, Mackay CE (2009) Distinct patterns of brain activity in young carriers of the APOE-ε4 allele. Proc Natl Acad Sci 106(17):7209–7214

Fletcher P, McKenna P, Friston K, Frith C, Dolan RJ (1999) Abnormal cingulate modulation of fronto-temporal connectivity in schizophrenia. NeuroImage 9(3):337–342

Fox MD, Snyder AZ, Vincent JL, Corbetta M, Van Essen DC, Raichle ME (2005) The human brain is intrinsically organized into dynamic, anticorrelated functional networks. Proc Natl Acad Sci 102(27):9673–9678

Friston KJ (1998) The disconnection hypothesis. Schizophr Res 30(2):115–125

Friston KJ (1999) Schizophrenia and the disconnection hypothesis. Acta Psychiatr Scand 99:68–79

Garrity AG, Pearlson GD, McKiernan K, Lloyd D, Kiehl KA, Calhoun VD (2007) Aberrant "default mode" functional connectivity in schizophrenia. Am J Psychiatr 164(3):450–457

Ghasemi M, Foroutannia A, Babajani-Feremi A (2021) Characterizing resting-state networks in Parkinson's disease: a multi-aspect functional connectivity study. Brain and behavior 11(5): e02101

Ghumman S, Fortin D, Noel-Lamy M, Cunnane S, Whittingstall K (2016) Exploratory study of the effect of brain tumors on the default mode network. J Neuro-Oncol 128(3):437–444

Gili T, Cercignani M, Serra L, Perri R, Giove F, Maraviglia B, Caltagirone C, Bozzali M (2011) Regional brain atrophy and functional disconnection across Alzheimer's disease evolution. J Neurol Neurosurg Psychiatry 82(1):58–66

Giraldo-Chica M, Woodward ND (2017) Review of thalamocortical resting-state fMRI studies in schizophrenia. Schizophr Res 180:58–63

Gold BT, Johnson NF, Powell DK, Smith CD (2012) White matter integrity and vulnerability to Alzheimer's disease: preliminary findings and future directions. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease 1822(3):416–422

Grady CL, McIntosh AR, Beig S, Keightley ML, Burian H, Black SE (2003) Evidence from functional neuroimaging of a compensatory prefrontal network in Alzheimer's disease. J Neurosci 23(3):986–993

Greicius MD, Krasnow B, Reiss AL, Menon V (2003) Functional connectivity in the resting brain: a network analysis of the default mode hypothesis. Proc Natl Acad Sci 100(1):253–258

Greicius MD, Srivastava G, Reiss AL, Menon V (2004) Default-mode network activity distinguishes Alzheimer's disease from healthy aging: evidence from functional MRI. Proc Natl Acad Sci 101(13):4637–4642

Grieder M, Wang DJJ, Dierks T, Wahlund L-O, Jann K (2018) Default mode network complexity and cognitive decline in mild Alzheimer's disease. Front Neurosci 12:770

Guo X, Duan X, Suckling J, Chen H, Liao W, Cui Q, Chen H (2019) Partially impaired functional connectivity states between right anterior insula and default mode network in autism spectrum disorder. Hum Brain Mapp 40(4):1264–1275

Gusnard DA, Akbudak E, Shulman GL, Raichle ME (2001) Medial prefrontal cortex and self-referential mental activity: relation to a default mode of brain function. Proc Natl Acad Sci 98(7):4259–4264

Gusnard DA, Raichle ME (2001) Searching for a baseline: functional imaging and the resting human brain. Nat Rev Neurosci 2(10):685–694

Hafkemeijer A, van der Grond J, Rombouts SA (2012) Imaging the default mode network in aging and dementia. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease 1822(3): 431–441

Haghighat H, Mirzarezaee M, Araabi B, Khadem A (2021) Functional networks abnormalities in autism Spectrum disorder: age-related hypo and hyper connectivity. Brain Topogr 34:1–17

Harris RJ, Bookheimer SY, Cloughesy TF, Kim HJ, Pope WB, Lai A, Nghiemphu PL, Liau LM, Ellingson BM (2014) Altered functional connectivity of the default mode network in diffuse gliomas measured with pseudo-resting state fMRI. J Neuro-Oncol 116(2):373–379

Heise V, Filippini N, Ebmeier K, Mackay C (2011) The APOE ε4 allele modulates brain white matter integrity in healthy adults. Mol Psychiatry 16(9):908–916

Holt DJ, Cassidy BS, Andrews-Hanna JR, Lee SM, Coombs G, Goff DC, Gabrieli JD, Moran JM (2011) An anterior-to-posterior shift in midline cortical activity in schizophrenia during self-reflection. Biol Psychiatry 69(5):415–423

Hsiao F-J, Yu H-Y, Chen W-T, Kwan S-Y, Chen C, Yen D-J, Yiu C-H, Shih Y-H, Lin Y-Y (2015) Increased intrinsic connectivity of the default mode network in temporal lobe epilepsy: evidence from resting-state MEG recordings. PLoS One 10(6):e0128787

Hu C-Y, Gao X, Long L, Long X, Liu C, Chen Y, Xie Y, Liu C, Xiao B, Hu Z-Y (2017) Altered DMN functional connectivity and regional homogeneity in partial epilepsy patients: a seventy cases study. Oncotarget 8(46):81475

Inglese M, Petracca M (2018) MRI in multiple sclerosis: clinical and research update. Curr Opin Neurol 31(3):249–255

Ismail MM, Keynton RS, Mostapha MM, ElTanboly AH, Casanova MF, Gimel'farb GL, El-Baz A (2016) Studying autism spectrum disorder with structural and diffusion magnetic resonance imaging: a survey. Front Hum Neurosci 10:211

Jack CR Jr, Knopman DS, Jagust WJ, Petersen RC, Weiner MW, Aisen PS, Shaw LM, Vemuri P, Wiste HJ, Weigand SD (2013) Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. The Lancet Neurology 12(2):207–216

Jones D, Machulda M, Vemuri P, McDade E, Zeng G, Senjem M, Gunter J, Przybelski S, Avula R, Knopman D (2011) Age-related changes in the default mode network are more advanced in Alzheimer disease. Neurology 77(16):1524–1531

Jovicich J, Minati L, Marizzoni M, Marchitelli R, Sala-Llonch R, Bartrés-Faz D, Arnold J, Benninghoff J, Fiedler U, Roccatagliata L, Picco A, Nobili F, Blin O, Bombois S, Lopes R, Bordet R, Sein J, Ranjeva J-P, Didic M, Gros-Dagnac H, Payoux P, Zoccatelli G, Alessandrini F, Beltramello A, Bargalló N, Ferretti A, Caulo M, Aiello M, Cavaliere C, Soricelli A, Parnetti L, Tarducci R, Floridi P, Tsolaki M, Constantinidis M, Drevelegas A, Rossini PM, Marra C, Schönknecht P, Hensch T, Hoffmann K-T, Kuijer JP, Visser PJ, Barkhof F, Frisoni GB (2016) Longitudinal reproducibility of default-mode network connectivity in healthy elderly participants: a multicentric resting-state fMRI study. NeuroImage 124: 442–454

Kenna H, Hoeft F, Kelley R, Wroolie T, DeMuth B, Reiss A, Rasgon N (2013) Fasting plasma insulin and the default mode network in women at risk for Alzheimer's disease. Neurobiol Aging 34(3):641–649

Kennedy DP, Redcay E, Courchesne E (2006) Failing to deactivate: resting functional abnormalities in autism. Proc Natl Acad Sci 103(21):8275–8280

Knyazev GG, Slobodskoj-Plusnin JY, Bocharov AV, Pylkova LV (2011) The default mode network and EEG alpha oscillations: an independent component analysis. Brain Res 1402:67–79

Kocher M, Jockwitz C, Caspers S, Schreiber J, Farrher E, Stoffels G, Filss C, Lohmann P, Tscherpel C, Ruge MI, Fink GR, Shah NJ, Galldiks N, Langen K-J (2020) Role of the default mode resting-state network for cognitive functioning in malignant glioma patients following multimodal treatment. NeuroImage. Clinical 27:102287–102287

Lawrence KE, Hernandez LM, Bookheimer SY, Dapretto M (2019) Atypical longitudinal development of functional connectivity in adolescents with autism spectrum disorder. Autism Res 12(1):53–65

Li P, Fan TT, Zhao RJ, Han Y, Shi L, Sun HQ, Chen SJ, Shi J, Lin X, Lu L (2017) Altered brain network connectivity as a potential endophenotype of schizophrenia. Sci Rep 7(1):1–9

Li Q, Cao W, Liao X, Chen Z, Yang T, Gong Q, Zhou D, Luo C, Yao D (2015) Altered resting state functional network connectivity in children absence epilepsy. J Neurol Sci 354(1–2):79–85

Li T, Liao Z, Mao Y, Hu J, Le D, Pei Y, Sun W, Lin J, Qiu Y, Zhu J, Chen Y, Qi C, Ye X, Su H, Yu E (2021) Temporal dynamic changes of intrinsic brain activity in Alzheimer's disease and mild cognitive impairment patients: a resting-state functional magnetic resonance imaging study. Annals of translational medicine 9(1):63–63

Li W, Mai X, Liu C (2014) The default mode network and social understanding of others: what do brain connectivity studies tell us. Front Hum Neurosci 8:74

Li Y-T, Chang C-Y, Hsu Y-C, Fuh J-L, Kuo W-J, Yeh J-NT, Lin F-H (2020) Impact of physiological noise in characterizing the functional MRI default-mode network in Alzheimer's disease. J Cereb Blood Flow Metab 41(1):166–181

Liang X, He Y, Salmeron BJ, Gu H, Stein EA, Yang Y (2015) Interactions between the salience and default-mode networks are disrupted in cocaine addiction. J Neurosci 35(21):8081–8090

Liu D, Hu X, Liu Y, Yang K, Xiao C, Hu J, Li Z, Zou Y, Chen J, Liu H (2019) Potential intra-or cross-network functional reorganization of the triple unifying networks in patients with frontal glioma. World Neurosurg 128:e732–e743

Louapre C, Perlbarg V, García-Lorenzo D, Urbanski M, Benali H, Assouad R, Galanaud D, Freeman L, Bodini B, Papeix C, Tourbah A, Lubetzki C, Lehéricy S, Stankoff B (2014) Brain networks disconnection in early multiple sclerosis cognitive deficits: an anatomofunctional study. Hum Brain Mapp 35(9):4706–4717

Lynch CJ, Uddin LQ, Supekar K, Khouzam A, Phillips J, Menon V (2013) Default mode network in childhood autism: posteromedial cortex heterogeneity and relationship with social deficits. Biol Psychiatry 74(3):212–219. https://doi.org/10.1016/j.biopsych.2012.1012.1013. Epub 2013 Jan 1031

Ma N, Liu Y, Fu X-M, Li N, Wang C-X, Zhang H, Qian R-B, Xu H-S, Hu X, Zhang D-R (2011) Abnormal brain default-mode network functional connectivity in drug addicts. PLoS One 6(1): e16560

Maesawa S, Bagarinao E, Fujii M, Futamura M, Motomura K, Watanabe H, Mori D, Sobue G, Wakabayashi T (2015) Evaluation of resting state networks in patients with gliomas: connectivity changes in the unaffected side and its relation to cognitive function. PLoS One 10(2): e0118072

Mak LE, Minuzzi L, MacQueen G, Hall G, Kennedy SH, Milev R (2017) The default mode network in healthy individuals: a systematic review and meta-analysis. Brain Connect 7(1): 25–33

Makris N, Biederman J, Valera EM, Bush G, Kaiser J, Kennedy DN, Caviness VS, Faraone SV, Seidman LJ (2007) Cortical thinning of the attention and executive function networks in adults with attention-deficit/hyperactivity disorder. Cereb Cortex 17(6):1364–1375

Maniar Y, Peck K, Jenabi M, Gene M, Holodny A (2021) Functional MRI shows altered deactivation and a corresponding decrease in functional connectivity of the default mode network in patients with gliomas. Am J Neuroradiol 42(8):1505–1512

Mars R, Neubert F-X, Noonan M, Sallet J, Toni I, Rushworth M (2012) On the relationship between the "default mode network" and the "social brain". Front Hum Neurosci 6:189

Martino M, Magioncalda P, El Mendili MM, Droby A, Paduri S, Schiavi S, Petracca M, Inglese M (2020) Depression is associated with disconnection of neurotransmitter-related nuclei in multiple sclerosis. Mult Scler J 27(7):1102–1111

Mash LE, Linke AC, Olson LA, Fishman I, Liu TT, Müller RA (2019) Transient states of network connectivity are atypical in autism: a dynamic functional connectivity study. Hum Brain Mapp 40(8):2377–2389

Mevel K, Chételat G, Eustache F, Desgranges B (2011) The default mode network in healthy aging and Alzheimer's disease. Int J Alzheimers Dis 2011:535816

Nejad-Davarani SP, Chopp M, Peltier S, Li L, Davoodi-Bojd E, Lu M, Bagher-Ebadian H, Budaj J, Gallagher D, Ding Y (2016) Resting state fMRI connectivity analysis as a tool for detection of abnormalities in five different cognitive networks of the brain in multiple sclerosis patients. Clinical case reports and reviews 2(9):464

Nomi JS, Uddin LQ (2015) Developmental changes in large-scale network connectivity in autism. NeuroImage: Clinical 7:732–741

Oblak AL, Rosene DL, Kemper TL, Bauman ML, Blatt GJ (2011) Altered posterior cingulate cortical cyctoarchitecture, but normal density of neurons and interneurons in the posterior cingulate cortex and fusiform gyrus in autism. Autism Res 4(3):200–211

Ostrom QT, Bauchet L, Davis FG, Deltour I, Fisher JL, Langer CE, Pekmezci M, Schwartzbaum JA, Turner MC, Walsh KM, Wrensch MR, Barnholtz-Sloan JS (2014) The epidemiology of glioma in adults: a "state of the science" review. Neuro-Oncology 16(7):896–913

Padmanabhan A, Lynch CJ, Schaer M, Menon V (2017) The default mode network in autism. Biological Psychiatry: Cognitive Neuroscience and Neuroimaging 2(6):476–486

Palmqvist S, Schöll M, Strandberg O, Mattsson N, Stomrud E, Zetterberg H, Blennow K, Landau S, Jagust W, Hansson O (2017) Earliest accumulation of β-amyloid occurs within the default-mode network and concurrently affects brain connectivity. Nat Commun 8(1):1–13

Pinter D, Beckmann C, Koini M, Pirker E, Filippini N, Pichler A, Fuchs S, Fazekas F, Enzinger C (2016) Reproducibility of resting state connectivity in patients with stable multiple sclerosis. PLoS One 11(3):e0152158

Raichle ME (2015) The brain's default mode network. Annu Rev Neurosci 38:433–447

Raichle ME, MacLeod AM, Snyder AZ, Powers WJ, Gusnard DA, Shulman GL (2001) A default mode of brain function. Proc Natl Acad Sci 98(2):676–682

Riccitelli GC, Pagani E, Meani A, Valsasina P, Preziosa P, Filippi M, Rocca MA (2020) Cognitive impairment in benign multiple sclerosis: a multiparametric structural and functional MRI study. J Neurol 267(12):3508–3517

Riehemann S, Volz HP, Stützer P, Smesny S, Gaser C, Sauer H (2001) Hypofrontality in neuroleptic-naive schizophrenic patients during the Wisconsin card sorting test-a fMRI study. Eur Arch Psychiatry Clin Neurosci 251(2):66–71

Rocca M, Riccitelli G, Rodegher M, Ceccarelli A, Falini A, Falautano M, Meani A, Comi G, Filippi M (2010a) Functional MR imaging correlates of neuropsychological impairment in primary-progressive multiple sclerosis. Am J Neuroradiol 31(7):1240–1246

Rocca MA, Valsasina P, Absinta M, Riccitelli G, Rodegher ME, Misci P, Rossi P, Falini A, Comi G, Filippi M (2010b) Default-mode network dysfunction and cognitive impairment in progressive MS. Neurology 74(16):1252–1259

Romero-Garcia R, Suckling J, Owen M, Assem M, Sinha R, Coelho P, Woodberry E, Price SJ, Burke A, Santarius T, Erez Y, Hart MG (2021) Memory recovery in relation to default mode network impairment and neurite density during brain tumor treatment. J Neurosurg 136(2): 358–368

Ruppert MC, Greuel A, Freigang J, Tahmasian M, Maier F, Hammes J, van Eimeren T, Timmermann L, Tittgemeyer M, Drzezga A, Eggers C (2021) The default mode network and cognition in Parkinson's disease: a multimodal resting-state network approach. Hum Brain Mapp 42(8):2623–2641

Samson D, Apperly IA, Chiavarino C, Humphreys GW (2004) Left temporoparietal junction is necessary for representing someone else's belief. Nat Neurosci 7(5):499–500

Saxe R (2006) Uniquely human social cognition. Curr Opin Neurobiol 16(2):235–239

Scherr M, Utz L, Tahmasian M, Pasquini L, Grothe MJ, Rauschecker JP, Grimmer T, Drzezga A, Sorg C, Riedl V (2021) Effective connectivity in the default mode network is distinctively disrupted in Alzheimer's disease—a simultaneous resting-state FDG-PET/fMRI study. Hum Brain Mapp 42(13):4134–4143

Schneider FC, Royer A, Grosselin A, Pellet J, Barral F-G, Laurent B, Brouillet D, Lang F (2011) Modulation of the default mode network is task-dependant in chronic schizophrenia patients. Schizophr Res 125(2–3):110–117

Schouwenaars IT, de Dreu MJ, Rutten G-JM, Ramsey NF, Jansma JM (2020) A functional MRI study of presurgical cognitive deficits in glioma patients. Neuro-oncology practice 8(1):81–90

Schouwenaars IT, de Dreu MJ, Rutten G-JM, Ramsey NF, Jansma JM (2021) A functional MRI study of presurgical cognitive deficits in glioma patients. Neuro-oncology practice 8(1):81–90

Shine JM, O'Callaghan C, Halliday GM, Lewis SJ (2014) Tricks of the mind: visual hallucinations as disorders of attention. Prog Neurobiol 116:58–65

Shulman GL, Corbetta M, Buckner RL, Fiez JA, Miezin FM, Raichle ME, Petersen SE (1997) Common blood flow changes across visual tasks: I. increases in subcortical structures and cerebellum but not in nonvisual cortex. J Cogn Neurosci 9(5):624–647

Silberstein RB, Pipingas A, Farrow M, Levy F, Stough CK (2016) Dopaminergic modulation of default mode network brain functional connectivity in attention deficit hyperactivity disorder. Brain and behavior 6(12):e00582–e00582

Sjøgård M, Wens V, Van Schependom J, Costers L, D'Hooghe M, D'Haeseleer M, Woolrich M, Goldman S, Nagels G, De Tiège X (2021) Brain dysconnectivity relates to disability and cognitive impairment in multiple sclerosis. Hum Brain Mapp 42(3):626–643

Sonuga-Barke EJS, Castellanos FX (2007) Spontaneous attentional fluctuations in impaired states and pathological conditions: a neurobiological hypothesis. Neurosci Biobehav Rev 31(7): 977–986

Sridharan D, Levitin DJ, Menon V (2008) A critical role for the right fronto-insular cortex in switching between central-executive and default-mode networks. Proc Natl Acad Sci 105(34): 12569–12574

Tang C, Wei Y, Zhao J, Nie J (2018) Different developmental pattern of brain activities in ADHD: a study of resting-state fMRI. Dev Neurosci 40(3):246–257

Tepper S, Ashina M, Reuter U, Brandes JL, Doležil D, Silberstein S, Winner P, Leonardi D, Mikol D, Lenz R (2017) Safety and efficacy of erenumab for preventive treatment of chronic migraine: a randomised, double-blind, placebo-controlled phase 2 trial. The Lancet Neurology 16(6):425–434

Tessitore A, Santangelo G, De Micco R, Giordano A, Raimo S, Amboni M, Esposito F, Barone P, Tedeschi G, Vitale C (2017) Resting-state brain networks in patients with Parkinson's disease and impulse control disorders. Cortex 94:63–72

Tian L, Jiang T, Wang Y, Zang Y, He Y, Liang M, Sui M, Cao Q, Hu S, Peng M, Zhuo Y (2006) Altered resting-state functional connectivity patterns of anterior cingulate cortex in adolescents with attention deficit hyperactivity disorder. Neurosci Lett 400(1):39–43

Tijhuis FB, Broeders TAA, Santos FAN, Schoonheim MM, Killestein J, Leurs CE, van Geest Q, Steenwijk MD, Geurts JJG, Hulst HE, Douw L (2021) Dynamic functional connectivity as a neural correlate of fatigue in multiple sclerosis. NeuroImage Clinical 29:102556–102556

Tordjman M, Madelin G, Gupta PK, Cordova C, Kurz SC, Orringer D, Golfinos J, Kondziolka D, Ge Y, Wang RL (2021) Functional connectivity of the default mode, dorsal attention and fronto-parietal executive control networks in glial tumor patients. J Neuro-Oncol 152(2):347–355

Uddin LQ, Kelly AMC, Biswal BB, Margulies DS, Shehzad Z, Shaw D, Ghaffari M, Rotrosen J, Adler LA, Castellanos FX, Milham MP (2008) Network homogeneity reveals decreased integrity of default-mode network in ADHD. J Neurosci Methods 169(1):249–254

Van Dellen E, de Witt Hamer P, Douw L, Klein M, Heimans J, Stam C, Reijneveld J, Hillebrand A (2013) Connectivity in MEG resting-state networks increases after resective surgery for low-grade glioma and correlates with improved cognitive performance. NeuroImage: Clinical 2:1–7

van Dellen E, de Witt Hamer PC, Douw L, Klein M, Heimans JJ, Stam CJ, Reijneveld JC, Hillebrand A (2012) Connectivity in MEG resting-state networks increases after resective surgery for low-grade glioma and correlates with improved cognitive performance. NeuroImage. Clinical 2:1–7

van Eimeren T, Monchi O, Ballanger B, Strafella AP (2009) Dysfunction of the default mode network in Parkinson disease: a functional magnetic resonance imaging study. Arch Neurol 66(7):877–883

van Rooij D, Hartman CA, Mennes M, Oosterlaan J, Franke B, Rommelse N, Heslenfeld D, Faraone SV, Buitelaar JK, Hoekstra PJ (2015) Altered neural connectivity during response inhibition in adolescents with attention-deficit/hyperactivity disorder and their unaffected siblings. NeuroImage. Clinical 7:325–335

Vatansever D, Bozhilova NS, Asherson P, Smallwood J (2019) The devil is in the detail: exploring the intrinsic neural mechanisms that link attention-deficit/hyperactivity disorder symptomatology to ongoing cognition. Psychol Med 49(7):1185–1194

Villain N, Desgranges B, Viader F, De La Sayette V, Mézenge F, Landeau B, Baron J-C, Eustache F, Chételat G (2008) Relationships between hippocampal atrophy, white matter disruption, and gray matter hypometabolism in Alzheimer's disease. J Neurosci 28(24): 6174–6181

Wang L, Zang Y, He Y, Liang M, Zhang X, Tian L, Wu T, Jiang T, Li K (2006) Changes in hippocampal connectivity in the early stages of Alzheimer's disease: evidence from resting state fMRI. NeuroImage 31(2):496–504

Wang Q, He C, Wang Z, Zhang Z, Xie C (2020) Dynamic connectivity alteration facilitates cognitive decline in Alzheimer's disease Spectrum. Brain Connect 11(3):213–224

Wang Y, Qin Y, Li H, Yao D, Sun B, Li Z, Li X, Dai Y, Wen C, Zhang L (2019) Abnormal functional connectivity in cognitive control network, default mode network, and visual attention network in internet addiction: a resting-state fMRI study. Front Neurol 10:1006

Weil RS, Winston JS, Leyland LA, Pappa K, Mahmood RB, Morris HR, Rees G (2019) Neural correlates of early cognitive dysfunction in Parkinson's disease. Annals of clinical and translational neurology 6(5):902–912

Weissman DH, Roberts KC, Visscher KM, Woldorff MG (2006) The neural bases of momentary lapses in attention. Nat Neurosci 9(7):971–978

Wiggins JL, Peltier SJ, Ashinoff S, Weng S-J, Carrasco M, Welsh RC, Lord C, Monk CS (2011) Using a self-organizing map algorithm to detect age-related changes in functional connectivity during rest in autism spectrum disorders. Brain Res 1380:187–197

Woodward ND, Karbasforoushan H, Heckers S (2012) Thalamocortical dysconnectivity in schizophrenia. Am J Psychiatr 169(10):1092–1099

Worhunsky PD, Angarita GA, Matuskey D, Gallezot J-D, Malison RT, Carson RE, Potenza MN (2021) Multimodal investigation of dopamine D 2/D 3 receptors, default mode network suppression, and cognitive control in cocaine-use disorder. Neuropsychopharmacology 46(2): 316–324

Xu X, Yuan H, Lei X (2016) Activation and connectivity within the default mode network contribute independently to future-oriented thought. Sci Rep 6(1):21001

Yang S, Zhang Z, Chen H, Meng Y, Li J, Li Z, Xu Q, Zhang Q, Fan YS, Lu G (2021) Temporal variability profiling of the default mode across epilepsy subtypes. Epilepsia 62(1):61–73

Yao N, Shek-Kwan Chang R, Cheung C, Pang S, Lau KK, Suckling J, Rowe JB, Yu K, Ka-Fung Mak H, Chua SE (2014) The default mode network is disrupted in Parkinson's disease with visual hallucinations. Hum Brain Mapp 35(11):5658–5666

Yildirim E, Büyükişcan ES (2019) Default mode network connectivity in Alzheimer's disease. Turk J Psychiatry 30(4):279–286

Ypma RJ, Moseley RL, Holt RJ, Rughooputh N, Floris DL, Chura LR, Spencer MD, Baron-Cohen S, Suckling J, Bullmore ET (2016) Default mode hypoconnectivity underlies a sex-related autism spectrum. Biological Psychiatry: Cognitive Neuroscience and Neuroimaging 1(4):364–371

Zhang H-Y, Wang S-J, Liu B, Ma Z-L, Yang M, Zhang Z-J, Teng G-J (2010) Resting brain connectivity: changes during the progress of Alzheimer disease. Radiology 256(2):598–606

Zhang H, Shi Y, Yao C, Tang W, Yao D, Zhang C, Wang M, Wu J, Song Z (2016) Alteration of the intra- and cross- hemisphere posterior default mode network in frontal lobe glioma patients. Sci Rep 6(1):26972

Zhang X, Cao X, Xue C, Zheng J, Zhang S, Huang Q, Liu W (2021) Aberrant functional connectivity and activity in Parkinson's disease and comorbidity with depression based on radiomic analysis. Brain and behavior 11(5):e02103

Zhou Y, Liang M, Tian L, Wang K, Hao Y, Liu H, Liu Z, Jiang T (2007) Functional disintegration in paranoid schizophrenia using resting-state fMRI. Schizophr Res 97(1–3):194–205

Zhu X, Zhu Q, Jiang C, Shen H, Wang F, Liao W, Yuan F (2017) Disrupted resting-state default mode network in betel quid-dependent individuals. Front Psychol 8:84

# Neuroendocrine Carcinoma of Endometrium Convention Treatment Approach to Nanomedicine

15

Md Akbar, Hasan Ali, Shweta Srivastav, Kainat Alam, Shipra Daniel, and Faraat Ali

**Abstract**

Endometrial carcinoma is the fourth most common malignancy in the female population worldwide. Subsequent death due endometrial carcinoma is around 12,590 in the US in 2020. Patients with type II and advanced endometrial cancer do not respond well to the current treatments. Therefore, endometrial cancer should be better understood in order to develop more effective treatments. Neuroendocrine carcinoma (NECa) of the endometrium is an uncommon tumour. Identifying an appropriate therapeutic management for SCC of endometrium is challenging since these are extremely rare tumours. An optimal initial therapeutic approach to this rare disease, especially at an advanced stage, has not yet been clearly defined. However, in these a multidisciplinary therapy, including surgery, chemotherapy, and radiotherapy represent until this time the only therapeutic option. Neuroendocrine carcinomas (NEC) of the female genital tract are aggressive and rare tumours that usually involve the cervix and ovary, and are seen rarely in the endometrium in perimenopausal or postmenopausal women But, their prospective role in gNECs still remains uncertain because of the small prevalence. Few subjective proofs exist on the effectiveness of ICIs in gNECs and mostly comes from cervical gNECs Inception of more effective treatment

M. Akbar (✉)
Department of Pharmaceutical Medicine, Faculty of Pharmacy, Jamia Hamdard, New Delhi, India

H. Ali · S. Srivastav · F. Ali
Kanpur Institute of Technology, Kanpur, Uttar Pradesh, India

K. Alam
KKECS College of Nursing, Bengaluru, Karnataka, India

S. Daniel
Christian College of Nursing, Faculty of Health Sciences, Sam Higginbottom University of Agriculture, Technology & Sciences, Allahabad, Uttar Pradesh, India



M. Rahman et al. (eds.), *Hormone Related Cancer Mechanistic and Nanomedicines*,
https://doi.org/10.1007/978-981-19-5558-7_15

choices are intensely needed. In the generation of précised and accurate medicine and therapeutic systems, novel target focused treatments could change the pattern of care for these uncommon cancers, particularly in the recurrent situation. Though, a number of immunotherapies and targeted molecules have shown efficacy in other high-grade neuroendocrine tumours strategies focused on the HIF-1α pathway, the mTOR pathway as well as the immunotherapy. Nanomedicine showed great potential in endometrial cancer's early diagnosis, metastasis determination and treatment.

**Keywords**

Endometrial · Neuroendocrine carcinomas · Immunotherapies · Conventional · Nanomedicine

## 15.1 Introduction

Human body is made up of trillions of cells, and the cells are the basic building block of all living things. Normal cells undergo a regulated cell division process to form new cell when body requires them or when cells grow old, become damaged or they die. When this regulated process breaks down, and abnormal or damaged cells grow and multiply, then it leads to cancer. Cancer is a type of disorder in which the abnormal cells of body divide uncontrollably and move towards other body parts. These abnormal cells may form tumors, which are a mass of tissue. Tumors are of two types: one is malignant and the other is benign. Malignant tumors lead to cancer while benign tumors are non-cancerous and they do not spread to nearby tissue. Metastasis is a process by which cancer cell spreads to other parts of the body. Many types of cancer form solid tumors but blood cancer like leukemias do not form solid tumors. On removal of malignant tumors, there is chance of grow back but it usually do not grow in case of benign tumor. Sometimes benign tumors are large such as benign tumors in the brain and cause serious symptoms which may be fatal (National Cancer Institute 2022).

The diffuse neuroendocrine cell system gives rise to range of malignancies like neuroendocrine tumors. Its prognosis mainly depends on its histological subtype and its origin site. Well-differentiated neoplasms (i.e., carcinoid and atypical carcinoid) are clinically and morphologically different from high-grade or poorly differentiated neuroendocrine carcinoma (i.e., small cell and large cell). This high-grade neuroendocrine carcinoma is closely related to highly aggressive pulmonary small-cell carcinoma which is managed by platinum-based chemotherapy, a multimodality approach (Moertel et al. 1991).

Generic neuroendocrine marker expression, i.e., expression of the markers synaptophysin and chromogranin which is detected by immunohistochemistry and due to this, well and poorly differentiated neuroendocrine tumors are grouped together. The clinical and biological outcome of poorly differentiated neuroendocrine carcinomas, nonetheless, are boundlessly not the same as very much separated

from the well-differentiated neuroendocrine tumors. Recent investigation at Memorial Sloan Kettering had suggested that neuroendocrine carcinoma is correspondingly similar to de novo carcinoma and it has non-neuroendocrine cell lineage (Yao et al. 2008).

## 15.2 Neuroendocrine System

The neuroendocrine system exists in concordance in a combination of both the nervous system (central and peripheral) and the endocrine system. This is a type of homeostasis regulatory system. The cells of this neuroendocrine system are nerve like structure and are highly specialized. These cells in response to nervous and chemical input synthesize, store, and release neurohormones, thus acting like receptor-effector units. Neurohormones are made up of amino acid and are stored in synaptic like vesicles (neurosecretory granules), and it plays different roles from endocrine and paracrine to neurotransmitter and neuromodulatory (Rindi and Inzani 2020; DeLellis 2001).

The cellular expression of neuroendocrine, which is detected by immunohistochemistry, acts as a marker of NE differentiation. The cells of neuroendocrine are characterized by the expression of several NE markers such as CD56, synaptophysin (SYN), chromogranin-A (CgA), and neuron-specific enolase (NSE), which are most frequently employed for IHC detection (Tempfer et al. 2018).

Protein gene product 9.5 (PGP 9.5), CD57, synaptic vesicle protein 2 (SV2), and wide-spectrum cytokeratins (CK) are the other NE markers and the most sensitive markers are CD56 and SYN. CD56 lacks specificity; thus, its isolated finding is not sufficient to confirm NE differentiation while CgA lacks sensitivity, its expression is diagnostic of NE differentiation and thus it is considered as most specific NE marker (Kyriakopoulos et al. 2018; Eriksson et al. 2000). The neuroendocrine system is divided into two categories, i.e., confined and diffuse. The confined NES is organized in distinct organs including hypothalamus, neurohypophysis, adrenal medulla, ganglia, and paraganglia while the diffuse NES system dissipates the NE cells all over the body specially within lungs, gastrointestinal tract, pancreas, thyroid, thymus, and genitourinary system (Caruso et al. 2021).

## 15.3 Neuroendocrine Carcinoma

Cancer that begins in specialized cells are neuroendocrine tumors, and these specialized cells are known as neuroendocrine cells. These neuroendocrine tumors arise scarcely but it takes place anywhere in the body, mostly it occurs in lungs, appendix, small intestine, rectum, and pancreas. NE tumors are of different types; some grow slowly and some grow rapidly, some produce excess hormones (functional neuroendocrine tumors) while some of them don't release enough amount of hormones to cause symptoms (nonfunctional neuroendocrine tumors). Its diagnosis and treatment depend on the type of tumor, its location, whether it produces excess

hormones, how aggressive it is, and whether it has spread to other parts of the body (Mayo Clinic 2022).

These NE tumors firstly don't always cause sign and symptoms as their symptoms depend on their location and quantity of hormones they produce. Generally, the symptoms include growing lump under the skin and pain from that lump, feeling tired, and loss of weight without attempting. NE tumors which produces excess hormones may cause symptoms like skin flushing, diarrhoea, frequent urination, increase in thirst, shakiness, skin rash, and dizziness (Mayo Clinic 2022).

Particular reason of NE carcinoma is not yet known. These cancers emerge in neuroendocrine cells which have similar traits of nerve cell and hormones producing cell. These NE cells are found throughout the body, and when these cells cause mutation in their DNA, then these neuroendocrine tumor develops. The DNA contains all the necessary information required for the functioning of cell. The changes occur in DNA due to mutation tells the endocrine cell to multiply rapidly and form tumor. Some NE tumors grows very slowly while others are aggressive tumors that invade and destroy normal body tissue or spread (metastasize) to other parts of the body rapidly (Mayo Clinic 2022).

## 15.4 Endometrial Carcinoma

Endometrial neuroendocrine carcinoma (NECE) is an infrequent histological type of endometrial carcinoma, with the manifestation of high invasiveness and deprived prognosis. Generally, at diagnosis, majority of the cases are in the advanced stage of the disease without precise clinical presentations. However, most of subjects take appointment with the doctor for vaginal bleeding, and rest of the patients seek medical assistance for paraneoplastic syndrome (such as retinopathy and Cushing's syndrome). Nevertheless, post-surgery pathological check-ups confirm that the subject had neuroendocrine carcinoma of the endometrium (Atienza-Amores et al. 2014).

## 15.5 Diagnosis

Occurrence of cervical cancer is about 2% of total small cell carcinoma. The frequency of these tumors is increasing due to increase in its recognition and accuracy of diagnostic method. The median age of diagnosis is in fifth decades which lies down in a range of 21–87 years. Its salient symptoms are vaginal bleeding with frequentation on cervical mass is identified. On examination, abnormal pap smear test may occur. In some case, patients may exhibit evidence of abnormal hormone production, including vasopressin in SIADH, corticotropin in Cushing's syndrome, insulin in hypoglycaemia, parathormone in hypercalcemia, and serotonin in carcinoid syndrome. Cervical biopsy method is used as a diagnostic tool for cervical cancer, on obtaining an inadequate amount of tissue results in poorly

distinguished cervical carcinoma. Generally, the neuroendocrine component is recognized later which is followed by hysterectomy.

Staging of endometrial NECs follows for common cervical cancer. However, it is of utmost importance to know the augmented risk for lymph-vascular space invasion (LVSI) and increased rate of extrapelvic relapses which is associated with an unskilled prognosis. For instance, initial lymphatic extension to the local lymph nodes was noted in 40% of stage IB small cell carcinoma 3 cm in diameter, and 60% of small cell carcinomas demonstrated LVSI at the time of diagnosis. Average time to relapse was 19.9 months. Lungs supraclavicular lymph nodes and bones were the most common sites of extrapelvic disease spread. Radiographic assessment should usually include either a CT or PET/CT scan. PET/CT imaging may be better tool to consider, in case of high rate of distant metastatic spread. Head CT is not necessary on early assessment for small cell tumors of the cervix. In a 14-year retrospective study, no cranial metastatic cases were observed on initial patient presentation (Lee et al. 2009; Hoskins et al. 2003; Gardner et al. 2011).

## 15.6 Neuroendocrine Endometrium Carcinoma Management

Because of the infrequency of NECE and the insufficiency of potential data to direct the treatment strategies, there are no typical treatment recommendations or guideline for these types of cancers. Treatment of NECE is still based on the conventional treatments of endometrial cancer and small cell lung cancer, like chemotherapy surgical removal and radiation therapy (Sidibe et al. 2018; Posligua et al. 2008). Treatment contemplations for small cell and large cell NEC of the endometrium take into account of the treatment possibilities for endometrial cancer, and draw on the data for treating small cell lung carcinoma. New findings back the use of cisplatin with or without combination of etoposide in small cell and large cell NEC to increase the survival (Embry et al. 2011).

## 15.7 Chemoradiation

As such there are no prospective figures to compare surgical procedure with initial chemoradiation for small cervical NECs, which can be manoeuvred by surgery. For non-surgical or advanced stage condition of cancer, chemo-radiation is judicious. Subjects with sign of lymphadenopathy or FDG-avid nodal basins might also be a subject for initial chemoradiation. To deduce from lung tumor regimens, chemoradiation in combination with cisplatin/etoposide with simultaneous pelvic radiation is good. A fruitful management of cervical small cell carcinoma employing chemoradiation in combination with cisplatin/etoposide, for initial stage to advanced stage disease, has been reported Metastasis was the maximum about 28%, and clinical results associated with initial disease extent (Hoskins et al. 2003).

## 15.8 Chemotherapy

For initial stage disease, subjects with comprehensive surgical procedure should be given adjuvant chemotherapy. In a study, survival up to 5 years of about 68% of patients who were given vincristine, adriamycin, and cyclophosphamide rotating with cisplatin and etoposide (VAC/PE) regimen as compared to 33% for those treated with a combination of cisplatin, vinblastine, and bleomycin (PVB) (Chang et al. 1998). It has also been observed that chemotherapy after surgery, either with EP or VAC/PE, was connected with a noteworthy advantage of survival. Adjuvant chemotherapy is useful for the survival rate of subjects with or without lymph node metastasis. Moreover, there was no extra survival rate with the adjuvant radiotherapy (Boruta II et al. 2001). In a clinical study, extraordinarily alike advantages of adjuvant chemotherapy were reported in the subjects with initial stage of cancer, who received post-surgical EP, experienced an 83% 3-year recurrence-free survival as compared to 0% for subjects who did not underwent adjuvant chemotherapy. Remarkably, because of less toxicity, EP regimens are routinely preferred over VAC-containing regimens (Zivanovic et al. 2009).

## 15.9 Pelvic Radiation

In a clinical study on 14 subjects, with timely diagnosis and treatment in initial stage of the disease with surgical procedure in combination with radiation for positive nodes or other high-risk features shows traditional mode of treatment are not effective in early-stage neuroendocrine small-cell cervical carcinoma (Sheets et al. 1988). After the treatment, cancer relapsed in all subjects, among them two died at the time of the report. In another study, 12 patients went through the surgery with radiation as adjuvant therapy for positive lymph node metastatic carcinoma or close surgical margins (Sevin et al. 1996). Four out of five subjects who underwent radiation therapy died of the disease had pelvic relapses. The subjects with minor lesions who underwent adjuvant radiation therapy were only survived, of the whole study group. However, these retrospective studies did not comprise any adjuvant chemotherapy, and do not seem to support the use of adjuvant radiation. In another study, among the 23 subjects, adjuvant radiation did not increase the survival of the subjects (Chang et al. 1998). In a clinical study, patients who underwent adjuvant radiation therapy likely to had a poor prognosis than the patients who did not went through radiation therapy with a survival of 5 years. This finding was consistent even after excluding those patients with minor tumors (Lee et al. 2008).

## 15.10 Nanomedicine in Cancer Therapy

Cancer is still thought to be one of the most problematic health complications. However, there are number of approved therapeutic molecules that can be employed in the treatment of malignancies, with obstacles of the treatment being drug delivery

and drug resistance. Moreover, conventional cancer treatment is hindered due to the varied pathophysiological features of malignancies and their unusual blood circulation and blood vessel architecture and hence function. Consequently, looking for technological tools that can enhance the efficacy of the treatment such as nanoparticles (NPs) is of vital importance. NPs have many properties such as their small size, ability to load various drugs and large surface area, and ability to increase the absorption of conjugated. Hence, the NPs are considered as outstanding tumor-targeting carriers. In recent times, nanocarriers encompassing polymeric nanoparticles, liposomes, nanocapsules, magnetic nanoparticles, dendrimers, and lipid nanoparticles have been employed as conjugates. The AmBisome® (amphotericin B liposomal) and Doxil® (liposomal doxorubicin) are few instances of approved conjugated antineoplastic NPs. There are numerous other conjugated antineoplastic drugs, those are in different phases of clinical trials for effective management of various other cancers (El-Readi and Althubiti 2019).

The management of EMNECs is a big challenge in clinical settings due to its rarity, primarily and differentiation from other related tumors, secondarily. Consequently, no nanotechnological advancements are observed for the treatment of ENEMC. Additionally, a very less findings are observed in tumors of other endocrine origins. For instance, ligand-anchored targeted liposomes have the promising characteristics to improve the therapeutic efficacy of antitumor drugs. A study was performed to evaluate the capability of a peptide targeting agent, i.e., antagonist G, able to hinder the action of multiple neuropeptides. Long circulating liposomes, LCL, and stabilized antisense lipid particles containing ionizable amino lipid, SALP were synthesized in order to enhance the targeting and uptake to H69 and H82 small cell lung cancer (SCLC) cell lines. Antagonist G anchored LCL, and SALP were synthesized by two different techniques (one is by direct covalent link at activated PEG, grafted onto the liposomal surface and another by post insertion of DSPE-PEG antagonist-G-conjugates into pre-formed liposomes, i.e., ex situ synthesis). Uptake of the liposomes in SCLC target cells was exhibited by fluorescence microscopic technique employing fluorescence-anchored liposomes. It was confirmed quantitatively with the help of [$^{3}$H]-CHE-anchored liposomal formulation. An antisense oligodeoxynucleotide against the overexpressed oncogene *c-myc*(*as(c-myc)*) was proficiently entrapped into SALP, the entrapment efficiency reduced because of the insertion of the targeting ligand. Physico-chemical properties of *as(c-myc)* has substantial effect on the size of liposomes. The extent of the concentration of antagonist G anchored to the surface of the liposomes was affected by the conjugation method as well as lipid composition used. It was observed that covalent linking of antagonist G enhanced the cellular uptake and accumulation of liposomes through clathrin-dependent endocytosis, receptor-mediated internalization in SCLC cell lines. Tissue distribution studies in mice shown a better lung accumulation of antagonist G–targeted SALP as compared to the non-targeted one. Accumulation antagonist G–targeted SALP in lungs was up to three times higher after 24 h of administration, exhibiting the importance of their probable use as drug delivery carriers for SCLC treatment (Carvalheiro et al. 2022). The occurrence of pancreatic neuroendocrine tumor (PNET) is constantly increasing. Because of their sluggish

feature, PNET patients frequently manifested with incurable, metastatic malignancy. New types of treatment regimen are instantly required. It has been exhibited that Receptor for Hyaluronic Acid-Mediated Motility isoform B ($RHAMM^B$) and Bcl-xL are overexpressed in PNETs, interestingly both of them are found to be responsible for PNET metastasis. Since RHAMM is untraceable in majority of the adult tissues, it was assumed that $RHAMM^B$ could be a potential gateway for nano-drug delivery into PNETs. To assess this assumption, a $RHAMM^B$-targeting nanocarrier was developed. In this nanocarrier, siBcl-xL, a small interfering RNA (siRNA) against Bcl-xL and KLA, a mitochondria-fusing peptide were incorporated. It was observed that $RHAMM^B$-positive PNETs picked up the $RHAMM^B$-anchored nanocarriers. It was seen that either siBcl-xL or KLA alone destroyed approximately 30% of PNET cells. Whereas, a synergistic effect was exhibited by the co-delivery of siBcl-xL and KLA peptide in vitro. Unpredictably, siBcl-xL prompted cell death was seen before reducing Bcl-xL protein levels. Direct injection of $RHAMM^B$-anchored nanocarriers carrying siBcl-xL and KLA peptide, into the systemic circulation, substantially decreased the tumor size in mice bearing $RHAMM^B$-positive PNETs. Collectively, these outcomes showed that the $RHAMM^B$-targeting nanomedicine functions as a potential drug carrier system for PNET and probably other malignancies with upregulated $RHAMM^B$. The combination of siBcl-xL and KLA peptide could be a potential therapy for the management of PNET (Chen et al. 2021; Carvalheiro et al. 2022).

## 15.11 Targeted Therapies and Future Perspectives

The therapeutic management of gNECs is a challenging task in clinical practice. In spite of various surgery-based treatment modalities, still the CHT and RT prognosis is not up to the mark, in the maximum cases, the survival is still not more than two years (Gardner et al. 2011). Inception of more effective treatment choices are intensely needed. In the generation of précised and accurate medicine and therapeutic systems, novel target focused treatments could change the pattern of care for these uncommon cancers, particularly in the recurrent situation. However, a number of immunotherapies and targeted molecules have shown efficacy in other high-grade neuroendocrine tumors (Herrera-Martínez et al. 2019; Lee et al. 2017; Caruso et al. 2021; Cives et al. 2019). But, their prospective role in gNECs still remains uncertain because of the small prevalence. Because gNECs are morphologically alike that of SCLC, disease management contemplations may be inferred from cohort clinical studies conducted in SCLC subjects. In the recent times, targeting programmed cell death-1 (PD-1) or programmed death-ligand 1 (PD-L1) by immune checkpoint inhibitors (ICIs) has attained encouraging outcomes in numerous cancers, encompassing gynaecological cancers (Menderes et al. 2016; Kurnit et al. 2020; Grywalska et al. 2019). On the basis of the outcomes of the IM power-133 trial (Horn et al. 2018), the CheckMate-032 trial (Antonia et al. 2016), and the pooled analysis of KEYNOTE-158 and KEYNOTE-028 (Chung et al. 2020), the FDA has approved, atezolizumab (anti-PD-L1 antibody), nivolumab (anti-PD-1 antibody),

and pembrolizumab (anti-PD-1 antibody), respectively, for the management of SCLC. Similar to other malignancies, gNECs are capable to dodge the immune reconnaissance, though PD-1/PD-L1 manifestation and immune response are not yet known and the advantages of ICIs are still debateable. Only subjective proofs exist on the effectiveness of ICIs in gNECs and mostly comes from cervical gNECs (Morgan et al. 2019; Zhao et al. 2019; Paraghamian et al. 2017; Sharabi et al. 2017). Furthermore, a new study established that cervical NECs were microsatellite stable and tremendously negative for PD-L1 manifestations, raising major apprehensions about the efficacy of ICIs in this disease setting (Carroll et al. 2020). The somatostatin (SST) receptor denotes an important and effective target for the neuroendocrine tumors of the gut, it can be targeted with other SST analogues. A very few accounts of positivity SST receptor in gNECs, which consequently are improbable to respond to SST analogues (Kajiwara et al. 2009). Remarkably, neuroendocrine carcinomas from other different sites have shown promising effect on poli-ADP-ribose-polymerase (PARP) inhibitors. Various studies in recurrent SCLC have shown overexpression of PARP and inhibition of tumor growth with PARP inhibitors (Cardnell et al. 2013). In a recent research, it was studied that more than 90% of the cervical NECs tested expressed PARP-1, opening new vista for the prospective use of PARP inhibitors (Carroll et al. 2020). Additionally, as HPV 16 and 18 infections has been found implicated in about 50–100% of cervical NECs, therapeutic HPV vaccines, inhibitors of cell cycle, and gene therapy might be a promising target for the treatment of cervical NECs (Manchana et al. 2010).

There is an increasing attention in exploring the role of the other targeting agents, say for example, PTEN inhibitors, MEK inhibitors, antiangiogens (like sunitinib, bevacizumab, and sorafenib), and mTOR inhibitors (everolimus), which have exhibited potential results in other non-gynecological NECs, though there are no substantial data on their any part to play in patients with gNECs (Manchana et al. 2010). Implementation of synergistic combination treatment strategies should be included in the futuristic treatment. Numerous new treatment choices are presently under study, and will possibly become accessible in the near future. Initial considerable outcomes of preclinical and clinical data are hopeful. However, conclusive evidence of their efficacy on rate of survival is challenging to attain as large, randomized clinical trials are prerequisite, which though cannot amass an adequate number of subjects, because of the rareness of the disease. Wide-ranging genomic categorization and unique study designs, including umbrella, basket, and platform trials, will perhaps throw more light on the efficacy of targeted agents. A DART (ClinicalTrials.gov identifier: NCT02834013) phase II (basket trial) is underway to evaluate the combination of ipilimumab and nivolumab, for a number of rare malignancies. Fascinatingly, in a high-grade neuroendocrine cohort study, around more than 40% overall response rate was observed (Patel et al. 2020). Presently, two ongoing studies are registered on ClinicalTrials.gov: (1) the phase II trial NCT04635956, which is assessing the combination of cisplatin/paclitaxel/bevacizumab with camrelizumab (anti-PD-1 antibody) for advanced or recurring cervical NECs and (2) the observational study NCT04723095, which is gathering more data on patients with cervical NECs directing to understand the disease

comprehensively, its treatment, and results. One more clinical trial (NCT00626561) for estimating the effectiveness of paclitaxel and bevacizumab in subject with recurrence of cervical NECs is aborted because of very slow accumulation treatment subjects (Clinical Trials.gov 2022).

Owing to the deficiency of uniform and consistent guidelines for the treatment, primary gNECs pose a massive treatment challenge for gyneco-oncologists across the globe. In this chapter, we collect and analyze the main quality evidences available on this rare topic, although inadequate, in an effort to reinforce the clinical decision-making process. Nonetheless, there is a crucial requirement to improve and develop unconventional treatments, particularly in the cases of recurrence. Randomized clinical trials are discontinued or discouraged due to the scarcity of gNECs and the low probability of attaining adequate number of subject. In near future, research centres across the globe should enter into a compact collaboration and collect a sufficient amount of data, sharing and comparing their single-institution experiences. Certainly, estimating huge population data from global databases may lead to a real step forward in the management of these rare and baffling malignancies. Finally, new basket clinical trials could assist to better comprehend the genetic and molecular features of gNECs, opening new windows for the novel targeted therapies.

## References

Antonia SJ, Lopez-Martin JA, Bendell J, Ott PA, Taylor M, Eder JP, Jäger D, Pietanza MC, Le DT, de Braud F, Morse MA, Paolo A, Ascierto PA, Horn L, Amin A, Pillai RN, Evans J, Chau I, Bono P, Atmaca A, Sharma P, Harbison CT, Lin C, Christensen C, Calvo E (2016) Nivolumab alone and nivolumab plus ipilimumab in recurrent small-cell lung cancer (check mate 032): a multicentre, open-label, phase 1/2 trial. Lancet Oncol 17(7):883–895

Atienza-Amores M, Guerini-Rocco E, Soslow RA, Park KJ, Weigelt B (2014) Small cell carcinoma of the gynecologic tract: a multifaceted spectrum of lesions. Gynecol Oncol 134:410–418

Boruta DM II, Schorge JO, Duska LA, Crum CP, Castrillon DH, Sheets EE (2001) Multimodality therapy in early-stage neuroendocrine carcinoma of the uterine cervix. Gynecol Oncol 81:82–87

Cardnell RJ, Feng Y, Diao L, Fan YH, Masrorpour F, Wang J, Shen Y, Mills GB, Minna JD, Heymach JV, Byers LA (2013) Proteomic markers of DNA repair and PI3K pathway activation predict response to the PARP inhibitor BMN 673 in small cell lung cancer. Clin Cancer Res 19(22):6322–6328

Carroll MR, Ramalingam P, Salvo G, Fujimoto J, Solis Soto LM, Phoolcharoen N, Hillman RT, Cardnell R, Byers L, Frumovitz M (2020) Evaluation of PARP and PDL-1 as potential therapeutic targets for women with high-grade neuroendocrine carcinomas of the cervix. Int J Gynecol Cancer 30:1303–1307

Caruso G, Sassu CM, Tomao F, Di Donato V, Perniola G, Fischetti M, Panici PB, Palaia I (2021) The puzzle of gynecologic neuroendocrine carcinomas: state of the art and future directions. Crit Rev Oncol Hematol 162:103344

Carvalheiro M, Ferreira-Silva M, Holovanchuk D, Marinho HS, Moreira JN, Soares H, Corvo ML, Cruz MEM (2022) Antagonist G-targeted liposomes for improved delivery of anticancer drugs in small cell lung carcinoma. Int J Pharm 612:121380

Chang TC, Lai CH, Tseng CJ, Hsueh S, Huang KG, Chou HH (1998) Prognostic factors in surgically treated small cell cervical carcinoma followed by adjuvant chemotherapy. Cancer 83:712–718

Chen X, Lee SK, Song M, Zhang T, Han MS, Chen YT, Chen Z, Ma X, Tung CH, Du Y-CN (2021) RHAMM$^{B}$-mediated bifunctional nanotherapy targeting Bcl-xL and mitochondria for pancreatic neuroendocrine tumor treatment. Mol Ther Oncolytics 23(17):277–287

Chung HC, Piha-Paul SA, Lopez-Martin J, Schellens JHM, Kao S, Miller WH, Delord JP, Gao B, Planchard D, Gottfried M, Zer A, Jalal SI, Penel N, Mehnert JM, Matos I, Bennouna J, Kim DW, Xu L, Krishnan S, Norwood K, Ott PA (2020) Pembrolizumab after two or more lines of previous therapy in patients with recurrent or metastatic SCLC: results from the KEYNOTE-028 and KEYNOTE158 studies. J Thorac Oncol 15:618–627

Cives M, Pelle E, Quaresmini D, Rizzo FM, Tucci M, Silvestris F (2019) The tumor microenvironment in neuroendocrine tumors: biology and therapeutic implications. Neuroendocrinology 109: 83–99

Clinical Trials.gov (2022) Bethesda, Maryland, U.S. https://clinicaltrials.gov/

DeLellis RA (2001) The neuroendocrine system and its tumors: an overview. Am J Clin Pathol 115 (Suppl):S5–S16

El-Readi MZ, Althubiti MA (2019) Cancer Nanomedicine: a new era of successful targeted therapy. J Nanomater 2019:1–13

Embry JR, Kelly MG, Post MD, Spillman MA (2011) Large cell neuroendocrine carcinoma of the cervix: prognostic factors and survival advantage with platinum chemotherapy. Gynecol Oncol 120:444–448

Eriksson B, Oberg K, Stridsberg M (2000) Tumor markers in neuroendocrine tumors. Digestion 62 (Suppl 1):33–38

Gardner GJ, Reidy-Lagunes D, Gehrig PA (2011) Neuroendocrine tumors of the gynecologic tract: a Society of Gynecologic Oncology (SGO) clinical document. Gynecol Oncol 122:190–198

Grywalska E, Sobstyl M, Putowski L, Rolinski J (2019) Current possibilities of gynecologic cancer treatment with the use of immune checkpoint inhibitors. Int J Mol Sci 20:4705

Herrera-Martínez AD, Hofland J, Hofland LJ, Brabander T, Falm E, Galvez Moreno MA, Luque RM, Castaño JP, de Herder WW, Feelders RA (2019) Targeted systemic treatment of neuroendocrine tumors: current options and future perspectives. Drugs 79:21–42

Horn L, Mansfield AS, Szczęsna A, Havel L, Krzakowski M, Hochmair MJ, Huemer F, Losonczy G, Johnson ML, Nishio M, Reck M, Mok T, Lam S, Shames DS, Liu J, Ding B, Lopez-Chavez A, Kabbinavar F, Lin W, Sandler A, Liu SV (2018) First-line atezolizumab plus chemotherapy in extensive-stage small-cell lung cancer. N Engl J Med 379:2220–2229

Hoskins PJ, Swenerton KD, Pike JA, Lim P, Aquino-Parsons C, Wong F, Lee N (2003) Small-cell carcinoma of the cervix: fourteen years of experience at a single institution using a combined-modality regimen of involved-field irradiation and platinum-based combination chemotherapy. J Clin Oncol 21:3495–3501

Kajiwara H, Hirabayashi K, Miyazawa M, Nakamura N, Hirasawa T, Muramatsu T, Mikami T, Yasuda M, Osamura RY (2009 Apr) Immunohistochemical expression of somatostatin type 2A receptor in neuroendocrine carcinoma of uterine cervix. Arch Gynecol Obstet 279(4):521–525

Kurnit KC, Reid P, Moroney JW, Fleming GF (2020) Immune checkpoint inhibitors in women with gynecologic cancers: practical considerations. Gynecol Oncol 158:531–537

Kyriakopoulos G, Mavroeidi V, Chatzellis E, Kaltsas GA, Alexandraki KI (2018) Histopathological, immunohistochemical, genetic and molecular markers of neuroendocrine neoplasms. Ann Transl Med 6:252

Lee A, Chan DL, Wong MH, Li BT, Lumba S, Clarke SJ, Samra J, Pavlakis N (2017) Systematic review of the role of targeted therapy in metastatic neuroendocrine tumors. Neuroendocrinology 104:209–222

Lee YJ, Cho A, Cho BC, Yun M, Kim SK, Chang J, Moon JW, Park IK, Choi HJ, Kim JH (2009) High tumor metabolic activity as measured by fluorodeoxyglucose positron emission tomography is associated with poor prognosis in limited and extensive stage small-cell lung cancer. Clin Cancer Res 15:2426–2432

Lee JM, Lee KB, Nam JH, Ryu SY, Bae DS, Park JT, Kim S-C, Cha S-D, Kim K-R, Song S-Y, Kang S-B (2008) Prognostic factors in FIGO stage IB–IIA small cell neuroendocrine carcinoma

of the uterine cervix treated surgically: results of a multi-center retrospective Korean study. Ann Oncol 19:321–326
Manchana T, Ittiwut C, Mutirangura A, Kavanagh JJ (2010) Targeted therapies for rare gynaecological cancers. Lancet Oncol 11:685–693
Mayo Clinic (2022) Rochester, Minnesota U.S. https://www.mayoclinic.org/
Menderes G, Hicks C, Black JD, Schwab CL, Santin AD (2016) Immune checkpoint inhibitors in gynecologic cancers with lessons learned from nongynecologic cancers. Expert Opin Biol Ther 16:989–1004
Moertel C, Kvols L, O'Connell M, Rubin J (1991) Treatment of neuroendocrine carcinomas with combined etoposide and cisplatin: evidence of major therapeutic activity in the anaplastic variants of these neoplasms. Cancer 68:227–232
Morgan S, Slodkowska E, Parra-Herran C, Mirkovic J (2019) PD-L1, RB1 and mismatch repair protein immunohistochemical expression in neuroendocrine carcinoma, small cell type, of the uterine cervix. Histopathology 74:997–1004
National Cancer Institute (2022) National Institutes of Health (NIH). U.S. Department of Health and Human Services, Bethesda, Maryland. https://www.cancer.gov/about-cancer/understanding/what-is-cancer
Paraghamian SE, Longoria TC, Eskander RN (2017) Metastatic small cell neuroendocrine carcinoma of the cervix treated with the PD-1 inhibitor, nivolumab: a case report. Gynaecol Oncol Res Pract 4:3
Patel SP, Othus M, Chae YK, Giles FJ, Hansel DE, Singh PP, Fontaine A, Shah MH, Kasi A, Al Baghdadi T, Matrana M, Gatalica Z, Korn WM, Hayward J, McLeod C, Chen HX, Sharon E, Mayerson E, Ryan CW, Plets M, Blanke CD, Kurzrock R (2020) A phase II basket trial of dual anti–CTLA-4 and anti–PD-1 blockade in rare tumors (DART SWOG 1609) in patients with nonpancreatic neuroendocrine tumors. Clin Cancer Res 26:2290–2296
Posligua L, Malpica A, Liu J, Brown J, Deavers MT (2008) Combined large cell neuroendocrine carcinoma and papillary serous carcinoma of the endometrium with pagetoid spread. Arch Pathol Lab Med 132:1821–1824
Rindi G, Inzani F (2020) Neuroendocrine neoplasm update: toward universal nomenclature. Endocr Relat Cancer 27:R211–R218
Sevin BU, Method MW, Nadji M, Lu Y, Averette HA (1996) Efficacy of radical hysterectomy as treatment for patients with small cell carcinoma of the cervix. Cancer 77:1489–1493
Sharabi A, Kim SS, Kato S, Sanders PD, Patel SP, Sanghvi P, Weihe E, Kurzrock R (2017) Exceptional response to nivolumab and stereotactic body radiation therapy (SBRT) in neuroendocrine cervical carcinoma with high tumor mutational burden: management considerations from the center for personalized cancer therapy at UC san Diego Moores cancer center. The Oncol 22:631–637
Sheets EE, Berman ML, Hrountas CK, Liao SY, DiSaia PJ (1988) Surgically treated, earlystage neuroendocrine small-cell cervical carcinoma. Obstet Gynecol 71:10–14
Sidibe FM, Traore Z, Georgala A, Kanab R, Larsimont D, Awada A, Piccart-Gebhart M (2018) Small cell carcinoma of the endometrium: a clinicopathological study and management of three cases. Bull Cancer 105:842–846
Tempfer CB, Tischoff I, Dogan A, Hilal Z, Schultheis B, Kern P, Rezniczek GA (2018) Neuroendocrine carcinoma of the cervix: a systematic review of the literature. BMC Cancer 18:530
Yao JC, Hassan M, Phan A, Dagohoy C, Leary C, Mares JE, Abdalla EK, Fleming JB, Vauthey J, Rashid A, Evans DB (2008) One hundred years after “carcinoid”: epidemiology of and prognostic factors for neuroendocrine tumors in 35,825 cases in the United States. J Clin Oncol 26:3063–3072
Zhao P, Li L, Jiang X, Li Q (2019) Mismatch repair deficiency/microsatellite instability-high as a predictor for anti-PD-1/PD-L1 immunotherapy efficacy. J Hematol Oncol 12:54
Zivanovic O, Leitao MM Jr, Park KJ, Zhao H, Diaz JP, Konner J et al (2009) Small cell neuroendocrine carcinoma of the cervix: analysis of outcome, recurrence pattern and the impact of platinum-based combination chemotherapy. Gynecol Oncol 112:590–593

# Effective Luteinizing Hormone Drug Delivery by Nanocarriers in Hormonal Cancer Treatment

16

Rohit Tripathi, Mahfoozur Rahman, Prateek Pathak, and Amita Verma

**Abstract**

The luteinizing hormone (LH) secretes mainly two types of hormone, that is, LH and follicle-stimulating hormone (FSH). The receptors of luteinizing hormone-releasing hormone (LHRH) are overexpression in the majority of different types of cancers, while their expression in healthy tissues, apart from pituitary cells, is limited. In the current scenario, modern research studies recommended that LHRH peptides can be employed to efficiently guide anticancer and imaging agent means visualizing the internal organs directly to cancerous cells. As a result, the number of these compounds in tumour tissue increases, while normal cells are spared unneeded exposure. Nanoparticles can be employed for targeting anticancer drugs in which it is anticipated that nanocarriers would deliver the drug at the unhealthy cancerous tissues via two mechanisms, i.e., passive or active mechanism. However, several nano-medicines have been formulated to improve

R. Tripathi
Department of Pharmacy, Kamla Nehru Institute of Management and Technology, Sultanpur, India

Bioorganic and Medicinal Chemistry Research Laboratory, Department of Pharmaceutical Sciences, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, India

M. Rahman
Department of Pharmaceutical Sciences, Faculty of Health Sciences, SHUATS, Allahabad, Uttar Pradesh, India

P. Pathak
Laboratory of Computational Modeling of Drugs, Higher Medical and Biological School, South Ural State University, Chelyabinsk, Russia

A. Verma (✉)
Bioorganic and Medicinal Chemistry Research Laboratory, Department of Pharmaceutical Sciences, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, India
e-mail: amita.verma@shiats.edu.in



M. Rahman et al. (eds.), *Hormone Related Cancer Mechanistic and Nanomedicines*,
https://doi.org/10.1007/978-981-19-5558-7_16

the anticancer effect. Hence, synthesized nanoparticles could serve as a potential carrier with an advantage of improved biodegradability, biocompatibility, improved loading capacity, targeting ability, scalability, and stability.

**Keywords**

Receptors · Nanocarriers · Biodegradability · Biocompatibility · Improved loading · Targeting ability

## 16.1 Introduction

Cancer is one of the contemporary medicine's most difficult challenges, and it is still the top cause of mortality globally (WHO: Cancer 2012). The limited accessibility of available therapeutic and imaging agents to cancer cells, their lack of selectivity, rapid clearance from the blood circulation, and toxicity on healthy organs are the main causes for the high mortality rate among cancer patients (Allen et al. 1995; Minko et al. 2013; Torchilin VP Passive and active drug targeting 2010). As a result, a medication delivery strategy that is focused and selective to cancer cells has enormous potential to increase the efficacy of cancer detection and treatment (Allen et al. 1995; Torchilin VP Passive and active drug targeting 2010).

It is commonly acknowledged that two major techniques, passive and active targeting, can be used to deliver anticancer medicines to specific cancer areas (Bae and Park 2011; Allen et al. 1995). The ability of large molecules and nanoparticles ranging in size from 10 nanometers to several hundred nanometers to accumulate specifically in the tumour microenvironment by escaping from systemic circulation into the tumor interstitium through leaky tumour blood vessels is the basis for passive targeting (Bae and Park 2011; Torchilin VP Passive and active drug targeting 2010). Furthermore, the retention of penetrated macromolecules inside cancer tissues is due to a lack of lymphatic outflow. Another strategy based on the modification of anticancer agents and/or drug-loaded nanoparticles with targeting ligands that bind specifically to the receptors preferentially expressed or highly overexpressed by cancer cells is active targeting of cancer cells (Bae and Park 2011; Torchilin VP Passive and active drug targeting 2010; Maeda et al. 2000). Many cancer cells have overexpressed cell surface receptors for peptides, hormones, and vital nutrients as a result of their changed cellular nature, giving a large number of target options for active drug targeting to cancer cells (Minko et al. 2013; Torchilin VP Passive and active drug targeting 2010). The goal of this research is to look into current drug delivery system improvements and advancements. Whereas the active targeting characteristics that utilize the luteinizing hormone-releasing hormone (LHRH) receptor.

### 16.1.1 Hormonal Cancer

Cancer is found in different parts of the body but not all types of cancer are affected by hormones. But some types of cancer such as ovarian cancer, breast cancer, and prostate cancer, uterine or endometrial cancer need hormones as estrogen and progesterone to grow. Hormone can influence your weight, your internal body temperature level, and surprisingly your mindset. (Garrett 2008) They can likewise affect your disease hazard. Estrogens, a female sex hormone, are known as human cancer-causing agents. Albeit these hormones play fundamental physiological parts in the two: females and male, they have additionally been related with an expanded danger of cancer. For example, taking joined menopausal hormone treatment (estrogen in addition to progestin, which is an engineered adaptation of the female hormone progesterone) can build a female breast cancer. In cancer cells, LHRH controls the cell multiplication and abnormal growth of cancer cell. (Pike et al. 1983; Collaborative Group on Hormonal Factors in Breast Cancer 1996).

Types of Hormone-Sensitive Cancer.

Few types of cancer powered by hormone:

- Ovarian cancer: Ovarian cancer beginning in female is more difficult to treat. It can be affected by estrogen. For a long time, scientists have accepted that in the case of female ovarian cancer, the stage of metastasizes through a passive cell mechanism system by which ovarian malignant growth cells are shed from the essential cancer and conveyed by the physiological development of peritoneal liquid to the peritoneum and omentum. Ovarian cancer is a hormone-based cancer with estrogen receptor.
- Breast cancer: Breast cancer also begins in female organ and affected by progesterone and estrogen. HER2 or HER2/Neu receptor of breast cell is responsible for the development of cancer cell, whereas HER2 receptor works with cancer cell and develops cancer fast.
- Prostate cancer: Prostate cancer begins in male organ. Some male sex hormones such as testosterone and other male sex hormones that help to grow for cancer. The male prostate malignant growth is essentially portrayed on the hub of androgen hormone and its intellectual receptor, the nuclear receptor (NR) and androgen receptor (AR), which assumes parts in carcinogenesis, malignancy advancement, illness movement, and treatment obstruction in the male prostate cancer. Prostate cancer progresses with androgen level.
- Uterine or endometrial cancer: Uterine cancer also begins in female organ and some female sex hormones such as estrogen and progesterone that help to grow for cancer. In the case of female uterine cancer, both estrogen and progesterone female sex hormone apply their impact through intra- and extra-nuclear receptors mechanism. The estrogen receptor and progesterone receptor are decidedly connected with the anticipation of endometrial malignant growth, including the endurance rate and endurance time.

## 16.2 Nanoparticles (NPs)

The term "nano" is derived from the Greek word meaning "Dwarf" which implies that nanoparticles size is generally in the range from 1 to 500 nm. NPs are of great scientific interest as they serve as a bridge between bulk materials and atomic molecules/structures. Properties of the substance vary completely when it is converted from bulk matter to nanomaterial. Bulk gold, for example, is a brilliant yellow colour, whereas nanogold seems red. Silver nanoparticles have a great deal of potential.

Different Types of Nanoparticles for Drug Delivery.

Nanoparticles in the size ranging from 1 to 500 nm are well known for their applications in various sectors, and specifically, healthcare system is increasing and replacing conventional systems. Various nanoparticles are involved in drug delivery systems.

1. Polymeric nanoparticles
2. Dendrimers
3. Carbon nanotubes
4. Nanocapsules
5. Liposomes
6. Solid lipid nanoparticles
7. Self-nanoemulsifying drug delivery system
8. Silica nanoparticles
9. Fullerenes
10. Metallic nanoparticles

### 16.2.1 Drug Delivery System

Drug delivery is an interesting field of pharmaceutical research which mainly encompasses the process of releasing the drug/pay loads at the specified cite and at a specific rate. Recent advances have proved that the research in targeted drug delivery is very crucial to improve therapeutic efficacy and reduce drug toxicity. It also attracts the attention of pharmaceutical industry to expand commercial drug markets. TDDS serves various advantages such as (Jing-Liang et al. 2009; Sperling et al. 2008; Kim et al. 2004):

1. Improved patience compliance
2. Improved product shelf life
3. Reduced costing
4. Reduced drug toxicity
5. Better therapeutic efficacy

Therefore, developments of techniques which are focused on TDDS are on rise in the present pharmaceutical drug delivery research. Emergence of nanotechnology in

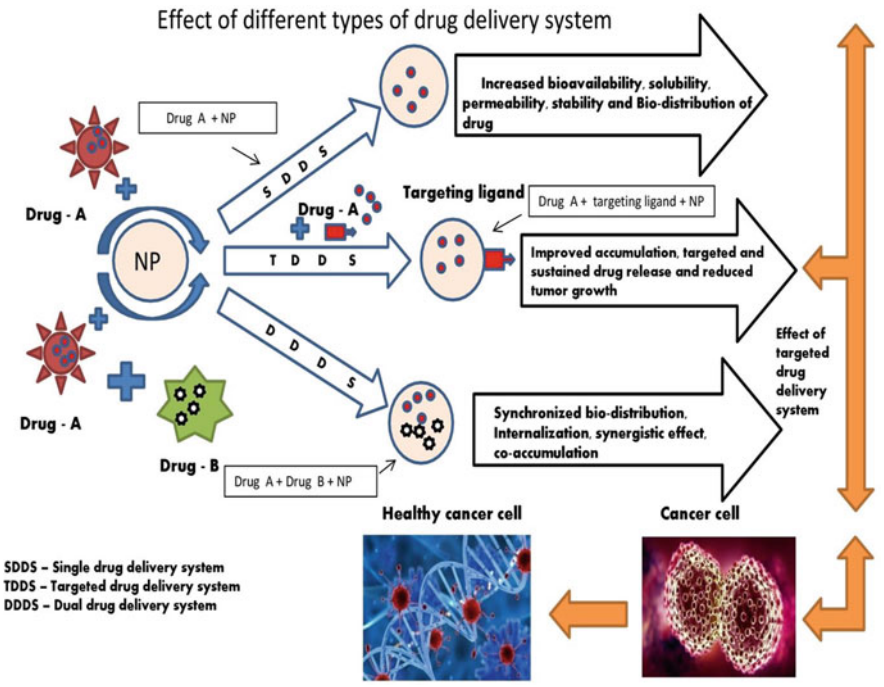



**Fig. 16.1** Schematic representation of different types of drug delivery system by nano carriers

the field of drug delivery is well known for its potential to effectively deliver the drug at targeted cites and is well proved for its improved therapeutic and diagnostic potential.

#### 16.2.1.1 Single Drug Delivery System (SDDS)

Fig. 16.1 represents that the SDDF increased bioavailability, solubility, permeability, stability, and bio-distribution of drug.

#### 16.2.1.2 Targeted Drug Delivery System (TDDS)

The aim of TDDS is to reach at the desired site after administration of it. Improved accumulation targeted and sustained drug release and reduced tumor growth (Fig. 16.1).

The two approaches explaining targeting mechanisms are as follows:

1. Active targeting
2. Passive targeting

Active targeting involves active ligands (drug molecule, peptides, proteins, and genes, DNA or RNA) loaded on the carrier surface for exact recognition by cell surface receptors. On the other hand, passive targeting depends on the concentration

of the drug molecules in unhealthy cancerous tissues due to extravasation via leaky gaps (approx. 600 nm) in blood vessels (Raghunandan et al. 2009; Naik et al. 2002).

#### 16.2.1.3 Dual Drug Delivery System (DDDS)

Dual drug delivery system stacked nanoparticles showed essentially improved cancer cell inhibitory impact. Figure 16.1 shows that the DDDS synchronized bio-distribution, internalization, synergistic effect, and co-accumulation of drug.

## 16.3 LHRH Drug Delivery by Nano Carriers

Gonadotropin-releasing hormone (GnRH) LHRH stimulates the pituitary to release LH and FSH, which regulate gonadal sex steroid synthesis in both males and females. LHRH binding to its receptors (LHRH-R) appears to cause receptor micro aggregation and peptide internalization (Keller et al. 2005a; Emons et al. 1996). It is well known that LRHR-R is expressed not only in the pituitary but also in cancer tissues. Although the precise biological role of LHRH-R in cancer tumours has yet to be determined, many studies show that LHRH peptides may act as local tumour growth regulators. The binding of LHRH activates mitogenic signal transduction pathways involving growth factor receptors and tyrosine kinase activity, as well as antimetastatic signal transduction (Emons et al. 1996; Moretti et al. 1996). Overexpression of the LHRH-R gene was found in hormone-dependent cancer tissues such as breast cancer, endometrial cancer, ovarian cancer, and prostate cancer, as well as hormone-independent cancer tissues like pancreatic cancer, lung cancer, melanoma, and glioblastoma. Furthermore, LHRH-R expression has been observed to be common in a variety of malignancies. LHRH-R is expressed in 86% of prostate cancers, 80% of human endometrial and ovarian cancers, 80% of renal malignancies, 50% of breast cancers, and 32–50% of pancreatic cancers. LHRH-R expression is much higher in several malignancies than in normal tissues, including tissues of the reproductive organs. Previous research also found that LHRH-R expression in lymph node metastases was comparable to or even greater than that in original cancer tumours (Keller et al. 2005a; Emons et al. 1996). The patients who received neo adjuvant LHRH agonist therapy, there was no substantial influence on receptor expression on cancer cells when pituitary LHRH receptors were down regulated (Keller et al. 2005b; Minko 2013). With the maximum effectiveness, nanoparticles conjugated with LHRH. These qualities have the potential to usher in a new era of tailored imaging and treatment, as well as expand nanoparticle uses in the future (Moretti et al. 1996; Needham et al. 2000).

### 16.3.1 Inorganic-Based Nano Carrier in Drug Delivery

For LHRH-targeted DDS, inorganic nanocarriers have also been developed. The production of nanocarriers from metallic and semimetallic materials for the possible application of medication delivery is referred to as inorganic nanomedicine. Due to

**Table 16.1** Advantages and disadvantages of different types of nanocarrier in drug delivery

| S. No. | Drug carrier nanomaterials | Targeted drug delivery therapy | |
|---|---|---|---|
| | | Advantage | Disadvantages |
| 1. | Inorganic based nano carrier in drug delivery | • Nontoxic in nature<br>• Hydrophilic in nature<br>• Highly stable biocompatible | • Toxicity |
| 2. | Dendrimers nano carrier in drug delivery | • Incorporate both hydrophobic and hydrophilic molecules<br>• High drugs carriage | • Low hydro solubility<br>• Cytotoxic |
| 3. | Liposomes and lipid-based nano carrier in drug delivery | • Wide range of drug delivery system | • Cationic lipids cause toxicity |
| 4. | Polymers nano carrier in drug delivery | • Biocompatibility, biodegradability<br>• Non-toxicity<br>• Hydrophilicity | • Toxic degradation<br>• Toxic monomers aggregation |
| 5. | Carbon nanotubes Nano carrier in drug delivery (CNTs) | • Better flow<br>• Improved hydrophilic properties | • Potential material toxicity<br>• Lack of solubility in aqueous media |

their diverse features, chemically modified inorganic nanoparticles are given as another choice for cellular delivery breakthrough. 39 Controlled release, various functions, good biocompatibility, and the ability to facilitate targeted drug delivery with imaging capabilities are among these qualities (Xu et al. 2006; Bauer et al. 2004). LHRH-targeted inorganic nanoparticles have also been investigated as PET and MRI contrast agents. By encapsulating hydrophobic $Mn_3O_4$ nanocrystals with lipid-PEG molecules, water soluble manganese oxide ($Mn_3O_4$) nanoparticles were created. Nano carrier of face-centered cubic (fcc) FePt NPs with an LHRH peptide were found to bind and highly toxic to the human ovarian cancer cell line. Nanocarrier of LHRH-targeted super paramagnetic iron oxide nanoparticles (SPION) increased accumulation of SPION in cancer cells (Table 16.2). The inorganic-based nano carrier has advantages such as high stability, biocompatibility, nontoxic in nature, and hydrophilic in nature and disadvantage of toxic in nature sometimes (Table 16.1).

### 16.3.2 Dendrimers Nano Carrier in Drug Delivery

Dendrimers are nanometer-sized drug delivery devices made from synthetic polymeric macromolecules. They are made up of several conspicuously branching monomers that protrude outwards from a central core. Because of their changeable surfaces, monodisperse size, hydrophilic interior chambers, and multivalences, dendrimer-based drug delivery devices have a lot of unique qualities (Li et al. 2018). Scaffold systems made on poly(amidoamine) dendrimers coupled with cisplatin are well-known. (Wang et al. 2009) Dendrimers are unique multifunctional

**Table 16.2** Summary of LHRH-targeted nano drug delivery system

| Drug delivery system | Carrier composition of nano drug | Type of cancer | Reference |
|---|---|---|---|
| Inorganic based nano carrier in drug delivery | Face-centered cubic nanoparticles (FCC-FEPT-NPs) | Ovarian | Xu et al. (2009) |
| | Super paramagnetic iron oxide (SPION-NPs) | Ovarian | Li et al. (2018) |
| Dendrimers nano carrier in drug delivery | Poly propylene imine (PPI-NPs) | ` | ` |
| | Poly-amido-amine (PAMAM-NPs) | Ovarian | Wang et al. (2009); Luo et al. (2019) |
| Liposomes and lipid-based nano carrier in drug delivery | Liposome-NPs | Breast | Schurmann et al. (1994) |
| Polymers nano carrier in drug delivery | Poly(ethylene glycol) (PEG-NPs) | Ovarian | Gasco (1997, 1993) |
| | Poly(ethylene glycol)-poly (methyl methacrylate) (PEGG-PMAA-NPs) | Ovarian | Vauthier and Bouchemal (2009) |
| | Dextran-NPs | Breast | Qiu and Bae (2006) |
| | Human serum albumin (HAS-NPs) | Breast | Qiu and Bae (2006) |
| Carbon nanotubes (CNTs) nano carrier in drug delivery | Cisplatin (CDDP—NPs) | Breast | Qiu and Bae (2006); Prakash et al. (2021) |
| | Multiwalled carbon nanotubes cisplatin (MWCNT-COOH-CDDP—NPs) | Breast | Prodana et al. (2011) |
| | Multiwalled carbon nanotubes (MWCNTs-NPs) | Breast | Shaffer and Koziol (2002) |

drug delivery devices because their single adjustable exterior property allows them to be conjugated with many molecules at the same time. LHRH-designated poly propyleneimine(PPI) dendrimers stacked with the CD44siRNA that have been additionally used to beat the cancer prevention agent protection instrument in ovarian malignancy cells, which thus essentially upgraded the remedial impact of photodynamic therapy. In option to the PPI dendrimers, an siRNA conveyance framework dependent on the LHRH focused on polyamidoamine (PAMAM) dendrimer was likewise evolved. The subsequent LHRHPAMAM forms proficiently complexed siRNA into circular nanoparticles and just the designated edifices essentially downregulated the designated quality in LHRH positive malignant growth cells (Table 16.2) (Luo et al. 2019). Advantages of dendrimers nano carriers are incorporate both hydrophobic and hydrophilic molecules, high drugs carriage with disadvantage of low hydro solubility, sometimes cytotoxic in nature. The impact of dendrimers loaded nanocarrier on cancer cell results good as shown in Fig. 16.2.

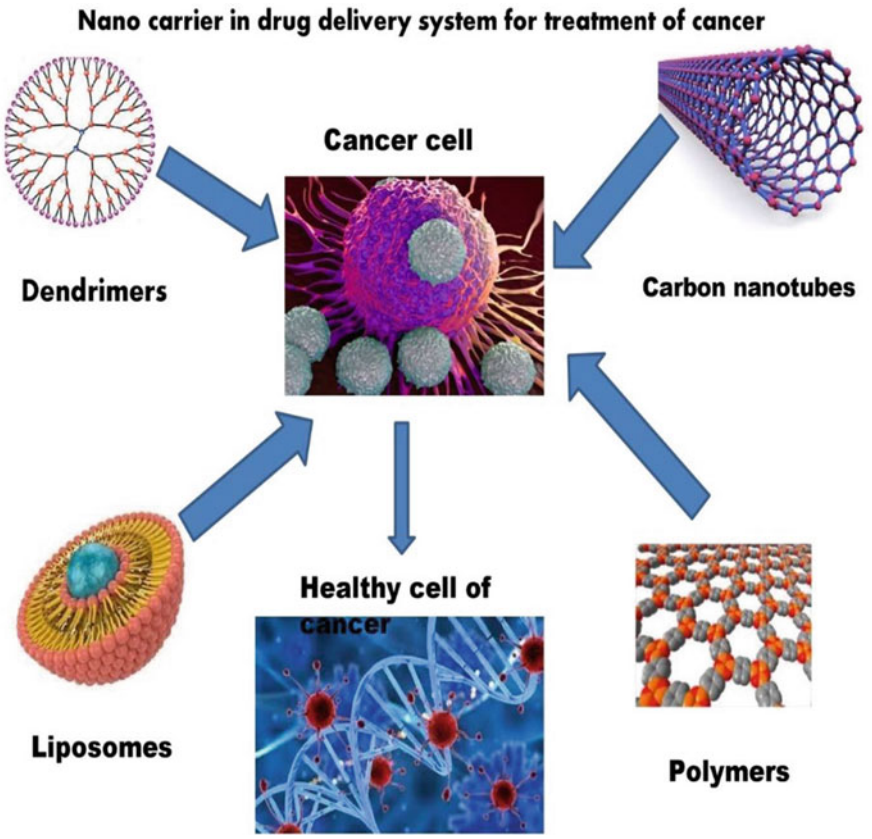



**Fig. 16.2** Schematic representations of different types of nano carrier in drug delivery system

### 16.3.3 Liposomes and Lipid-Based Nano Carrier in Drug Delivery

Liposomes have a wide range of features in terms of size, surface charge, and lipid content, and their ability to incorporate practically any medication regardless of its water solubility making them suitable for drug administration. Song and colleagues developed an LHRH-targeted liposome method to deliver the anticancer medication mitoxantrone (Mit). A thioether bond was used to link gonadorelin, a peptide homolog of LHRH, to PEGylated liposomes. At a dosage of 1.0 mg/mL, the loading of MIT was 98%. Targeted liposomes showed increased uptake and cytotoxicity than non-targeted liposomes in in vitro tests on LHRH-R high expressing MCF-7 cells (Gasco 1997). This increased performance, however, was Mit dose-dependent, and the difference between targeted and non-targeted liposomes may not be significant at some dosage ranges. This study also discovered that the tested formulations of targeted liposomes containing Mit did not achieve the same threshold of toxicity

as free Mit. According to the scientists, the release of encapsulated Mit from endocytosed liposomes and subsequent escape from endosomes or lysosomes was less than that of free Mit, which is consistent with earlier research. Liposomes have a lengthy history of use as a drug delivery vehicle for APIs with poor pharmacokinetics, bioavailability, or solubility (Schurmann et al. 1994; Gasco 1993, 1997).

Nanostructured lipid carriers (NLCs) are a new class of lipid nanoparticles that combine the benefits of several nanocarriers, such as liposomes, multifunctional NLC DDS for pulmonary co-delivery of an anticancer medication and siRNA via inhalation. This NLC has an analogue of LHRH on the surface, an encapsulated anticancer drug (doxorubicin or paclitaxel), and electrostatically bound siRNAs targeted to MRP1 mRNA as a suppressor of drug efflux pumps (pump drug resistance), as well as siRNA targeted to BCL2 mRNA as a suppressor of antiapoptotic defence (nonpump drug resistance). The suggested NLC efficiently delivered its payload to lung cancer cells after inhalation in mouse orthotopic models of human lung cancer, although healthy lung tissues and organs were much less exposed when compared to intravenous injection. The results showed effective tumour growth reduction and no harmful effects on healthy organs, confirming the great efficacy of NLC DDS for tumor-targeted delivery of anticancer medicines and siRNA mixes directly to lung cancer cells by inhalation (Schroit et al. 1986; Wang et al. 2004; Terada et al. 2006) (Table 16.2). Advantages of liposomes nano carrier are wide range of drug delivery system with disadvantage of cationic lipids causing toxicity (Table 16.1). The impact of liposomes loaded nanocarrier on cancer cell results good as shown in Fig. 16.2.

### 16.3.4 Polymers Nano Carrier in Drug Delivery

Polymeric nanoparticles are colloidal materials of nanoscale dimensions that can encapsulate, adsorb, or covalently bind pharmaceuticals. Minko and colleagues attached an LRHR homolog to one end of PEG and an anticancer medication called camptothecin (CPT) to the other (LHRH-PEG-CPT) (Vauthier and Bouchemal 2009; Qiu and Bae 2006). When compared to free CPT and PEG-CPT conjugates, the conjugation considerably increased cytotoxicity in human ovarian cancer cells. On mice with xenografts of human ovarian cancer, the LHRH-PEG-CPT compound was also tested. When compared to CPT alone and PEG-CPT, LHRH-PEG-CPT dramatically reduced tumour size. The targeting polymer (LHRH-PEG) accumulated primarily in the ovaries (endogenous LHRH-R) of mice without tumours and in the cancer tissues (specific targeting) of mice bearing human ovarian carcinoma xenografts, whereas the non-targeting conjugate (PEG) accumulated in the liver and in the tumour tissues (enhanced permeability retention effect of nanocarriers) (Lestrell et al. 2019). The tumor-targeted polymer accumulated at about twice the rate of PEG alone, suggesting the anticancer drug's precise targeting effect and improved efficacy in this LRHR-targeting DDS. As a result, several molecules of the targeting peptide and anticancer medicines were covalently coupled with bis

(2-carboxyethyl)-PEG using citric acid as a multivalent spacer (Prakash et al. 2021). In vitro cytotoxicity and in vivo antitumor activity of conjugates containing up to three copies of the LHRH targeting moiety and the anticancer drug, and found that conjugates containing multiple copies of the targeting molecule and drug had amplified in vitro and in vivo activity. In addition to the anticancer medication CPT and the LHRH targeting moiety, one or more copies of the BCL2 Homology 3 domain (BH3) peptide were linked to PEG via a citric acid spacer to reduce cellular anti-apoptotic defence, which is principally responsible for ovarian cancer cell treatment resistance. This multifunctional DDS elicited a robust apoptotic response in human ovarian cancer cell lines, and numerous treatments with DDS led to nearly full regression of the primary tumour and prevented the formation of malignant ascites in nude mice with the human ovarian cancer xenograft.

PEG polymer, dendrimers, and liposomes have typical sizes of 30 nm, 5 nm, and 100 nm, respectively. The LHRH analogue, paclitaxel (TAX) anticancer medication, and near-infrared cyanine Cy5.5 imaging agent were all used as targeted DDSs in this work. Adding the LHRH peptide to DDS increased their anticancer activity to a level that was comparable across all three types of nanocarriers. As a result, tumour targeting reduces the disparities in anticancer efficacy and unfavourable side effects on healthy tissues between DDS of diverse architecture, size, molecular mass, and composition. The in vivo bio distribution of these three nanocarriers differed significantly, despite the fact that the major accumulation site in each case was a tumour. Furthermore, the size variation between these nanocarriers was caused by their composition, which should be taken into account. Overall, this study contributed to the field of targeted drug delivery by establishing that the most important component in DDS design for high therapeutic yield is tumor-specific receptor targeting Table 16.2). Advantages of polymer nano carriers are biocompatibility, biodegradability on-toxicity hydrophilicity with disadvantage toxic degradation, toxic monomers aggregation (Table 16.1). The impact of polymer loaded nanocarrier on cancer cell results good as shown in Fig. 16.2.

### 16.3.5 Carbon Nanotubes (CNTs) Nano Carrier in Drug Delivery

Carbon nanotubes (CNTs) have been used in the delivery of medicinal agents such as peptides, proteins, nucleic acids, genes, and vaccines, as well as in the regeneration of bone and neural tissue. LHRH-R expression is much higher in several malignancies than in normal tissues, including tissues of the reproductive organs. a CNTs can be loaded with bioactive peptides, proteins, nucleic acids, and medicines before being delivered to cells and organs (Prodana et al. 2011; Shaffer and Koziol 2002). The multiple rolled layer carbon nanotubes (MWCNTs) are basically used in the transporters for a medication dependent on platinum metal for the best treatment of breast cancer. This technique for functionalization includes the carboxyl functionalization of CNTs and exemplification of cisplatin loaded (CDDP) into MWCNTs includes for the cancer as ovarian, cervical, oesophageal, and types of

cancer. Advantages of carbon nano carriers are better flow, improved hydrophilic properties with disadvantage potential material toxicity, and lack of solubility in aqueous media (Table 16.1). The impact of carbon loaded nanocarrier on cancer cell results good as shown in Fig. 16.2.

## 16.4 Conclusion and Future Perspectives

LHRH-targeted DDS (LHRH-PEG-Camptothecin) do not have effect on the plasma concentration of luteinizing hormone (LH) following systemic administration. It improves drug efficacy and decreases side effects; LHRH receptors have been discovered in many types of cancer like lung, breast, and ovarian, etc. LHRH-targeted drug delivery system has been shown to be effective carriers in treatment of different types of cancer. LHRH-targeted-DDS for anticancer improves drug efficacy and decreases side effects. Different types of nanoparticles have proven useful in imaging and delivery of therapeutics; it includes increasing tumor penetration, increasing circulation time, enabling non-invasive tracking of nanoparticles over time, and optimizing selective release and targeting of contents within desired regions.

## References

Allen TM, Hansen CB, Demenezes DEL (1995) Pharmacokinetics of long-circulating liposomes. Adv Drug Deliv Rev 16(2–3):267–284

Bae YH, Park K (2011) Targeted drug delivery to tumors: myths, reality and possibility. J Control Release 153:198–205

Bauer LA, Birenbaum NS, Meyer GJ (2004) Biological applications of high aspect ratio nanoparticles. J Mater Chem 14(4):517–526

Collaborative Group on Hormonal Factors in Breast Cancer (1996) Breast cancer and hormonal contraceptives: collaborative reanalysis of individual data on 53 297 women with breast cancer and 100 239 women without breast cancer from 54 epidemiological studies. Lancet 347(9017): 1713–1727

Emons G, Muller V, Ortmann O, Grossmann G, Tautner U, von Stuckrad B, Schulz K, Schally A (1996) Luteinizing hormone releasing hormone agonist triptorelin antagonizes signal transduction and mitogenic activity of epidermal growth factor in human ovarian and endometrial cancer cell lines. Int J Oncol 9:1129–1137

Garrett A, Quinn MA (2008) Hormonal therapies and gynaecological cancers. Best Pract Res Clin Obstet Gynaecol 22(2):407–421

Gasco MR (1993) Method for producing solid lipid microspheres having a narrow size distribution. US188837

Gasco MR (1997) Solid lipid nanospheres from warm microemulsions. Pharm Tech Eur 9:52–58

Jing-Liang L, Wang L, Xiang-Yang L, Zhang Z, Hong-Chen G, Wei-Min L et al (2009) In vitro cancer cell imaging and therapy using transferrin-conjugated gold nanoparticles. Cancer Lett 27(2):319

Keller G, Schally A, Gaiser T, Nagy A, Baker B, Halmos G, Engel JB (2005a) Receptors for LHRH expressed in human non-Hodgkins lymphomas can be targeted for therapy with the cytotoxic LHRH analogue AN 207. Eur J Cancer 41:2196–2202

Keller G, Schally A, Gaiser T, Nagy A, Baker B, Westphal G, Halmos G, Engel J (2005b) Human malignant melanomas express receptors for luteinizing hormone releasing hormone allowing targeted therapy with cytotoxic luteinizing hormone releasing hormone analogue. Cancer Res 65:5857–5863

Kim F, Connor S, Song H, Kuykendall T, Yang P (2004) Platonic gold nanocrystals. Angew Chem 116:3759

Lestrell E, Patolsky F, Voelcker NH, Elnathan R (2019) Engineered nano-bio interfaces for intracellular delivery and sampling: applications, agency and artefacts mater. Today 33:87–104

Li L, Wang J, Kong H, Zeng Y, Liu G (2018) Functional biomimetic nanoparticles for drug delivery and theranostic applications in cancer treatment. Sci Tech Adv Materials 19(1):771–790. https://doi.org/10.1080/14686996.2018.1528850109

Luo Y, Yin X, Yin X et al (2019) Dual pH/redox-responsive mixed polymeric micelles for anticancer drug delivery and controlled release. Pharmaceutics 11(4):176

Maeda H et al (2000) Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. J Control Release 65(1–2):271–284

Minko T (2013) Nanotechnology and drug resistance. Adv Drug Deliv Rev 65:1665–1666

Minko T, Rodriguez-Rodriguez L, Pozharov V (2013) Nanotechnology approaches for personalized treatment of multidrug resistant cancers. Adv Drug Deliv Rev 65:1880–1895

Moretti R, Marelli M, Dondi D (1996) LHRH agonists interfere with the stimularory actions of egf in human prostate cancer cell lines LNCaP and DU 145. J Clin Endocrinol Metab 81:3930–3937

Naik RR, Stringer SJ, Agarwal G, Jones SE, Stone MO (2002) Bio-mimetic synthesis and patterning of silver nanoparticles. Nat Mater 1:169–172

Needham D et al (2000) A new temperature-sensitive liposome for use with mild hyperthermia: characterization and testing in a human tumor xenograft model. Cancer Res 60(5):1197–1201

Pike MC, Krailo MD, Henderson BE, Casagrande JT, Hoel DG (1983) 'Hormonal'risk factors,'breast tissue age'and the age-incidence of breast cancer. Nature 303:5

Prakash J, Shekhar H, Yadav SR, Dwivedy AK, Patel VK, Tiwari S, Vishwakarma NK (2021) Green synthesis of silver nanoparticles using Eranthemum Pulchellum (blue sage) aqueous leaves extract: characterization, evaluation of antifungal and antioxidant properties. BBRJ 5:222

Prodana M, Ionita D, Ungureanu C, Bojin D, Demetrescu I (2011) Enhancing antibacterial effect of multiwalled carbon nanotubes using silver nanoparticles. Dig J Nanomater Biostruct 6:549–556

Qiu LY, Bae YH (2006) Polymer architecture and drug delivery. Pharm Res 23:1–30

Raghunandan D, Basavaraja S, Mahesh B, Balaji S, Manjunath SY, Venkataraman A (2009) Biosynthesis of stable polyshaped gold nanoparticles from micro¬wave-exposed aqueous extracellular anti-malignant guava (Psidium guajava) leaf extract. Nanobiotechnol 5:34–41

Schroit AJ, Madsen J, Nayar R (1986) Liposome–cell interactions: in vitro discrimination of uptake mechanism and in vivo targeting strategies to mononuclear phagocytes. Chem Phys Lipids 40(2–4):373–393

Schurmann D, Dormann A, Grunewald T, Ruf B (1994) Successful treatment of AIDS-related pulmonary Kaposi's sarcoma with liposomal daunorubicin. Eur Respir J 7:824–825. https://doi.org/10.1183/09031936.94.07040824

Shaffer MSP, Koziol K (2002) Polystyrene grafted multi-walled carbon nanotubes. Chem Commun 18:2074–2075. https://doi.org/10.1039/b205806p

Sperling RA, Gil PR, Zhang F, Zanella M, Parak WJ (2008) Biological applications of gold nanoparticles. Chem Soc Rev 37:1896

Terada T et al (2006) Novel PEG-matrix metalloproteinase-2 cleavable peptide–lipid containing galactosylated liposomes for hepatocellular carcinoma-selective targeting. J Control Release 111(3):333–342

Torchilin VP Passive and active drug targeting (2010) Drug delivery to tumors as an example. Handb Exp Pharmacol 197:3–53

Vauthier C, Bouchemal K (2009) Methods for the preparation and manufacture of polymeric nanoparticles. Pharm Res 26:1025–1058

Wang X, Wang Y, Chen ZG, Shin DM (2009) Advances of cancer therapy by nanotechnology. Cancer Res Treatment 41(1):1

Wang YW et al (2004) Photoacoustic tomography of a nanoshell contrast agent in the in vivo rat brain. Nano Lett 4(9):1689–1692

WHO: Cancer (2012). Available from: http://www.who.int/mediacentre/factsheets/fs297/en/

Xu CJ, Yuan ZL, Kohler N, Kim JM, Chung MA (2009) Sun SH FePt nanoparticles as an Fe reservoir for controlled fe release and tumor inhibition. J Am Chem Soc 131:15346–15351

Xu ZP, Zeng QH, Lu GQ, Yu AB (2006) Inorganic nanoparticles as carriers for efficient cellular delivery. Chem Eng Sci 61(3):1027–1040

# Regulatory Landscapes in Approval of Cancer Vaccines

17

Shubham Mule, Mayank Handa, and Rahul Shukla

**Abstract**

Cancer vaccines are hypothesized to trigger an immunological reaction against cancerous tissues. The scope of expanded clinical activities in the cancer vaccine research programmes can be acknowledged by the fact that around 2000 trials are registered under clinical cancer vaccines programme. The research activities in the cancer vaccine research area have gained a boost following the marketing authorization of Sipuleucel-T—the very first cancer vaccine in the US and EU. Though the regulatory guidelines for already existing cancer therapies like chemotherapy, radiotherapy are well established. Recently, the guidelines regarding regulatory aspects for cancer vaccines are developed. However, these guidelines are advisory in nature about the clinical requirements. However, the cancer vaccine development is relatively new area of research. There exists a huge scope for innovative strategies in this field. Hence, bilateral talks with the regulatory body are mandatory requirement to discuss and deliberate the clinical development plan on case-by-case basis. Thereby, the specific issues related to the quality of product under development are taken into consideration. All such regulatory aspects pertaining to the development and approval of cancer vaccines are discussed hereby in this chapter.

**Keywords**

T-cells · Antigens · Cancer vaccines · Immune system

S. Mule · M. Handa · R. Shukla (✉)
Department of Pharmaceutics, National Institute of Pharmaceutical Education and Research-Raebareli, Lucknow, U.P, India
e-mail: rahul.shukla@niperraebareli.edu.in



M. Rahman et al. (eds.), *Hormone Related Cancer Mechanistic and Nanomedicines*,
https://doi.org/10.1007/978-981-19-5558-7_17

## 17.1 Introduction

Cancer is amongst the most serious health concerns among all age groups around the globe. Current available treatment approaches for cancer have serious toxicity concerns. Hence, a relatively non-toxic approach that neutralizes cancerous growth by the immune system of the patients is a recent subject of interest for researchers. The domain of research and innovation in this area expands with high profile success owing to the advances in research methodologies and diagnostic techniques. However, to overcome regulatory hurdles, it is essential to demonstrate proof of concept and evidence supporting safety and efficacy. Furthermore, it must clarify the proposed mechanism of action. Hence, the clinical development plan must be constructed keeping the same in mind. However, to demonstrate mechanism of action in non-clinical program, a different approach should be considered. One must bear vast knowledge regarding regulatory guidelines regarding clinical development plan and clear plan for its justification and ensured regulatory input.

In some instances, the immune system has exhibited the ability to demonstrate the immune response against tumor. A successful, specific, and effective immune response would be the one which destroys not only the tumor tissue specifically but also toxicity to normal tissues and risk of second malignancy. Long-lasting immune memory and the specificity of cancer vaccine would induce destruction of existing tumor (therapeutic benefits) or identification of antigens on premalignant cells by immune system (prophylactic benefits).

The approaches in cancer immunotherapy include passive immunization (monoclonal antibodies (MABs) specific to tumor cell antigen), utilization of tumor specific cells (e.g., lymphocytes that penetrate melanoma tumor), silencing of immune check points to enhance immune response (anti-PD-1 and CLTA4), and CAR (chimeric antigen receptor) T cell engager technology (Heelan 2014). Recent findings in tumor pathophysiology and associated immunity have opened a broad gateway of better understanding of balance among immune cell activation, tumor cell proliferation, and escape mechanisms. This information can be utilized to assert the need for safe, effective, and successful cancer vaccines. Existing regulatory guidelines regarding antineoplastic medicinal products in the EU (European Union) and US (United States) mostly consider MABs and cytotoxic treatment approach. However, later on guidelines pertaining to cancer vaccine have been included. As different approaches are undertaken for different types of tumors, it becomes very cumbersome for both the drug developers and the regulatory personnel to ensure adequate quality, clinical and non-clinical attributes.

## 17.2 Overview of Cancer Vaccines and its Types

### 17.2.1 Cellular-Based Strategies

Preclinical studies indicate that CD8+ T cells specific to tumor play a significant therapeutic role. On this preclinical observation, majority of the cancer vaccines attempt to stimulate these cells. The most widely utilized immunization techniques for activating CD8+ T lymphocytes have relied on major histocompatibility complex (MHC) class I restricted peptide epitopes on tumor associated antigens (TAAs). They enhance presentation by endogenous antigen presenting cells (APCs) after administration with several adjuvant preparations (containing cytokines and toll-like receptors ligands). In order to evaluate protein amino acid sequences for candidate MHC class I members, peptide vaccines use computer-based algorithms. These candidature epitopes are evaluated in vitro for their ability to bind with human leucocyte antigen molecules, to be processed and presented naturally by cancer cells, as well as immunogenicity (able to stimulate CD8+ cells). Most of these epitopes have also been evaluated in mice models (to either extremely comparable human antigens, mouse self-antigen homologs, transgenic, or transfected models) and prove to possess tumor rejection antigen therapeutic characteristics. Another frequent strategy for stimulating CD8+ T cells specific to TAA is to prepare TAA-based vaccine containing autologous APCs like dendritic cells. This method considers the fact that tumors might impart a deleterious impact on endogenous APCs (Kiertscher et al. 2000). Tailored APCs can successfully stimulate antitumor T cells. However, these tailored APCs must be free of tumor-derived substances but should possess appropriate growth factor and immunological signals.

Another technique employs tumor cells (a complicated and incompletely defined antigens array, mutant antigens specific to tumor), which are frequently modified using cytokines like granulocyte-macrophage colony-stimulating factor (GM-CSF) or manufactured using adjuvants (or both). The whole set of mutations specific to tumor as well as all the MHC molecules are present on these cells. Another technique makes use of viruses' inherent effective infectivity to infect tumor antigens encoded cells. Oncolytic viruses are also used in viral strategies. It comprises autologous antigen-presenting cells that have already been loaded with GM-CSF and TAA prostatic acid phosphatase. It received approval for prostate cancer (metastatic) based on evidence from phase III clinical studies that showed the increased survival rate by around 4 months (Kawalec et al. 2012).

### 17.2.2 Peptide-Based Strategies

The most prevalent ways of cancer vaccination are to give epitopes of MHC class I restricted peptide produced from common TAAs to stimulate rare particular CD8+ T cell clones which respond to self-antigens (Kissick et al. 2014). Preclinical studies show the possibility of such vaccinations having a significant clinical utility (Komita et al. 2008; Zhao et al. 2012). Peptides crafted in adjuvants even without cytokines

like interferon γ and TLR agonists or GM-CSF have demonstrated therapeutic benefit in clinical trials (Slingluff et al. 2013; Pollack et al. 2014). Vaccines relied on a peptide or perhaps many peptides can be administered either individually or with Montanide ISA-51 in conjunction with a cytokine to stimulate APCs, additional adjuvants (like a particular TLR ligand to stimulate and mature APCs), or the peptides pulsed on to autologous APCs or a combination of these approaches. TAA-specific cytotoxic T cells were observed to be activated in mice models upon administration of IFA like oil-in-water emulsion of Montanide ISA-51(Fourcade et al. 2008; Kenter et al. 2008; Schwartzentruber et al. 2011). Data revealed that adjuvanticity of Montanide were also altered when olive oil source was used instead of beef source in manufacturing of Montanide (Rosenberg et al. 2010). A current issue related to IFA is that it may cause T cell buildup at immunization sites rather than fostering systemic immunity (Hailemichael et al. 2013). As peptide-based vaccines are tried in preclinical as well as clinical trials, appropriate vaccine formulations and adjuvants are constantly being developed (Bezu et al. 2018). Peptide-based techniques offer economic advantages in terms of cost of production as nine to 10 amino acid peptides are conveniently as well as inexpensively produced. Large-scale production is feasible, also the stability of peptides remains robust upon storage and transportation. Tragically, those who fail to express typical HLA cannot be tackled with this kind of vaccines due to HLA restrictions. Furthermore, CD4+ helper T cells cannot be triggered by the typical MHC class I binding short peptides, limiting the activity of CD8+ cytotoxic T cells. Although no data on the type of the "aid" provided by heterologous helper peptides exists, the insertion of non-tumor specific aid like tetanus, keyhole limpet hemocyanin (KLH), or pan-DR binding synthetic helper (PADRE) peptides has addressed this obstacle.

Another clinically effective method is to use synthetic peptides that are capable to encompass numerous epitopes of MHC class I and II (Welters et al. 2010). These subcutaneously injected long peptides with 23–45 amino acids have demonstrated better efficacy, presumably due to effective processing, and presentation route, ultimately leading to greater T cell activation (Rosalia et al. 2013). The data obtained in phase III clinical trials suggest that complete tumor antigen protein method allows for the absorption, processing, as well as the presentation of several antigen peptides of MHC class II and MHC class I (although with maybe lesser efficiency) (Dreno et al. 2018). Despite being formulated with an improved adjuvant, this vaccine contains full-length protein but still to achieve clinical objectives (Hirschler 2014). Several peptides can be administered simultaneously, targeting multiple antigens and T cell clones at the same time (Slingluff 2011). A study that combines cyclophosphamide (pre-vaccine) with GM-CSF and various peptides discovered that better survival was correlated with antigenic range of response and decreased myeloid-derived suppressor cells (MDSCs) and suppressive circulating regulatory T cells (Tregs) (Walter et al. 2012). Studies on mouse models support the use of multiple-antigen peptide vaccines in combination with chemotherapy (Disis et al. 2013). Peptide vaccines may potentially be effective in preventing pre-malignant lesions from advancing to cancer. A mucin 1 TAA peptide-based method was evaluated in a recent clinical study to prevent the development of colon adenoma

to colorectal cancer (Kimura et al. 2013). Nearly half of the 39 individuals tested exhibited high immunogenicity developed by vaccine. Notably, in more than half of the individuals having advanced colonic adenoma, a significant number of immunosuppressive MDSCs were already present. This suggests that circulating MDSCs could serve as a biomarker to access the response to vaccine and that previous vaccination should be investigated, since systemic immunosuppression may even be caused by premalignant lesions.

### 17.2.3 APC-Based Strategies

A diverse population of APCs comprises dendritic cells which may effectively absorb antigens and subsequently process and present them to CD4+, CD8+ T cells, additionally include immune response modifying signals such as the release of cytokines. Interferon, tumor necrosis factor, and IL-2 mediate a type 1 response, which enhances the stimulation of cytotoxic CD8+ T cells (Palucka et al. 2011; Palucka and Banchereau 2013).

#### 17.2.3.1 Dendritic Cells

Clinical studies of dendritic cell-based vaccines are often one-of-a-kind, including personalized patient immunization procedures and just one clinical trial arm. As a result, comparing trials and drawing clear inferences regarding the efficacy or different methods is challenging. CD34+ progenitor cells and monocytes were examined, and antigens such as complex tumor lysates possessing non-malignant tumor-related antigens, in addition synthetic MHC class I restricted peptides, were employed. Vaccines were administered intravenously, subcutaneously, and intradermally. All these variables might have an impact on the clinical outcomes observed. The dendritic cell-based vaccines were found to be safe practical, and immunogenic, and in certain individuals, they could produce clinically meaningful shrinkage of tumor (Hsu et al. 1996; Banchereau et al. 2001). Numerous significant clinical studies using dendritic cell vaccines have recently been reported. In a study with dendritic cells pulsed with heterologous PADRE peptides and mucin 1-based peptide administered subcutaneously in individuals having renal cell cancer, objective immunologic as well as clinical results were observed (Wierecky et al. 2006). Mucin 1 was a high-ranking antigen in the National Cancer Institute's prioritization study (Cheever et al. 2009). A modest dose of IL-2 was given in conjugation with these dendritic cell vaccines. In another trial, individuals suffering from acute myeloid leukemia were administered vaccines containing WT-1 TAA mRNA loaded dendritic cells. These individuals were under remission following normal therapy. These individuals demonstrated immune stimulation and enhanced clinical response. Another study that looked at dendritic cell-tumor fusions in participants prior to and after the transplantation of autologous stem cell discovered antitumor autoimmune response as well as disease decrease (Rosenblatt et al. 2013). Surprisingly, all of these studies employed a dendritic cell vaccine combination method, in which

systemic cytokine therapy and standard care therapies were combined with vaccines loaded with antigens (Galluzzi et al. 2012).

### 17.2.4 Tumor-Based Strategies

Early research focused non-development of cancer vaccine discovered that the tumor cells that had been destroyed as well as modified to produce immunostimulatory cytokines such as GM-CSF may be injected into the mice (Dranoff et al. 1993; McBride et al. 1992). Autologous tumor cells-based approach of cancer vaccine preparation was suitable but quite complex. The evidence supporting clinical testing of autologous, syngeneic, or allogeneic tumor cells expressing increased levels of GM-CSF due to transfection justified clinical study with some clinical and immunological responses (Soiffer et al. 1998; Nemunaitis et al. 2004; Luiten et al. 2005).

#### 17.2.4.1 Cell Lines

Allogeneic cell lines had been investigated, either with autologous tumor cells or alone. The G-Vax platform is still being investigated, and it is an element of a "prime boost" combined vaccine research which includes participants having pancreatic cancer. These participants are administered with recombinant Listeria bacteria encoding TAA mesothelin, alone or with a G-Vax consisting of two cell lines of allogeneic pancreatic cancer (Le et al. 2012a, b). Furthermore, numerous injections are achievable without being hampered by induced neutralization by antibody, along with that the addition of bacteria mimics many elements of a genuine infection by activating foreign pathogen pattern recognition receptors or TLRs. Other individualized approaches utilizing autologous tumor antigens involve the use of tumor lysates for loading APCs ex vivo and the fusion of tumor cells with autologous APCs. Many of them are evaluated in initial stage clinical studies, with encouraging results. In rare situations, immune responses to unidentified foreign helper proteins and tumor lysates have been shown (Chakraborty et al. 1998; Geiger et al. 2000).

#### 17.2.4.2 Autologous Tumor Cells

Transfection of APCs with tumor genomic DNA can also be done using autologous tumor cells (Kim and Chopra 2006). This permits unidentified altered tumor related unique gene products to be produced as well as given to the immune system for activation.

### 17.2.5 Virus-Based Strategies

As previously stated, if the pathogens are introduced in cancer vaccines, they can significantly boost immune response as tumor antigens are presented. Although TLR ligands like polyIC/polyICLC or CpG molecules are used in peptide-based vaccines, pathogens possess complex arrays comprising molecules capable of activating

several immune stimulation channels. Vaccines against Gardasil and human papillomavirus (HPV) Cervarix are approved to prevent cervical cancer caused by the HPV. They function by inducing humoral immunity towards viral capsid proteins that have been organized into viral particles non-infectious in nature. However, they are ineffective against malignancies developed in infected people. Viruses, particularly adenovirus, were employed as vectors for the purpose of active immunization with tumor antigens by injecting into muscle tissue with rapid transfection (Meng et al. 2001; Butterfield et al. 2014), leveraging their intrinsic infectivity. To transduce antigens into APCs, viruses have been utilized earlier (Arthur et al. 1997; Schumacher et al. 2004). These transduced cells exhibited activation or inhibition as a set of consequences due to each virus. Direct delivery of viral vectors can neutralize antibody responses preventing recurrent usage; however, following vaccination using ex vivo transduced viral APCs, the production of neutralizing antibodies is limited. But, clinical utilization of viral vectors can be complicated logistically, including the need for therapeutic grade virus manufacturing. Some of these restrictions can be overcome by using virus-related prime boost using viral backbones of heterologous nature encoding tumor antigens. This strategy has been demonstrated by the fowl pox virus and vaccinia virus prime boost for prostate cancer, expressing the TAA and PSA and the intercellular adhesion molecule 1 (CD54), lymphocyte function-associated antigen 3 (CD58), and costimulatory markers B7–1 (CD80) (Madan et al. 2012). This intriguing technique has enhanced survival in individuals with prostate cancer, and it is still being explored in late-stage clinical studies (Smith and Kantoff 2010; Gulley et al. 2010). This team is also working on viruses that encode mucin 1 and CEA rather than PSA to be used against various types of cancer. Oncolytic viruses drive the trans-activator early genes like E1b as well as E1a, as well as viral amplification from a tumor-specific promoter (which includes promoters driving TAAs such as human telomerase reverse transcriptase (hTERT), PSA, and fetoprotein) (Kanerva et al. 2013). Vaccinia viruses are also employed for this purpose. To increase tumor specificity for viral multiplication and neutralization, one approach has induced viral serpin genes mutations (Guo et al. 2005). Additional customization is being explored, including the use of chemokine-encoding genes or combinations with co-stimulation (John et al. 2012; Li et al. 2012). Herpesviruses have additionally been utilized in cancer vaccination as oncolytic viruses. A possible approach has been to include GM-CSF as an adjuvant or APC growth factor into herpesvirus vectors that proliferate. T-VEC, one of such vectors, had recently been evaluated in melanoma sufferers in a phase III experiment (Kaufman and Bines 2010). The experiment estimated a 26% rate of objective response and an 11% rate of full clinical response in individuals carrying stage IIIB-IV melanoma (Ross et al. 2014). However, it will be critical to explore immune response mechanisms.

## 17.3 Regulatory Considerations

The regulatory body ensures quality safety and efficacy before granting marketing approval to a medicinal product. This function of regulatory body ensures that the medicinal product has positive benefit to risk ratio in an indicated set of population. Even after marketing approval clinical safety data are continuously collected which helps to ensure that benefit to risk ratio stays positive after marketing approval also. Rare adverse effects undetected in initial clinical experiments on limited number of people can be detected in this way. Like all other products, cancer vaccines also have to comply to the need of regulatory and pharmacovigilance requirements along with proper risk management plan. Here, regulatory requirements mean the requirements for the benefit: risk ratio to remain positive.

The US-FDA (Food and Drug Administration) and EMA (European Medicines Agency) which are regulatory bodies for the United States and Europe, respectively, regularly keep watch on quality, clinical, and non-clinical aspects by releasing guidelines regarding them. ICH guidelines cover regulatory as well as multidisciplinary aspects along with quality safety and efficacy issues. The ICH (International Council for Harmonisation) releases harmonized guidelines in the US, Japan, and European Union; hence, they are very useful for drug developers as they provide guiding principles for multi-regional drug development approach.

### 17.3.1 Quality Considerations

EMA has issued guidelines for variety of products like gene therapy products, cell therapy products, etc.; but due to continuously growing knowledge of tumor pathophysiology as well as immune system along with novel therapeutic options such as ATMPs (Advanced Therapy Medicinal Product), it becomes unattainable to accommodate all scenarios in any fixed single guideline. Although guidelines have regulatory purpose, they should not be mandatorily followed if not feasible. Sometimes, if a new understanding about the disease emerges and if the drug developers can firmly justify their position based on this new understanding, they should consult the regulatory body regarding the differences between existing guidelines and the findings of drug development program. The drug developers must have a good quality data and sound rationale to support their claims. Quality assessment of cancer vaccines is more complex and challenging. Hence, for some cancer vaccines, case-by-case release specifications must be agreed upon provided that these specifications must be in accordance with the product's mechanism of action.

### 17.3.2 Non-clinical Considerations

For non-clinical trials, selection of non-clinical model is governed by the target tumor and cancer vaccine type. Various products like proteins, peptides, and cells, e.g., tumor cells, T cells, and dendritic cells are tested. In-vitro manipulated cells,

genetically modified cells, expanded cells, and fused cells were also tested. Vaccines combinations with adjuvants, vectors, cytokines, and immune checkpoint inhibitors have also been tested. However, in some instances, due to unavailability of suitable non-clinical model, demonstration of mechanism of action and proof of concept may be difficult or impossible. This problem arises more prominently during development of autologous personalized vaccines. In this case, while planning the experiments, use of pre-clinical models would vary from case to case.

In some cases, the murine model can demonstrate the proof of concept which extends the base for clinical development; however, in these cases also, differences arise due to differences of patients in comparison to the murine model. Generally, young mice are utilized for the experiments. Therapeutic vaccines are used in patients who might be having tumor for long period of time. Owing to prior radiotherapy or chemotherapy and tumor specific suppression, such patients may have immunosuppression and tolerance to tumor. In case if non-human primates are used, prominent immune system related inter-species differences are observed (ICH n.d.; Sathish et al. 2013). Hence, non-human primates are not recommended.

Human tissues are tested in vitro to check how TAA are distributed and to support the desired outcomes of modifying candidate antigen (Badaracco et al. 1981). Antigen expression can be visualized using tumor cell lines and tumor tissues. Proof of concept can be established by checking cell count and activation status of tumor penetrating cells like CD4+ and CD8+ T cells, MDSCs, dendritic cells, and tumor infiltrating myeloid cells (Horn et al. 2021). Proof of concept can be further supported if it is possible to test the tumor sequentially after vaccination.

A separate section on cancer vaccines is available under anticancer products in EMA guidelines (European Medicines Agency 2012). The EMA guideline states that "Non-clinical in vitro and in vivo proof-of-concept should be presented to justify the planned starting dose and phase 1 studies." However, there is some limitation for instances in which relevant non-clinical model is unavailable. This caveat considers the challenges in planning non-clinical program for few types of cancer vaccines. In such cases, proof of concept can be acceptably established by in vitro experimentations with human cells.

### 17.3.3 Clinical Considerations

In case of few cancer vaccines, due to limitations in non-clinical study plan, human in vivo studies are undertaken to establish the mechanism of action of cancer vaccine product.

#### 17.3.3.1 Immune Status Pre- and Post-Vaccination

Immune system baseline considerations may differ in animal models from human patients. Prior therapy and older age may be the primary reasons for immune function reduction. The process called immunoediting is the defensive mechanism for tumor to befool the immune system and to evade detection and elimination by the immune system (Dunn et al. 2004). In addition to this, it is observed in early

tumorigenesis that tumor microenvironment also exhibits immunosuppression (Predina et al. 2013).

All these factors affect the individual patient's response to the cancer vaccine. CD4+ cells, CD8+ cells, serum Igs, dendritic cells, MDSCs, and tumor antigen associated specific T cells must be measured during a clinical program as they indicate the baseline immune status. These parameters affect the prognosis as well as individual patient's response to the vaccine. If early phase studies indicate a correlation between baseline status of immune system and patient's response to vaccine, these data can be utilized to design the clinical trial program.

#### 17.3.3.2 Changes Following Vaccination

Periodic in vitro testing gives idea about therapeutic efficacy of vaccine and can be the source of crucial data in early development clinical phase (Pagès et al. 2009). Ex-vivo method of direct peripheral blood analysis can produce more satisfying outcomes as compared to in vitro testing of expanded cells from peripheral blood. Although it remains well-established fact that the changes in immune cells within the tumor cannot be entirely indicated by peripheral blood analysis, this can be a preferred method to assess pharmacodynamic effect in absence of tumor biopsies. Assessment of peripheral blood for tumor antigen associated T cells is very relevant method to establish pharmacodynamic effect. However, additional phenomena like epitope spreading that might be an element of the effect proposed thereby cannot be fully reflected by this method. Establishing the pharmacodynamic effect strengthens the proposed mechanism of action, supports the proof of concept, and helps in determining the dose. This information is very crucial in determining optimum treatment duration.

To establish proof of concept, all the available clinical data are recommended to be utilized as the non-clinical data may not provide sufficient evidence in this regard.

However, major concern about cancer vaccines is the paradox that they can sometimes affect the tumor infiltrating cells and demonstrate enhanced tumor-specific immunosuppression which raises the concern about safety of cancer vaccines (Zhou et al. 2006). Hence, it becomes very crucial to establish proof of concept and pharmacodynamic readout after vaccination. If these types of evidence are available in human, they should be well understood before planning pivotal investigations.

Prophylactic vaccines on the other hand offer lesser obstacles as far as overcoming of immune suppression is concerned. In this case, the subjects under question are not cancer patients; they are rather high-risk individuals with pre-cancerous lesions (Finn 2014). Hence, selection of patients and assigning of suitable endpoints in clinical studies may differ for prophylactic vaccine than for therapeutic vaccine. In such cases, the need for development as well as validation of surrogate end points arises (Gilbert and Hudgens 2008). As the regulatory guidelines are not very well established and very limited knowledge and experience is available in this area, it becomes essential to have two-way communication with regulatory body. This is recommended to be done after acquiring good initial clinical information and a strong basis for future development. In case of therapeutic cancer

vaccines, studies for clinical dose determination with reference to periodic monitoring of immune functions are required. The guidelines also assert the importance of descriptively elaborating the analytical assays performed during the development of cancer vaccine. In cases wherever possible, serial tumor biopsies are performed but the outcomes can provide marker for anticancer action. In such case, the proof of concept can be established from the data generated in early clinical studies wherein few numbers of subjects are subjected to serial tumor biopsies. Although it is not mentioned in guidelines, imaging techniques can also be used to establish evidence for such response in case of tumors for which safe biopsies are not possible.

The patients with large tumors and late-stage disease will show immune suppression and limited life expectancy which makes it difficult to select group of patients for pivotal clinical studies. Hence, EMA guidelines in this regard suggest selecting those having a low or minimum illness burden.

Newly detected cases are not pretreated; immune stimulation approach in such patients may provide greater likelihood of success. Nevertheless, a satisfactory justification for use of such patients must be provided owing to existence of alternative therapies. If a good proof of concept and a sound rationale supports this approach, then it is advised to discuss with regulatory body in order to develop suitable clinical plan. Delayed response for efficacy read-out is allowed for cancer vaccines as per the guidelines which suggest that "revised criteria defining progression is accepted if properly justified." This is with reference to revised RECIST (Response Evaluation Criteria for Solid Tumors) criteria highlighting the possibility of time lag in producing effective immune response hence delayed tumor response in contrast to anticancer agents (Wolchok et al. 2009). EMA recommends overall survival (OS) as the core efficacy endpoint.

While overall survival (OS) is the clear objective, it may be acceptable if the sufficient proof of concept and significantly increased progression-free survival (PFS) is demonstrated by a cancer vaccine in comparison to a suitable comparator. Additional information on post-licensing OS, on the other hand, will be extremely crucial in these scenarios to assure that there is no trace of any significant consequences on OS.

A double-blind trial is employed for evaluating PFS. When double blinding is not practicable, the trials should employ blinded effectiveness assessment. The blinded evaluators should review the radiological examinations if they are the primary measures of effectiveness. The FDA guidance to industry on clinical aspects of therapeutic cancer vaccines (Guidance for industry n.d.) covers a more extensive overview, including the design of companion diagnostics. Yet, as with the EU guidelines, the concerns of the patient group to be investigated as well as determination of realistic endpoint remains unresolved. If the patient group selected has no or little residual illness, the effectiveness objective of illness recurrence will need a longer period of monitoring. The FDA considers immune response monitoring to be experimental, and relevance of such measures is acknowledged as valuable in proof of concept, dosage determination, and probable association with clinical effectiveness. For proof-of-concept purposes, the FDA encourages the utility of exploratory biomarkers as well as offers some recommendations on multi-antigen and adjuvants

treatment. Given the variety of therapeutic vaccination approaches, the guideline suggests that the major clinical outcomes have clinical meaningfulness and reviewed with the FDA.

Following a study of the guidelines of FDA and EMA, it is obvious that the essential standards for safety, efficacy, and quality stays the same as that for other products, and the pathway to marketing authorization would be case-by-case. When available guidelines do not cover the strategy employed in the development of new drug, as is intended for therapeutic as well as all prophylactic cancer vaccines, it is recommended that discussions with regulatory bodies be held to reach consensus on the grounds of a well-justified methodology and well-thought-out program for clinical development.

There are yet no regulatory guidelines for prophylactic cancer vaccines. It is crucial to consider this form of prophylactic vaccine in a different light since the participants engaged in studies will be healthy, as opposed to those getting cancer vaccines. As a result, safety would be a greater issue in such scenario, mandating a bigger patient-safety group than would be required for a therapeutic vaccine administered in advanced cancer sufferers. Another issue to consider is that the uncertainty around the safety of a gene therapy product will be greater than that of, say, a peptide vaccine. Drug developers should perceive the lack of guidance for prophylactic cancer vaccines as a chance to influence regulatory decision-making rather than an obstacle. Regulators appreciate such early communication, which can take the shape of a meeting with the EMA's innovation task force (ITF). Although regulatory clearance is obliged, it does not promise the success with regards to patient access and reimbursement. Hence, considerations for rapid commercial utilization are essential. Consultation with Health Technology Assessment (HTA) bodies is indeed recommended to verify that endpoints specified by regulatory agencies are agreeable to both the payers and HTAs. In November 2011, the EMA organized a workshop on EMA/HTA-body parallel scientific guidance in drug development. The documents and presentations can be accessed from the EMA website. While this workshop is not indication-specific, the underlying conclusion is obvious; whatever the program, it is best to get early involvement of HTA/payer and regulatory authority. Such a strategy is likely to lower the chance of failure at the post-licensing reimbursement phase. Such collaborative consulting practices are also accessible at the national level, and in the UK, concurrent scientific advice conferences with MHRA and NICE are provided.

## 17.4 Personalized Cancer Vaccines

Owing to the emergence of next-generation sequencing (NGS) to recognize tumor mutations, the concept of developing vaccines capable of targeting specific tumor neoantigens was conceived. Complete exome sequencing of tumor as well as healthy cell DNA from specific patient is used to identify non-synonymous somatic mutations. The mutations are then graded based on their probability of manifestation and affinity adherence of the neoantigen to autologous MHC (major

histocompatibility complex) molecules, that may be anticipated with bioinformatic technologies such as NetMHCpan or IEDB (Ott et al. 2017; Sahin et al. 2017).

Since central tolerance is unlikely to remove cytotoxic T cell clones with high affinity for these antigen, neo-antigens arising from tumor specific mutations are strongly immunogenic. This strategy was tested in a phase 1 trial employing six patients who had melanoma of stage III and IV following surgical resection. Subcutaneous vaccination of synthetic long peptides designed to target up to 20 specific neoantigens per patient were administered, together with the TLR 3 and melanoma differentiation linked protein 5 agonist poly-ICLC as an immunostimulant. After 25 months of follow-up, four patients were free of tumor relapse (Ott et al. 2017). A phase 1 trial is now underway in glioblastoma individuals to probe a tailored personalized vaccine based on mutations. The vaccine is made up of numerous peptides that are tailored to individual patient's unique tumor sequence. The vaccine is administered following chemotherapy and radiation, during the temozolomide maintenance phase, and in tumor-treating fields (NCT03223103). Some other phase 1 trial is employing the personalized peptide vaccine strategy in individuals suffering from severe pancreatic colorectal or pancreatic cancer in conjunction with the checkpoint inhibitor pembrolizumab (NCT02600949). The use of mRNA-based cancer vaccines is by far the most current approach (Pardi et al. 2018). The IVAC-mutanome trial, a phase 1 trial that involved 13 participants having late melanoma (Sahin et al. 2017). Ten mutations had been specified for each patient, and couple of synthetic RNA molecules encoding five (27 mer) peptides showing the position 14 mutation were produced in vitro. Following that, the RNA molecules were coupled to an MHC trafficking signal peptide for improved routing and presentation to MHC and injected into inguinal lymph nodes of the patients. IFN-ELISpot was used to assess immunogenicity in CD4+ and CD8+ T cells from both before and post-vaccination leukapheresis samples. Without in-vitro stimulation, blood responses to one-fifth of the mutations could be detected. Two of the five patients with advanced illness demonstrated clear vaccine-related responses. The removal of an individual's lymph node metastases verified the presence of vaccine-induced T cells specific to neoepitopes in the tumor (Sahin et al. 2017).

A phase 1 clinical trial is now underway in patients with solid tumors to investigate an intravenous preparation of an RNA-based personalized vaccine in conjunction with the PD-L1 specific drug atezolizumab (NCT03289962) (Liao and Zhang 2021).

## 17.5 Challenges in Personalized Vaccines

### 17.5.1 Selecting the Right Antigen: Improving Bioinformatics

Owing to the advent of parallel sequencing, a new era in antigen selection emerged. It is difficult to determine mutations that will have the greatest in vivo immunogenicity. Bioinformatic forecasting methods strive to rate antigen immunogenicity based on the expected epitope's binding affinity to molecules of MHC, the chances

of presentation, clonality, and the amount of expression of the related RNA. But subsequent studies revealed that CD8+ responses to predicted high-affinity binders were as low as 29%, highlighting the necessity of improved algorithms (Sahin et al. 2017).

### 17.5.2 Selecting the Right Combination

Because tumor cells have developed several immune escape strategies, combined therapies are required to re-establish anticancer immunity. Antigen release by tumor cell death can be aided by traditional approaches such as chemotherapy and radiation. By inhibiting the negative regulatory route employed by tumors, checkpoint inhibitors put a stop to endogenous T cells. They have demonstrated effectiveness across many cancer types on their own; however, less effectiveness was gained in tumors free of penetrating lymphocytes (Hegde et al. 2016). The absence of invading T cells may be due to cancer cells creating a tumor suppressive milieu via the production of immunosuppressive cytokines, recruitment of regulatory T cells, and MDSCs. An increased level of indoleamine-pyrrole 2,3-dioxygenase (IDO) expression in cancerous cells results in immunosuppression via depletion of tryptophan, that promotes T regulator cells (Elia et al. 2008; Moon et al. 2015; Braun et al. 2005). T cell migration via the vascular endothelium at the tumor site requires the expression of vascular adhesion molecules (VCAM-1) and intercellular adhesion molecules (ICAM-1). Angiogenic chemicals, such as vascular endothelial growth factor (VEGF), restrict endothelial adhesion molecule production and hence T cell movement in the tumor microenvironment (Bouzin et al. 2007; Motz and Coukos 2013). Combination therapy with TGF-ß inhibitors, VEGF inhibitors, or newer immunomodulators such as IDO inhibitors may be beneficial in overcoming the tumor-suppressive microenvironment and are now being studied in clinical investigations (NT02873962, NCT03347123, and NCT02423343) (Moon et al. 2015). Traditional chemotherapy, such as cyclophosphamide, can also be used to deplete Tregs.

### 17.5.3 Choosing the Right Time: Adjuvant Vs. Palliative

Tumor immunosuppression frequently corresponds to tumor burden, lowering the benefits of immunotherapy in patients with advanced illness. Immunologic responsiveness rates to vaccinations in clinical trials are frequently greater during adjuvant treatment than in palliative care, providing support for the use of vaccines at an initial phase of disease (Gulley et al. 2011). Moreover, existing personalized vaccine techniques are demanding, and the manufacturing duration of few months may be difficult for individuals with severe illness. Again, the combination approaches might be utilized to strike a balance between vaccine production and application.

### 17.5.4 Tumor Evolution and Loss of Antigen

Along with the tumor growth, additional mutations arise, that can render neo-epitope vaccines ineffective owing to mutation and loss of neoepitope's antigenicity (Ott et al. 2017; Sahin et al. 2017). T cells rely on processing and presentation of antigen via MHC proteins to recognize targets. Downregulation of MHC class I proteins in cancerous cells leads to diminished antigen presentation, which favors immune evasion (Seliger et al. 2001). MHC class I protein downregulation is seen in a variety of cancer types. It can occur either genetically or because of a protein synthesis deficiency (Reeves and James 2017). Antigens are normally broken into the fragments of peptide by immunoproteasomes in the cytoplasm of cells in order to ensure their attachment to MHC class I. Downregulation of proteasome complex subunits has been correlated to tumor proliferation and metastasis. The endoplasmic reticulum is where tiny peptide fragments are coupled to MHC class I. A deficiency in the antigen processing associated transporters (TAP) in the endoplasmic reticulum or a loss of the endoplasmic aminopeptidases (ERAP 1 and ERAP2) can lead to even lower antigen expression (Mehta et al. 2009) (Table 17.1).

## 17.6 Conclusion

The reported success stories of vaccines have provided an explosion of rejuvenation approaches like tailored approaches and renewing of techniques specifically for vaccine development. But till date, there is no vaccine in market that act as pillar to neutralize progression of cancer. Many clinical trials at early stages indicate the potential of vaccines. Furthermore, advancement in molecular techniques has paved way for much advanced protocol designing and preparation of target-based vaccines. Hopefully, the next decade expects to provide market with high throughput vaccines that will act as boon to eradicate the mass tumor cases. Further advancements are required for precision-based prediction about modeling, algorithms, and simulation factors. This era must focus on the development of validated simplified complex models for pharmacological platforms that play a rational role in acceptance of these vaccines. Overcoming of these hurdles will pave a way for optimized vaccine with a tagline of "one short to curb cancer menace."

**Disclosures** There is no conflict of interest and disclosures associated with the manuscript.

**Acknowledgments** The authors acknowledge Department of Pharmaceuticals, Ministry of Chemical and Fertilizers, Govt. of India, for their support. The NIPER-R communication number is NIPER-R/Communication/276.

**Table 17.1** Cancer vaccines in clinical development

| Clinicaltrials.gov identifier | Phase of study | Type of cancer | Treatment | Mode of action |
|---|---|---|---|---|
| NCT03328026 | Phase 2 | Breast carcinoma | Intradermal SV-BR-1-GM, ipilumab, interferon, cyclophosphamide, pembrolizumab | Vaccine containing cells secreting GM-CSF, anti-CLTA-4, cytokine, chemotherapy, anti-PD-1 |
| NCT03152565 | Phase1/2 | Colorectal cancer | Autologous dendritic cell (ADC) (intradermal) in combination with avelumab | ADC vaccine, anti-PD-1 |
| NCT03029403 | Phase 2 | Fallopian tube cancer, epithelial ovarian carcinoma | DPX survivac (subcutaneous), Pembrolizumab, cyclophosphamide | Peptide vaccine targeting surviving, anti-PD-1, chemotherapy |
| NCT02499835 | Phase-1 | Carcinoma of prostate | pTVG-HP (intradermal), Pembrolizumab | pTVG-HP plasmid DNA vaccine encoding prostatic acid phosphate, anti-PD-1 |
| NCT03164772 | Phase 1/2 | Non-small cell lung cancer | BI 1361849 (intradermal), Durvalumab alone or in combination with tremelimumab | m-RNA vaccine, Anti-PD-1, anti-CLTA-4 |
| NCT03406715 | Phase 2 | Small cell lung cancer | Ad.p53-DC (intradermal) in combination with ipilimumab and nivolumab | Autologous dendritic cell based p53 vaccine, anti-CLTA-4, anti-PD-1 |
| NCT03289962 | Phase 1 | Solid tumors | RO7198457 (intravenous), atezolizumab | Personalized RNA mutanome vaccine, anti-PD-L1 |
| NCT03162224 | Phase 1/2 | Head and neck cancer | MEDI0457 (intramuscular), Durvalumab | HPV DNA vaccine, anti-PD-L1 |
| NCT03260023 | Phase 1/2 | HPV related carcinoma | TG4001 (subcutaneous), Avelumab | Anakinra virus vaccinia encoding IL-2 and HPV-16, anti-PD-L1 |
| NCT02451982 | Phase 2 | Pancreatic adenocarcinoma | GVAX (intradermal), Cyclophosphamide alone or in combination with nivolumab | Vaccine based on whole tumor cell secreting GM-CSF, chemotherapy, anti-PD-1 |

(continued)

**Table 17.1** (continued)

| Clinicaltrials.gov identifier | Phase of study | Type of cancer | Treatment | Mode of action |
|---|---|---|---|---|
| NCT03047928 | Phase 1/2 | Metastatic melanoma | PD-L1/IDO vaccine (subcutaneous), nivolumab | Peptide vaccine, anti-PD-1 |
| NCT02808143 | Phase 1 | Bladder carcinoma | Pembrolizumab, BCG | BCG, anti-PD-1 |
| NCT03199040 | Phase 1 | Triple negative breast cancer | DNA vaccine (intramuscular) individually or with durvalumab | DNA vaccine, anti-PD-L1 |

Note. The data shown in following table have been retrieved from https://clinicaltrials.gov

## References

Arthur JF, Butterfield LH, Roth MD, Bui LA, Kiertscher SM, Lau R, Dubinett S, Glaspy J, McBride WH, Economou JS (1997) A comparison of gene transfer methods in human dendritic cells. Cancer Gene Ther 4:17–25

Badaracco G, Corsi A, Maisto A, Natali PG, Starace G, Zupi G (1981) Expression of tumor-associated antigens and kinetic profile of two in vitro human melanoma cell lines. Cytometry 2: 63–69. https://doi.org/10.1002/cyto.990020205

Banchereau J, Palucka AK, Dhodapkar M, Burkeholder S, Taquet N, Rolland A, Taquet S, Coquery S, Wittkowski KM, Bhardwaj N, Pineiro L, Steinman R, Fay J (2001) Immune and clinical responses in patients with metastatic melanoma to CD34(+) progenitor-derived dendritic cell vaccine. Cancer Res 61:6451–6458

Bezu L, Kepp O, Cerrato G, Pol J, Fucikova J, Spisek R, Zitvogel L, Kroemer G, Galluzzi L (2018) Trial watch: peptide-based vaccines in anticancer therapy. Onco Targets Ther 7:e1511506. https://doi.org/10.1080/2162402X.2018.1511506

Bouzin C, Brouet A, De Vriese J, Dewever J, Feron O (2007) Effects of vascular endothelial growth factor on the lymphocyte-endothelium interactions: identification of caveolin-1 and nitric oxide as control points of endothelial cell anergy. J Immunol 178:1505–1511. https://doi.org/10.4049/jimmunol.178.3.1505

Braun D, Longman RS, Albert ML (2005) A two-step induction of indoleamine 2,3 dioxygenase (IDO) activity during dendritic-cell maturation. Blood 106:2375–2381. https://doi.org/10.1182/blood-2005-03-0979

Butterfield LH, Economou JS, Gamblin TC, Geller DA (2014) Alpha fetoprotein DNA prime and adenovirus boost immunization of two hepatocellular cancer patients. J Transl Med 12:86. https://doi.org/10.1186/1479-5876-12-86

Chakraborty NG, Sporn JR, Tortora AF, Kurtzman SH, Yamase H, Ergin MT, Mukherji B (1998) Immunization with a tumor-cell-lysate-loaded autologous-antigen-presenting-cell-based vaccine in melanoma. Cancer Immunol Immunother 47:58–64. https://doi.org/10.1007/s002620050504

Cheever MA, Allison JP, Ferris AS, Finn OJ, Hastings BM, Hecht TT, Mellman I, Prindiville SA, Viner JL, Weiner LM, Matrisian LM (2009) The prioritization of cancer antigens: a national cancer institute pilot project for the acceleration of translational research. Clin Cancer Res An Off J Am Assoc Cancer Res 15:5323–5337. https://doi.org/10.1158/1078-0432.CCR-09-0737

Disis ML, Gad E, Herendeen DR, Lai VP, Park KH, Cecil DL, O'Meara MM, Treuting PM, Lubet RA (2013) A multiantigen vaccine targeting neu, IGFBP-2, and IGF-IR prevents tumor

progression in mice with preinvasive breast disease., cancer Prev. Res (Phila) 6:1273–1282. https://doi.org/10.1158/1940-6207.CAPR-13-0182

Dranoff G, Jaffee E, Lazenby A, Golumbek P, Levitsky H, Brose K, Jackson V, Hamada H, Pardoll D, Mulligan RC (1993) Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity. Proc Natl Acad Sci U S A 90:3539–3543. https://doi.org/10.1073/pnas.90.8.3539

Dreno B, Thompson JF, Smithers BM, Santinami M, Jouary T, Gutzmer R, Levchenko E, Rutkowski P, Grob J-J, Korovin S, Drucis K, Grange F, Machet L, Hersey P, Krajsova I, Testori A, Conry R, Guillot B, Kruit WHJ, Demidov L, Thompson JA, Bondarenko I, Jaroszek J, Puig S, Cinat G, Hauschild A, Goeman JJ, van Houwelingen HC, Ulloa-Montoya F, Callegaro A, Dizier B, Spiessens B, Debois M, Brichard VG, Louahed J, Therasse P, Debruyne C, Kirkwood JM (2018) MAGE-A3 immunotherapeutic as adjuvant therapy for patients with resected, MAGE-A3-positive, stage III melanoma (DERMA): a double-blind, randomised, placebo-controlled, phase 3 trial. Lancet Oncol 19:916–929. https://doi.org/10.1016/S1470-2045(18)30254-7

Dunn GP, Old LJ, Schreiber RD (2004) The three Es of cancer Immunoediting. Annu Rev Immunol 22:329–360. https://doi.org/10.1146/annurev.immunol.22.012703.104803

Elia AR, Cappello P, Puppo M, Fraone T, Vanni C, Eva A, Musso T, Novelli F, Varesio L, Giovarelli M (2008) Human dendritic cells differentiated in hypoxia down-modulate antigen uptake and change their chemokine expression profile. J Leukoc Biol 84:1472–1482. https://doi.org/10.1189/jlb.0208082

European Medicines Agency (2012) 'Guideline on the evaluation of anticancer medicinal products in man'. EMA/CHMP/205/95/Rev.4. https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-evaluation-anticancer-medicinal-products-man-revision-4_en.pdf

Finn OJ (2014) Vaccines for cancer prevention: a practical and feasible approach to the cancer epidemic, cancer. Immunol Res 2:708–713. https://doi.org/10.1158/2326-6066.CIR-14-0110

Fourcade J, Kudela P, Andrade Filho PA, Janjic B, Land SR, Sander C, Krieg A, Donnenberg A, Shen H, Kirkwood JM, Zarour HM (2008) Immunization with analog peptide in combination with CpG and montanide expands tumor antigen-specific CD8+ T cells in melanoma patients. J Immunother 31:781–791. https://doi.org/10.1097/CJI.0b013e318183af0b

Galluzzi L, Senovilla L, Vacchelli E, Eggermont A, Fridman WH, Galon J, Sautès-Fridman C, Tartour E, Zitvogel L, Kroemer G (2012) Trial watch: dendritic cell-based interventions for cancer therapy. Onco Targets Ther 1:1111–1134. https://doi.org/10.4161/onci.21494

Geiger J, Hutchinson R, Hohenkirk L, McKenna E, Chang A, Mulé J (2000) Treatment of solid tumours in children with tumour-lysate-pulsed dendritic cells. Lancet (London, England) 356:1163–1165. https://doi.org/10.1016/S0140-6736(00)02762-8

Gilbert PB, Hudgens MG (2008) Evaluating candidate principal surrogate endpoints. Biometrics 64:1146–1154. https://doi.org/10.1111/j.1541-0420.2008.01014.x

Guidance for industry: clinical considerations for therapeutic cancer vaccines. 2011, (n.d.). https://www.fda.gov/media/82312/download

Gulley JL, Arlen PM, Madan RA, Tsang K-Y, Pazdur MP, Skarupa L, Jones JL, Poole DJ, Higgins JP, Hodge JW, Cereda V, Vergati M, Steinberg SM, Halabi S, Jones E, Chen C, Parnes H, Wright JJ, Dahut WL, Schlom J (2010) Immunologic and prognostic factors associated with overall survival employing a poxviral-based PSA vaccine in metastatic castrate-resistant prostate cancer. Cancer Immunol Immunother 59:663–674. https://doi.org/10.1007/s00262-009-0782-8

Gulley JL, Madan RA, Schlom J (2011) Impact of tumour volume on the potential efficacy of therapeutic vaccines. Curr Oncol 18:e150–e157. https://doi.org/10.3747/co.v18i3.783

Guo ZS, Naik A, O'Malley ME, Popovic P, Demarco R, Hu Y, Yin X, Yang S, Zeh HJ, Moss B, Lotze MT, Bartlett DL (2005) The enhanced tumor selectivity of an oncolytic vaccinia lacking the host range and antiapoptosis genes SPI-1 and SPI-2. Cancer Res 65:9991–9998. https://doi.org/10.1158/0008-5472.CAN-05-1630

Hailemichael Y, Dai Z, Jaffarzad N, Ye Y, Medina MA, Huang X-F, Dorta-Estremera SM, Greeley NR, Nitti G, Peng W, Liu C, Lou Y, Wang Z, Ma W, Rabinovich B, Sowell RT, Schluns KS, Davis RE, Hwu P, Overwijk WW (2013) Persistent antigen at vaccination sites induces tumor-specific $CD8^+$ T cell sequestration, dysfunction and deletion. Nat Med 19:465–472. https://doi.org/10.1038/nm.3105

Heelan BT (2014) Regulatory considerations for clinical development of cancer vaccines. Hum Vaccines Immunother 10:3409–3414. https://doi.org/10.4161/21645515.2014.982999

Hegde PS, Karanikas V, Evers S (2016) The where, the when, and the how of immune monitoring for cancer immunotherapies in the era of checkpoint inhibition. Clin Cancer Res an Off J Am Assoc Cancer Res 22:1865–1874. https://doi.org/10.1158/1078-0432.CCR-15-1507

Hirschler B (2014) 2-GSK cancer vaccine fails again but testing continues, Reuters. https://www.reuters.com/article/gsk-vaccine-idUSL6N0MH1IR20140320

Horn LA, Fousek K, Hamilton DH, Hodge JW, Zebala JA, Maeda DY, Schlom J, Palena C (2021) Vaccine increases the diversity and activation of Intratumoral T cells in the context of combination immunotherapy. Cancers (Basel) 13. https://doi.org/10.3390/cancers13050968

Hsu FJ, Benike C, Fagnoni F, Liles TM, Czerwinski D, Taidi B, Engleman EG, Levy R (1996) Vaccination of patients with B-cell lymphoma using autologous antigen-pulsed dendritic cells. Nat Med 2:52–58. https://doi.org/10.1038/nm0196-52

ICH guideline M3(R2) on non-clinical safety studies for the conduct of human clinical trials and marketing authorisation for pharmaceuticals, (n.d.). https://www.ema.europa.eu/en/documents/scientific-guideline/ich-guideline-m3r2-non-clinical-safety-studies-conduct-human-clinical-trials-marketing-authorisation_en.pdf

John LB, Howland LJ, Flynn JK, West AC, Devaud C, Duong CP, Stewart TJ, Westwood JA, Guo ZS, Bartlett DL, Smyth MJ, Kershaw MH, Darcy PK (2012) Oncolytic virus and anti-4-1BB combination therapy elicits strong antitumor immunity against established cancer. Cancer Res 72:1651–1660. https://doi.org/10.1158/0008-5472.CAN-11-2788

Kanerva A, Nokisalmi P, Diaconu I, Koski A, Cerullo V, Liikanen I, Tähtinen S, Oksanen M, Heiskanen R, Pesonen S, Joensuu T, Alanko T, Partanen K, Laasonen L, Kairemo K, Pesonen S, Kangasniemi L, Hemminki A (2013) Antiviral and antitumor T-cell immunity in patients treated with GM-CSF-coding oncolytic adenovirus. Clin Cancer Res an Off J Am Assoc Cancer Res 19:2734–2744. https://doi.org/10.1158/1078-0432.CCR-12-2546

Kaufman HL, Bines SD (2010) OPTIM trial: a phase III trial of an oncolytic herpes virus encoding GM-CSF for unresectable stage III or IV melanoma. Future Oncol 6:941–949. https://doi.org/10.2217/fon.10.66

Kawalec P, Paszulewicz A, Holko P, Pilc A (2012) Sipuleucel-T immunotherapy for castration-resistant prostate cancer. A systematic review and meta-analysis. Arch Med Sci 8:767–775. https://doi.org/10.5114/aoms.2012.31610

Kenter GG, Welters MJP, Valentijn ARPM, Lowik MJG, Berends-van der Meer DMA, Vloon APG, Drijfhout JW, Wafelman AR, Oostendorp J, Fleuren GJ, Offringa R, van der Burg SH, Melief CJM (2008) Phase I immunotherapeutic trial with long peptides spanning the E6 and E7 sequences of high-risk human papillomavirus 16 in end-stage cervical cancer patients shows low toxicity and robust immunogenicity. Clin Cancer Res an Off J Am Assoc Cancer Res 14:169–177. https://doi.org/10.1158/1078-0432.CCR-07-1881

Kiertscher SM, Luo J, Dubinett SM, Roth MD (2000) Tumors promote altered maturation and early apoptosis of monocyte-derived dendritic cells. J Immunol 164:1269–1276. https://doi.org/10.4049/jimmunol.164.3.1269

Kim TS, Chopra A, O-Sullivan IS, Cohen EP (2006) Enhanced immunity to breast cancer in mice immunized with fibroblasts transfected with a complementary DNA expression library from breast cancer cells: enrichment of the vaccine for immunotherapeutic cells. J Immunother 29:261–273. https://doi.org/10.1097/01.cji.0000197097.46100.bb

Kimura T, McKolanis JR, Dzubinski LA, Islam K, Potter DM, Salazar AM, Schoen RE, Finn OJ (2013) MUC1 vaccine for individuals with advanced adenoma of the colon: a cancer

immunoprevention feasibility study. Cancer Prev Res (Phila) 6:18–26. https://doi.org/10.1158/1940-6207.CAPR-12-0275

Kissick HT, Sanda MG, Dunn LK, Arredouani MS (2014) Immunization with a peptide containing MHC class I and II epitopes derived from the tumor antigen SIM2 induces an effective CD4 and CD8 T-cell response. PLoS One 9:1–7. https://doi.org/10.1371/journal.pone.0093231

Komita H, Zhao X, Taylor JL, Sparvero LJ, Amoscato AA, Alber S, Watkins SC, Pardee AD, Wesa AK, Storkus WJ (2008) CD8+ T-cell responses against hemoglobin-beta prevent solid tumor growth. Cancer Res 68:8076–8084. https://doi.org/10.1158/0008-5472.CAN-08-0387

Le DT, Brockstedt DG, Nir-Paz R, Hampl J, Mathur S, Nemunaitis J, Sterman DH, Hassan R, Lutz E, Moyer B, Giedlin M, Louis J-L, Sugar EA, Pons A, Cox AL, Levine J, Murphy AL, Illei P, Dubensky TWJ, Eiden JE, Jaffee EM, Laheru DA (2012b) A live-attenuated listeria vaccine (ANZ-100) and a live-attenuated listeria vaccine expressing mesothelin (CRS-207) for advanced cancers: phase I studies of safety and immune induction. Clin Cancer Res an Off J Am Assoc Cancer Res 18:858–868. https://doi.org/10.1158/1078-0432.CCR-11-2121

Le DT, Dubenksy TWJ, Brockstedt DG (2012a) Clinical development of listeria monocytogenes-based immunotherapies. Semin Oncol 39:311–322. https://doi.org/10.1053/j.seminoncol.2012.02.008

Li J, O'Malley M, Sampath P, Kalinski P, Bartlett DL, Thorne SH (2012) Expression of CCL19 from oncolytic vaccinia enhances immunotherapeutic potential while maintaining oncolytic activity. Neoplasia 14:1115–1121. https://doi.org/10.1593/neo.121272

Liao J-Y, Zhang S (2021) Safety and efficacy of personalized cancer vaccines in combination with immune checkpoint inhibitors in cancer treatment. Front Oncol 11. https://doi.org/10.3389/fonc.2021.663264

Luiten RM, Kueter EWM, Mooi W, Gallee MPW, Rankin EM, Gerritsen WR, Clift SM, Nooijen WJ, Weder P, van de Kasteele WF, Sein J, van den Berk PCM, Nieweg OE, Berns AM, Spits H, de Gast GC (2005) Immunogenicity, including vitiligo, and feasibility of vaccination with autologous GM-CSF-transduced tumor cells in metastatic melanoma patients. J Clin Oncol Off J Am Soc Clin Oncol 23:8978–8991. https://doi.org/10.1200/JCO.2005.01.6816

Madan RA, Bilusic M, Heery C, Schlom J, Gulley JL (2012) Clinical evaluation of TRICOM vector therapeutic cancer vaccines. Semin Oncol 39:296–304. https://doi.org/10.1053/j.seminoncol.2012.02.010

McBride WH, Thacker JD, Comora S, Economou JS, Kelley D, Hogge D, Dubinett SM, Dougherty GJ (1992) Genetic modification of a murine fibrosarcoma to produce interleukin 7 stimulates host cell infiltration and tumor immunity. Cancer Res 52:3931–3937

Mehta AM, Jordanova ES, Corver WE, van Wezel T, Uh H-W, Kenter GG, Jan Fleuren G (2009) Single nucleotide polymorphisms in antigen processing machinery component ERAP1 significantly associate with clinical outcome in cervical carcinoma. Genes Chromosomes Cancer 48:410–418. https://doi.org/10.1002/gcc.20648

Meng WS, Butterfield LH, Ribas A, Dissette VB, Heller JB, Miranda GA, Glaspy JA, McBride WH, Economou JS (2001) Alpha-fetoprotein-specific tumor immunity induced by plasmid prime-adenovirus boost genetic vaccination. Cancer Res 61:8782–8786

Moon YW, Hajjar J, Hwu P, Naing A (2015) Targeting the indoleamine 2,3-dioxygenase pathway in cancer. J Immunother Cancer 3:51. https://doi.org/10.1186/s40425-015-0094-9

Motz GT, Coukos G (2013) Deciphering and reversing tumor immune suppression. Immunity 39:61–73. https://doi.org/10.1016/j.immuni.2013.07.005

Nemunaitis J, Sterman D, Jablons D, Smith JW, Fox B, Maples P, Hamilton S, Borellini F, Lin A, Morali S, Hege K (2004) Granulocyte-macrophage colony-stimulating factor gene-modified autologous tumor vaccines in non-small-cell lung cancer. J Natl Cancer Inst 96:326–331. https://doi.org/10.1093/jnci/djh028

Ott PA, Hu Z, Keskin DB, Shukla SA, Sun J, Bozym DJ, Zhang W, Luoma A, Giobbie-Hurder A, Peter L, Chen C, Olive O, Carter TA, Li S, Lieb DJ, Eisenhaure T, Gjini E, Stevens J, Lane WJ, Javeri I, Nellaiappan K, Salazar AM, Daley H, Seaman M, Buchbinder EI, Yoon CH, Harden M, Lennon N, Gabriel S, Rodig SJ, Barouch DH, Aster JC, Getz G,

Wucherpfennig K, Neuberg D, Ritz J, Lander ES, Fritsch EF, Hacohen N, Wu CJ (2017) An immunogenic personal neoantigen vaccine for patients with melanoma. Nature 547:217–221. https://doi.org/10.1038/nature22991

Pagès F, Kirilovsky A, Mlecnik B, Asslaber M, Tosolini M, Bindea G, Lagorce C, Wind P, Marliot F, Bruneval P, Zatloukal K, Trajanoski Z, Berger A, Fridman W-H, Galon J (2009) In situ cytotoxic and memory T cells predict outcome in patients with early-stage colorectal cancer. J Clin Oncol Off J Am Soc Clin Oncol 27:5944–5951. https://doi.org/10.1200/JCO.2008.19.6147

Palucka K, Banchereau J (2013) Dendritic-cell-based therapeutic cancer vaccines. Immunity 39:38–48. https://doi.org/10.1016/j.immuni.2013.07.004

Palucka K, Ueno H, Fay J, Banchereau J (2011) Dendritic cells and immunity against cancer. J Intern Med 269:64–73. https://doi.org/10.1111/j.1365-2796.2010.02317.x

Pardi N, Hogan MJ, Porter FW, Weissman D (2018) mRNA vaccines–a new era in vaccinology. Nat Rev Drug Discov 17:261–279. https://doi.org/10.1038/nrd.2017.243

Pollack IF, Jakacki RI, Butterfield LH, Hamilton RL, Panigrahy A, Potter DM, Connelly AK, Dibridge SA, Whiteside TL, Okada H (2014) Antigen-specific immune responses and clinical outcome after vaccination with glioma-associated antigen peptides and polyinosinic-polycytidylic acid stabilized by lysine and carboxymethylcellulose in children with newly diagnosed malignant brainstem and. J Clin Oncol Off J Am Soc Clin Oncol 32:2050–2058. https://doi.org/10.1200/JCO.2013.54.0526

Predina J, Eruslanov E, Judy B, Kapoor V, Cheng G, Wang L-C, Sun J, Moon EK, Fridlender ZG, Albelda S, Singhal S (2013) Changes in the local tumor microenvironment in recurrent cancers may explain the failure of vaccines after surgery. Proc Natl Acad Sci U S A 110:E415–E424. https://doi.org/10.1073/pnas.1211850110

Reeves E, James E (2017) Antigen processing and immune regulation in the response to tumours. Immunology 150:16–24. https://doi.org/10.1111/imm.12675

Rosalia RA, Quakkelaar ED, Redeker A, Khan S, Camps M, Drijfhout JW, Silva AL, Jiskoot W, van Hall T, van Veelen PA, Janssen G, Franken K, Cruz LJ, Tromp A, Oostendorp J, van der Burg SH, Ossendorp F, Melief CJM (2013) Dendritic cells process synthetic long peptides better than whole protein, improving antigen presentation and T-cell activation. Eur J Immunol 43:2554–2565. https://doi.org/10.1002/eji.201343324

Rosenberg SA, Yang JC, Kammula US, Hughes MS, Restifo NP, Schwarz SL, Morton KE, Laurencot CM, Sherry RM (2010) Different adjuvanticity of incomplete freund's adjuvant derived from beef or vegetable components in melanoma patients immunized with a peptide vaccine. J Immunother 33:626–629. https://doi.org/10.1097/CJI.0b013e3181dac9de

Rosenblatt J, Avivi I, Vasir B, Uhl L, Munshi NC, Katz T, Dey BR, Somaiya P, Mills H, Campigotto F, Weller E, Joyce R, Levine JD, Tzachanis D, Richardson P, Laubach J, Raje N, Boussiotis V, Yuan YE, Bisharat L, Held V, Rowe J, Anderson K, Kufe D, Avigan D (2013) Vaccination with dendritic cell/tumor fusions following autologous stem cell transplant induces immunologic and clinical responses in multiple myeloma patients. Clin Cancer Res an Off J Am Assoc Cancer Res 19:3640–3648. https://doi.org/10.1158/1078-0432.CCR-13-0282

Ross MI, Andtbacka RHI, Puzanov I, Milhem MM, Collichio FA, Delman KA, Noyes RD, Zager JS, Cranmer LD, Spitler LE, Hsueh EC, Ollila DW, Amatruda T, Chen L, Gansert JL, Kaufman HL (2014) Patterns of durable response with intralesional talimogene laherparepvec (T-VEC): results from a phase III trial in patients with stage IIIb-IV melanoma. J Clin Oncol 32:9026. https://doi.org/10.1200/jco.2014.32.15_suppl.9026

Sahin U, Derhovanessian E, Miller M, Kloke B-P, Simon P, Löwer M, Bukur V, Tadmor AD, Luxemburger U, Schrörs B, Omokoko T, Vormehr M, Albrecht C, Paruzynski A, Kuhn AN, Buck J, Heesch S, Schreeb KH, Müller F, Ortseifer I, Vogler I, Godehardt E, Attig S, Rae R, Breitkreuz A, Tolliver C, Suchan M, Martic G, Hohberger A, Sorn P, Diekmann J, Ciesla J, Waksmann O, Brück A-K, Witt M, Zillgen M, Rothermel A, Kasemann B, Langer D, Bolte S, Diken M, Kreiter S, Nemecek R, Gebhardt C, Grabbe S, Höller C, Utikal J, Huber C, Loquai C,

Türeci Ö (2017) Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. Nature 547:222–226. https://doi.org/10.1038/nature23003

Sathish JG, Sethu S, Bielsky M-C, de Haan L, French NS, Govindappa K, Green J, Griffiths CEM, Holgate S, Jones D, Kimber I, Moggs J, Naisbitt DJ, Pirmohamed M, Reichmann G, Sims J, Subramanyam M, Todd MD, Van Der Laan JW, Weaver RJ, Park BK (2013) Challenges and approaches for the development of safer immunomodulatory biologics. Nat Rev Drug Discov 12:306–324. https://doi.org/10.1038/nrd3974

Schumacher L, Ribas A, Dissette VB, McBride WH, Mukherji B, Economou JS, Butterfield LH (2004) Human dendritic cell maturation by adenovirus transduction enhances tumor antigen-specific T-cell responses. J Immunother 27:191–200. https://doi.org/10.1097/00002371-200405000-00003

Schwartzentruber DJ, Lawson DH, Richards JM, Conry RM, Miller DM, Treisman J, Gailani F, Riley L, Conlon K, Pockaj B, Kendra KL, White RL, Gonzalez R, Kuzel TM, Curti B, Leming PD, Whitman ED, Balkissoon J, Reintgen DS, Kaufman H, Marincola FM, Merino MJ, Rosenberg SA, Choyke P, Vena D, Hwu P (2011) gp100 peptide vaccine and Interleukin-2 in patients with advanced melanoma. N Engl J Med 364:2119–2127. https://doi.org/10.1056/NEJMoa1012863

Seliger B, Ritz U, Abele R, Bock M, Tampé R, Sutter G, Drexler I, Huber C, Ferrone S (2001) Immune escape of melanoma: first evidence of structural alterations in two distinct components of the MHC class I antigen processing pathway. Cancer Res 61:8647–8650

Slingluff CLJ (2011) The present and future of peptide vaccines for cancer: single or multiple, long or short, alone or in combination? Cancer J 17:343–350. https://doi.org/10.1097/PPO.0b013e318233e5b2

Slingluff CLJ, Lee S, Zhao F, Chianese-Bullock KA, Olson WC, Butterfield LH, Whiteside TL, Leming PD, Kirkwood JM (2013) A randomized phase II trial of multiepitope vaccination with melanoma peptides for cytotoxic T cells and helper T cells for patients with metastatic melanoma (E1602). Clin Cancer Res an Off J Am Assoc Cancer Res 19:4228–4238. https://doi.org/10.1158/1078-0432.CCR-13-0002

Smith MR, Kantoff PW (2010) Changes in PSA kinetics after DNA vaccine therapy-not so fast! J Clin Oncol Off J Am Soc Clin Oncol 28:e58; author reply e59. https://doi.org/10.1200/JCO.2009.26.3111

Soiffer R, Lynch T, Mihm M, Jung K, Rhuda C, Schmollinger JC, Hodi FS, Liebster L, Lam P, Mentzer S, Singer S, Tanabe KK, Cosimi AB, Duda R, Sober A, Bhan A, Daley J, Neuberg D, Parry G, Rokovich J, Richards L, Drayer J, Berns A, Clift S, Cohen LK, Mulligan RC, Dranoff G (1998) Vaccination with irradiated autologous melanoma cells engineered to secrete human granulocyte-macrophage colony-stimulating factor generates potent antitumor immunity in patients with metastatic melanoma. Proc Natl Acad Sci U S A 95:13141–13146. https://doi.org/10.1073/pnas.95.22.13141

Walter S, Weinschenk T, Stenzl A, Zdrojowy R, Pluzanska A, Szczylik C, Staehler M, Brugger W, Dietrich P-Y, Mendrzyk R, Hilf N, Schoor O, Fritsche J, Mahr A, Maurer D, Vass V, Trautwein C, Lewandrowski P, Flohr C, Pohla H, Stanczak JJ, Bronte V, Mandruzzato S, Biedermann T, Pawelec G, Derhovanessian E, Yamagishi H, Miki T, Hongo F, Takaha N, Hirakawa K, Tanaka H, Stevanovic S, Frisch J, Mayer-Mokler A, Kirner A, Rammensee H-G, Reinhardt C, Singh-Jasuja H (2012) Multipeptide immune response to cancer vaccine IMA901 after single-dose cyclophosphamide associates with longer patient survival. Nat Med 18:1254–1261. https://doi.org/10.1038/nm.2883

Welters MJP, Kenter GG, de Vos PJ, van Steenwijk MJG, Löwik DMA, der Meer F, Essahsah LFM, Stynenbosch APG, Vloon TH, Ramwadhdoebe SJ, van der Piersma JM, Hulst ARPM, Valentijn LM, Fathers JW, Drijfhout KLMC, Franken J, Oostendorp GJ, Fleuren CJM, van der Melief SH (2010) Burg, Success or failure of vaccination for HPV16-positive vulvar lesions correlates with kinetics and phenotype of induced T-cell responses. Proc Natl Acad Sci 107:11895–11899. https://doi.org/10.1073/pnas.1006500107

Wierecky J, Müller MR, Wirths S, Halder-Oehler E, Dörfel D, Schmidt SM, Häntschel M, Brugger W, Schröder S, Horger MS, Kanz L, Brossart P (2006) Immunologic and clinical responses after vaccinations with peptide-pulsed dendritic cells in metastatic renal cancer patients. Cancer Res 66:5910–5918. https://doi.org/10.1158/0008-5472.CAN-05-3905

Wolchok JD, Hoos A, O'Day S, Weber JS, Hamid O, Lebbé C, Maio M, Binder M, Bohnsack O, Nichol G, Humphrey R, Hodi FS (2009) Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. Clin Cancer Res 15:7412–7420. https://doi.org/10.1158/1078-0432.CCR-09-1624

Zhao X, Bose A, Komita H, Taylor JL, Chi N, Lowe DB, Okada H, Cao Y, Mukhopadhyay D, Cohen PA, Storkus WJ (2012) Vaccines targeting tumor blood vessel antigens promote CD8(+) T cell-dependent tumor eradication or dormancy in HLA-A2 transgenic mice. J Immunol 188: 1782–1788. https://doi.org/10.4049/jimmunol.1101644

Zhou G, Drake CG, Levitsky HI (2006) Amplification of tumor-specific regulatory T cells following therapeutic cancer vaccines. Blood 107:628–636. https://doi.org/10.1182/blood-2005-07-2737