Chapter 6
Anti-cancer Drugs – Discovery, Development and Therapy

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Abstract The most widely used treatments for cancer are surgery, radiotherapy and chemotherapy. Chemotherapy is the only option for metastatic cancers, where the treatment has to be systemic. The most frequently used chemotherapy drugs have been identified empirically without any pre-existing knowledge regarding the molecular mechanism of action of the drugs. Despite the remarkable progress achieved in cancer care and research over the past several decades, the treatment options for the majority of epithelial cancers have not changed much. However, a critical mass of knowledge has been accumulated that may transform cancer treatments from cytotoxic regimens towards the rapidly dividing cells into personalized targeted therapies. This chapter will provide an overview of currently used chemotherapeutics and will explore the impact of the molecular understanding of cancer on modern drug discovery, drug development and cancer therapy.

Keywords Cancer · Chemotherapy · Targeted therapy · Drug discovery and development · Preclinical development · Clinical trials

The most widely used treatments for cancer are surgery, radiotherapy and chemotherapy. Chemotherapy is the only option for metastatic cancers, where the treatment has to be systemic. The most frequently used chemotherapy drugs have been identified empirically without any pre-existing knowledge regarding the molecular mechanism of action of the drugs. Despite the remarkable progress achieved in

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R. A. De Mello et al. (eds.), *International Manual of Oncology Practice*,
https://doi.org/10.1007/978-3-030-16245-0_6

cancer care and research over the past several decades, the treatment options for the majority of epithelial cancers have not changed much. However, a critical mass of knowledge has been accumulated that may transform cancer treatments from cytotoxic regimens towards the rapidly dividing cells into personalized targeted therapies. This chapter will provide an overview of currently used chemotherapeutics and will explore the impact of the molecular understanding of cancer on modern drug discovery, drug development and cancer therapy.

6.1 Introduction

Despite significant progress in the understanding of cancer biology there is a persistent lack of progress in curing most metastatic forms of cancer. Among the standard treatment options for human cancers which include surgery, radiation therapy, immunotherapy and chemotherapy, the latter one is often the only option for treatment of metastatic disease where treatment has to be systemic throughout the entire body. Chemotherapy is the use of chemical agents for the treatment of cancer. Most chemotherapeutic agents exert their cytotoxic effect by modifying DNA, by acting as fraudulent mimics of DNA components, by inhibiting enzymes involved in DNA synthesis or by blocking cell division. Traditional chemotherapy kills cells that are rapidly dividing, regardless if they are cancer cells or not. Therefore standard chemotherapy damages healthy tissues, especially those that display a high replacement rate. Over the past few decades efforts in cancer research has paved the way for better therapies that interfere with specific targeted molecules. These treatments are called targeted therapies and hold promise to improve clinical outcomes without the toxicity associated with traditional chemotherapy. The transformation of the accumulated knowledge in cancer biology into clinical practice represents a major challenge for the scientific community and pharmaceutical industry.

6.2 Conventional Chemotherapy

6.2.1 The Origin of Chemotherapy

The origin of chemotherapy dates back to the early 1940s when the toxic action of nitrogen mustard-based war gas on cells of the haematopoietic system was discovered. Researchers at Yale University demonstrated the anticancer activity of mustard agents in a murine lymphoma model and then in a patient who had non-Hodgkin's lymphoma. The results of these studies conducted in 1943 were published in 1946. Nitrogen mustards are DNA alkylating agents that attach an alkyl group (R-CH2) to the guanine base of DNA and interfere with DNA replication.

6.2.2 The Classification of Traditional Chemotherapy

Nowadays, many different alkylating agents are given as part of anticancer therapy regimes. In addition a broad range of non-alkylating drugs have been developed to treat cancer. All current chemotherapeutic drugs can be classified into several categories according to their mechanism of action: (1) DNA-modifying agents (alkylating agents and alkylating-like agents), (2) anti-metabolites (that imitate the role of purines or pyrimidines as building blocks of DNA), (3), spindle poisons (typically plant alkaloids and terpenoids that block cell division by inhibiting microtubule function), (4) topoisomerase inhibitors (preventing transcription and replication of DNA) and (5) cytotoxic antibiotics (for example anthracycline, that inhibit DNA and RNA synthesis thus block topoisomerase. Table 6.1 shows examples of each category. Chemotherapy agents can also be classified into cell cycle specific and cell cycle non-specific drugs. Most chemotherapeutic drugs are cell cycle-specific and act on cells undergoing division. Cell cycle-specific drugs can be subdivided into S-phase- G1-phase-, G2 phase- and M-phase-specific agents according to the phase of the cell cycle in which they are active. Antimetabolites are most active during the S phase of cell cycle because they exert their cytotoxic activity by inhibiting DNA synthesis. Conversely, vinca alkaloids which inhibit spindle formation and alignment of chromosomes are M-phase specific. Cell cycle-specific drugs are most effective for high growth fraction malignancies (e.g.: hematologic cancers). Their capability to kill cells displays a dose-related plateau and does not increase with further increased dosage, because at a certain time point only a subset of cells is fully drug sensitive. In contrast, cell cycle non-specific drugs such as alkylating

Table 6.1 Conventional chemotherapeutic agents classified according to their mode of action

Type of agent	Examples	Mode of action	Affected cell cycle phese
DNA-modifying agents			
Alkylating agents	Chlorambucil	Alkylation of DNA	Phase nonspecific
	Cyclophosphamide	Alkylation of DNA	Phase nonspecific
	Carmustine	Alkylation of DNA	Phase nonspecific
	Lomustine	Alkylation of DNA	Phase nonspecific
	Dacarbazine	Alkylation of DNA	Phase nonspecific
	Temozolomide	Alkylation of DNA	Phase nonspecific
Platinum complexes	Cisplatin	DNA adduct formation	Phase nonspecific
	Oxaliplatin	DNA adduct formation	Phase nonspecific
	Carboplatin	DNA adduct formation	Phase nonspecific
Anti-metabolites			
	Methotrexate	Folic acid antagonist	S-Phase
	6-Mercaptpurine	Inhibits nucleotide synthesis	S-Phase
	Fluorouracil	Inhibits synthesis of nucleic acids	S-Phase
	Gemcitabine	Incorporated into DNA/Interfere with DNA synthesis	S-Phase
Spindle poisons			
Vinca alkaloids	Vinblastine	Prevent microtubule assembly	M-Phase
	Vincristine	Prevent microtubule assembly	M-Phase
Taxanes	Paclitaxel	Prevent microtubule disassembly	M-Phase
	Docetaxel	Prevent microtubule disassembly	M-Phase
Topoisomerase inhibitors			
Topoisomerase I inhibitors	Camptothecin	Causes strand breaks/Inhibits DNA replication	G2 phase
Topoisomerase II inhibitors	Etoposide	Inhibits DNA replication	M-Phase
	Topotecan	Inhibits DNA replication	M-Phase
Antitumor antibiotics			
	Bleomycin	Causes DNA fragmentation	G2 phase
	Daunorubicin	intercalate with DNA/inhibit topoiosmerase II	S-Phase
	Doxorubicin	intercalate with DNA/inhibit topoiosmerase II	S-Phase

agents have a linear dose-response curve and affect cells regardless whether they are proliferating or resting. They are effective for both low and high growth fraction tumors.

6.2.3 The Limitations of Traditional Chemotherapy

The success of cancer chemotherapy is limited by problems with toxicity, efficacy and drug resistance. As most conventional chemotherapeutic agents also affect rapidly dividing cells in healthy tissues they can cause severe side effects, in particular myelosuppression, immunosuppression, alopecia, mucositis, nausea and vomiting, diarrhea and flu-like symptoms. The cytotoxic effect of conventional chemotherapy affects resting cells, e.g., cancer stem cells less effectively. Therefore, the drug might be very efficient against cells that form the bulk of the tumor, that are not able to form new cells but does not affect the rare subpopulation of cancer cells which can repopulate the tumor and cause relapse. In addition, traditional chemotherapeutic agents target cell proliferation with little effect on other important hallmarks of cancers such as angiogenesis, invasion and metastases. A major problem associated with anticancer drugs (traditional and targeted therapies) is drug resistance. Some tumors, in particular pancreatic cancer, renal cell cancer, brain cancer and melanoma exhibit absence of response on the first exposure to standard agents (primary resistance). Conversely, some drug-sensitive tumors acquire resistance during the course of the treatment (acquired resistance). Drug resistance can be classified into drug-specific resistance and multi-drug resistance. Whereas drug-specific resistance is usually mediated by specific genetic alterations, the multi-drug resistant phenotype is often associated with increased expression of P-glycoprotein which expels drugs from the cell.

6.2.4 Targeted Therapies

Targeted therapeutic agents interact with a specific molecular target to mediate their therapeutic effects. These molecular targets have been identified and validated through careful research as part of pathways and processes that drive tumor formation and progression. A therapeutic target is a cellular macromolecule that is involved in the pathogenesis of the disease, druggable (undergoes a specific interaction with a drug) and its pharmacological modulation has an effect on the course of the disease. There are four main types of drug targets: proteins, polysaccharides, lipids, and nucleic acids. Proteins are considered the best source of drug targets as most known drugs have been shown to interact with them.

Targeted therapeutic drugs can be classified into small molecules, antibodies, and vaccines. Small molecules are defined as molecules below a molecular weight of 900 Daltons. They rapidly diffuse across cell membranes and can reach intracel-

lular targets as well as targets located outside the cell. Several small-molecule kinase inhibitor have been approved for clinical use. Conversely, monoclonal antibodies cannot cross cell membranes and act on the outside of a cell. They can inhibit the interaction of signaling molecules and receptors or trigger an immune response to kill cancer cells. Alternatively, monoclonal antibodies coupled to toxic agents or radioactive molecules can be used to guide cytotoxicity specifically to cancer cells. Therapeutic cancer vaccines activate the body's immune system to attack cancer cells. These cancer vaccines usually contain antigens that are specific or overexpressed in cancer cells. As many of these antigens are also present on normal cells, self tolerance has to be suppressed to obtain an effective antitumor immune response. This strategy is viable as long as the normal tissue is nonessential. Examples include antigens such as tyrosinase, MART-1, gp100, and TRP-1, which are expressed on melanoma cells as well as normal melanocytes.

6.2.5 Imatinib (Gleevec)

The small molecule kinase inhibitor Imatinib emerged as a paradigm for molecularly targeted therapies. Gleevec was introduced in 2001 for the treatment of Chronic Myelogenous Leukaemia (CML). CML is a cancer of the white blood cells caused by the reciprocal translocation between chromosome 9 and chromosome 22. The resulting Philadelphia chromosome contains the fusion of the Bcr and Abl genes that gives rise to a constitutively active tyrosine kinase enzyme. Imatinib prevents signal transduction of BCR-ABL by binding to its ATP binding site. This prevents the transfer of phosphate groups from ATP to a protein substrate and suppresses cell growth and division. The success of Imatinib has proven that the concept of targeting specific molecular events in cancer can result in highly efficient anticancer therapies. Nevertheless, as CML is a genetically simple neoplasm caused by a single aberrant protein there is still substantial debate about whether the Imatinib-paradigm can be translated to other cancers which are caused by a multitude of complex interacting genetic and environmental factors.

6.2.6 Trastuzumab (Herceptin)

The monoclonal antibody Trastuzumab (Herceptin) inhibits the activity of the growth factor receptor HER-2 which is required for cell growth in normal breast tissue. HER-2 is overexpressed in 30% of breast cancer patients either by transcriptional activation or gene amplification contributing to cancerous cell growth. Trastuzumab binds to HER-2 at the cell surface and prevents HER-2 mediated growth stimulatory downstream signaling. As a result disease progression is slowed down. However, 70% of breast cancer patients (with HER-2 negative tumors) would not benefit from the treatment with Trastuzumab which is expensive and associated

with adverse effects. This is a good example for the fact that many targeted therapies require companion diagnostic biomarkers to identify the subset of patients that would benefit from the corresponding targeted drug. In the case of Trastuzumab, several companion diagnostic test that detect the overexpression of HER-2 by immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) have been approved by the US Food and Drug Administration (FDA).

6.2.7 The Limitations of Targeted Therapies

Targeted therapies have been introduced in recent years and at present the impact is limited to some specific types of cancer. These are still early days to judge whether targeted therapies will mark a true breakthrough in cancer treatment. The widespread optimism is not shared by everyone, however. It has been argued that most targeted therapies offer only marginal extensions of life and few cures. Considering the enormous costs of these treatments, gains are rather modest. Some researchers suggest that we should focus more on metabolic and oxidative vulnerabilities that arise as a consequence of the uncontrolled growth and proliferation capacities of all cancer cells, rather than on targeting molecular events specific only for a small subset of a given cancer type. It is important to note that intrinsic or acquired resistance still limits the efficacy of targeted therapies in cancer treatment. Selective pressure in combination with mutations, epigenetic alterations or changes in microenvironment lead to resistant cancer cells and in turn to tumor regrowth and clinical relapse. As the malignant phenotype is often regulated by multiple parallel pathways the cancer cell may start to use alternative rescue signaling, if the main route has been targeted by an inhibitor. Therefore it might be useful to block several supporting pathways using combination therapies with other anticancer agents to prevent resistance development. Importantly, the determination of resistance mechanisms can provide the basis for the design of second-generation therapies. This strategy has been successfully employed to inhibit BCR-ABL with imatinib resistant point mutations using the second-generation kinase inhibitor dasatinib (SPRYCEL).

6.2.8 Discovery and Development of Targeted Therapies

The important progress in the molecular understanding of cancer which has been made during the last three decades has profoundly transformed the way we identify and develop anticancer drugs. Nowadays, drug discovery and drug development is a long and expensive process. It takes an average of 12 years and costs about 800 million US dollars to get a new drug from the laboratory to the pharmacy shelf. The process consists of several sequential steps: (1) Target identification, (2) Target validation, (3) Lead identification, (4) Lead optimization, (5) Pre-clinical development and (6) Clinical development (Fig. 6.1).

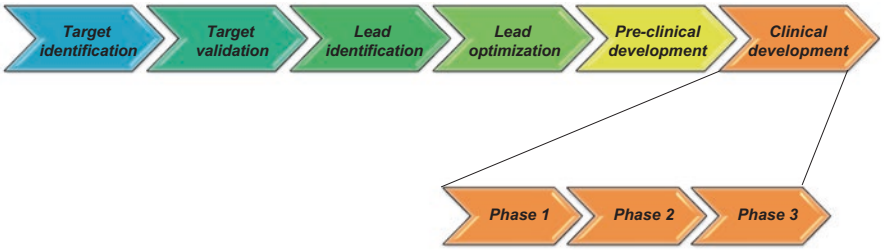

Fig. 6.1 Flow chart of the drug discovery and development process. The process consists of several sequential steps including target identification, target validation, lead identification, lead optimization, pre-clinical development and clinical development. Clinical development is carried out in three phases before a new drug can be approved for commercialization

6.2.9 Target Identification

The identification and validation of disease relevant targets are crucial for the development of molecularly targeted anticancer therapies. However, without a thorough understanding of the molecular events driving tumor formation and progression it is difficult to identify therapeutically useful targets. Therefore, these targets often emerge from research laboratories of the nonprofit and public sectors such as university and government laboratories. An ideal molecular target for an anticancer drug is specific and essential for the cancer cell. That means that it is absent in normal cells and necessary for tumor formation and progression just as the bacterial cell wall, as the target of penicillin is specific for the bacterium (not present in humans) and essential for its viability. As cancer cells evolve from normal cells most cancers do not possess molecular targets comparable to the bacterial cell wall. Therefore cancer research aims to identify targets that are to some degree essential and specific to cancer cells versus normal cells for example a protein that present an increased expression in cancer cells compared to normal cells (Table 6.2).

6.2.10 Target Validation

Protein overexpression in cancer cells might represent a defensive mechanism against tumorigenesis or occur completely unrelated. The fact that a correlation does not establish causation is illustrated by the following example: firemen are found at burning houses, but firemen are not found at normal houses. Therefore, firemen cause house fire and therefore, we should eliminate firemen to prevent fires. In order to confirm molecules as useful therapeutic targets the disease relevance has to be established. Target validation is the process of establishing a disease-causative effect and the therapeutic potential of a potential target. Target validation involves a variety of methods including genetic, cell-based, and animal models. TaqMan, in situ hybridization, western blotting and immunohistochemistry can be used to

Table 6.2 Targeted anticancer agents

Drug (Trade name)	Drug type	Target(s)	Disease indication
Alemtuzumab (Campath-1H®)	Antibody	CD52,	CLL, CTCL, T-cell lymphoma
Bevacizumab (Avastin®)	Antibody	VEGF	Glioblastoma and colorectal cancer
Bortezomib (Velcade®)	Small molecule	Proteasome	Multiple myeloma /MCL
Cetuximab (Erbitux®)	Antibody	EGFR	SCC and colorectal cancer
Dasatinib (Sprycel®)	Small molecule	BCR/ABL, Src family	CML and ALL
Erlotinib (Tarceva®)	Small molecule	EGFR	NSCLC and pancreatic cancer
Gefitinib (Iressa®)	Small molecule	EGFR	NSCLC
Gemtuzumab (Mylotarg®)	Antibody/immunotoxin	CD33	AML
Ibrutinib (Imbruvica®)	Small molecule	BTK	MCL, CLL
Imatinib (Gleevec®)	Small molecule	ABL and c-KIT	CML
Ipilimumab (YERVOY®)	Antibody	CTLA-4	Melanoma
Rituximab (Rituxan®)	Antibody	CD20	Non-Hodgkin lymphoma and CLL
Sorafenib (Nexavar®)	Small molecule	VEGFR, PDGFR and C-Raf	RCC
Temsirolimus (Torisel®)	Small molecule	mTOR	RCC
Tositumomab (Bexxar®)	Antibody /immunotoxin	CD20	Non-Hodgkin lymphoma
Trastuzumab (Herceptin®)	Antibody	HER2	Breast cancer
Vemurafenib (Zelboraf®)	Small molecule	BRAF V600E	Melanoma
Vismodegib (Erivedge®)	Small molecule	Smoothened (SMO)	BCC
Vorinostat (Zolinza®)	Small molecule	HDAC	CTCL

Abbreviations: *AML* acute myeloid leukemia, *ALL* acute lymphocytic leukaemia, *BCC* basal-cell carcinoma, *BTK* Bruton's tyrosine kinase, *CLL* chronic lymphocytic leukemia, *CTCL* cutaneousT-cell lymphoma, *CTLA-4* cytotoxicT-lymphocyte-associatedantigen-4, *GIST* gastrointestinal stromal tumor, *HDACs* histonede acetylases, *NSCLC* non-small cell lung cancer, *MCL* mantle cell lymphoma, *RCC* renal cell carcinoma, *SCC* squamous cell carcinoma, *VEGF* vascular endothelial growth factor

determine mRNA or protein expression of the target in normal vs. disease tissues. Direct modulation of target activity can be achieved by RNA interference, antibodies, peptides, and tool compounds and provides functional insights. In vivo target manipulation using transgenic and knock-out/knock-in mouse models is an essential approach for functional validation and to prove disease relevance. An important aspect of these experiments is to explore the potential adverse consequences of modulating the target In addition, population-based genetic studies can provide evidence for the significance of the target in the population where the disease occurs. Careful validation of the potential drug target is extremely important as any efforts expended on developing a drug on a poorly validated target will probably lead to its failure in clinical trials due to a lack of efficacy. A cancer drug target is only truly validated by demonstrating that a given therapeutic agent is clinically effective and acts through the target against which it was designed.

6.2.11 Lead Identification

Once the potential drug target has been validated, a biochemical or cell-based assay to monitor target activity is developed. Assay developers adapt the assay to a multiwell format to test many different treatments in parallel. The quality and consistency of the assay is determined by calculation the Z′ factor. This metric describes the available signal window for an assay in terms of the total separation between negative and positive controls minus the error associated with each type of control.

A Z′ value greater than 0.5 is considered as acceptable for high-throughput screening (HTS). Screening is the testing a random and large number of different molecules for biological activity. Many different collections of chemical compounds, called compound libraries for HTS are commercially available or owned by pharmaceutical companies. If the protein to be targeted is for example a kinase involved in a cancer signaling pathway, then rather than screening a complex library of diverse compounds, a focused chemical library would be constructed to target the ATP binding sites on the kinase enzyme. The active compounds from the primary screening known as hits are then analyzed in subsequent confirmation screens and counter screens to identify leads. This step in early drug discovery is referred to as the "hit-to-lead" process. A lead compound is a chemical molecule that demonstrates desired biological activity on a validated molecular target. Its chemical structure is used as a starting point for chemical modifications. In addition to the screening approach, there are several alternative strategies that can be used to identify lead compounds. A starting point is often an interesting bioactive compound which is chemically modified to improve its biological activity or pharmacokinetic properties or to strengthen intellectual property position. An increasingly important strategy in modern drug discovery is rational drug design. Rational drug design begins with the design of compounds that conform to specific requirements coming either from the 3D structure of biological target (structure -based drug design) or from structures of known active small molecules (ligand-based drug design). Lastly, even in modern drug discovery serendipity (luck) is still an important factor as the development of Viagra to treat erectile dysfunction illustrates.

6.2.12 Lead Optimization

The difference between a good ligand and a successful drug is that the latter is not only potent against the intended target (as a good ligand), but also exhibits good physical and chemical properties. The concept of druglikeness defines several structural features which determine whether a molecule is similar to known drugs. Assessment of druglikeness usually follows the Lipinski's rule of five (see Box 6.1). Newly identified compounds may have poor druglikeness and may require chemical modification to become drug-like enough to be tested biologically or clinically. During the lead optimization process medicinal chemists attempt to improve the physical and chemical properties of a lead compound introducing small structural modifications. Importantly, a successful drug must be absorbed into the bloodstream, distributed to the proper site of action in the body, metabolized efficiently and effectively and successfully excreted from the body. These pharmacokinetic or ADME (Absorption, Distribution, Metabolism and Excretion) properties describe the disposition of a compound within an organism and influence the activity of the compound as a drug. In modern drug discovery ADME properties of lead compounds are determined in early phases using relatively simple in vitro assays to

Box 6.1: Lipinski's Rule of Five
Lipinski's rule of five (there are only 4 rules) is a guideline to determine if a chemical compound has properties that would make it a likely orally active drug in humans. Christopher Lipinski, a medicinal chemist at Pfizer analyzed the physical and chemical properties of marketed drugs. He formulated the rule in 1997 based on the observation that most medication drugs are relatively small and lipophilic molecules. In fact most of them (87%) satisfy all Lipinski's rules:

1. <5 hydrogen bond donors (nitrogen or oxygen atoms with one or more hydrogen atoms)
2. <10 hydrogen bond acceptors (all nitrogen or oxygen atoms).
3. A molecular mass < 500 daltons.
4. log P (octanol-water partition coefficient) < 5

   All values are multiples of five (origin of the rule's name)

guide medicinal chemistry during lead optimization. Early ADME assays assess the solubility, lipophilicity, membrane permeability and metabolic stability of the lead compound as well as its capacity to bind plasma proteins and inhibit or induce enzymes that are essential for the metabolism of many drugs (indicative of possible drug-drug interactions). The lead optimization process consists of iterative cycles of chemical design and biological assessment aimed at the selection of a drug candidate for preclinical development.

6.2.13 Pre-clinical Development

Preclinical development is the process of taking an optimized lead through the stages necessary to allow human testing. Preclinical development includes in vitro and in vivo experiments to determine safety and efficacy of the drug candidate. During preclinical development, researchers must work out how to make large enough quantities of the drug for clinical trials. Efficacy evaluation of an anticancer drug candidate involves testing the impact on the viability of a broad variety of cancer cell lines, xenograft experiments in nude mice and experiments in more sophisticated genetically engineered mouse models. One of the major challenges in drug development is the accurate prediction of drug toxicity in humans. The standard approach to toxicity testing includes acute, subchronic, chronic exposure in three animal species. Regulatory authorities usually require that drugs are tested in both a rodent and a non-rodent mammalian species. Usually, these tests are carried out in mice, rats and dogs. Drugs with toxicity only in humans and not in non-human animals should be detected in the clinical trials. Unfortunately, due to

several limitations in the design of clinical trials this is not always the case. That is one of the reasons why 2.9% of the marketed drugs were withdrawn from the market during the last four decades. Pre-clinical studies must be conducted according to stringent good laboratory practices (GLPs), which require meticulous control and recording of processes. Before any clinical trial can begin, the sponsor, usually a pharmaceutical company must obtain permission to test the candidate drug in humans filing an Investigational New Drug (IND) application. The application is reviewed by regulatory authorities to make sure people participating in the clinical trials will not be exposed to unreasonable risks. Studies in humans can only begin after IND is approved.

6.3 Clinical Development

Clinical trials serve as the basis for evidence-based medicine and are conducted in three phases of development before a new drug can be approved for commercialization.

6.3.1 Phase 1 Clinical Trials

A phase 1 clinical trial (also called first in humans, FIH) is the first step in testing a new investigational drug or new use of a marketed drug in humans. Oncology phase 1 trials typically involve 20–80 patients with advanced cancer that has not responded to standard cancer treatments. In phase 1 clinical studies emphasis is put on drug safety. A principal goal of this phase is to establish a dose and/or schedule of a candidate drug for testing its efficacy in phase 2 trials. Trial participants are divided into small groups, known as cohorts. The first cohort receives a low dose of the new drug. In the absence of any major adverse side effects, the dose is escalated until pre-determined safety levels are reached, or intolerable side effects start showing up. Drug induced toxicity is analyzed relative to the dose and unexpected side effects are explored. Furthermore, researchers characterize the metabolism and routes of excretion of the candidate drug. Phase 1 clinical trials last about 1 year. About 70% of drugs pass this phase.

6.3.2 Phase 2 Clinical Trials

In Phase 2, the candidate drug is tested to see if it has any beneficial effect and to determine the dose level needed for this effect. Phase 2 clinical trials are clinical studies on a limited scale focused on efficacy. They typically involve 100–300 individuals who have the target disease and may be done at multiple sites to enhance

recruiting. As the success of targeted anticancer treatments depends on the presence of a specific molecular target, the selection of suitable patients is key for testing these agents in phase 2 clinical trials. Patients receiving the drug are compared to similar patients receiving a placebo or another drug. The efficacy of a candidate drug in clinical trials is measured by means of certain predetermined endpoints such as overall survival or progression free survival. An increasingly important aspect in phase 2 trials for targeted agents is the development of mechanism-based biomarker to determine if the candidate drug affects the intended target. Phase 2 clinical trials last about 2 years. About 33% of drugs pass this phase.

6.3.3 Phase 3 Clinical Trials

Phase 3 clinical trials are comparative studies on large number of patients to demonstrate that the candidate drug works. In order to generate statistically significant data about safety and efficacy phase 3 clinical trials are conducted as multi-center (conducted at more than one medical center), randomized (patients are randomly allocated to receive one or other of the alternative treatments) and double-blind (neither the participants nor the researchers know who is receiving a particular treatment) controlled studies. Phase 3 clinical trials typically involve 1000–3000 patients. The drug candidate is compared with existing treatments focused on safety and efficacy. Phase 3 clinical trials should characterize the effect of the candidate drug in different populations considering patient variations in genetics, life style and concomitant conditions such as liver impairment or pregnancy using different dosages as well as combined treatment with other drugs. Phase 3 clinical trials should confirm therapeutic efficacy in the target population and determine the safety profile. It also provides the basis for labeling instructions to ensure proper use of the drug. Phase 3 clinical trials last about 3 years. About 25–30% of drugs pass this phase.

6.3.4 Drug Approval

All new drugs have to be approved by regulatory authorities such as the Food and Drug Administration (FDA) in the United States or the European Medicines Agency (EMA) in the European Union. These agencies evaluate new drugs based on the evidence presented from the clinical studies. These data is provided by the sponsor in the so called “New Drug Application” (NDA). After NDA approval is obtained, the pharmaceutical company will market the drug. To be approved, a new drug has to be non-inferior or better than an approved drug. Non-inferior outcome ensures that a survival advantage associated with an approved drug will not be lost with a new agent.

6.4 Conclusions

A better molecular understanding of cancer has enabled the development of targeted therapies. Unlike conventional chemotherapeutic drugs that kill rapidly dividing cells by affecting DNA replication and cell division, targeted agents interfere with specific molecular targets that are critical for tumor formation and progression. The advent of targeted therapies has profoundly transformed the drug discovery and development process. The identification and rigorous validation of disease relevant molecular targets are among the most critical activities for successful development of targeted anti-cancer agents. The challenges associated with targeted therapies also apply to the subsequent phases of the drug development process. In particular, the development of companion diagnostic tests to identify patient populations that are most likely to benefit from the treatment are essential for the success in clinical efficacy studies. Emerging resistance to targeted therapies can be addressed by second-generation agents or combination therapies to prevent resistance or restore response.

1. **Which of the following drugs is an alkylating agent?**

   A. Paclitaxel
   B. 5-Fluorouracil
   C. Dacarbazine
   D. Doxorubicin
   E. Topotecan

2. **Chemotherapy agents can be classified into cell cycle specific and cell cycle non-specific drugs. Which of the following statements about vinca alkaloids is correct?**

   A. Vinca alkaloids block cell division by inhibiting microtubule function and are G1-phase specific
   B. Vinca alkaloids which inhibit spindle formation and alignment of chromosomes are M-phase specific
   C. Vinca alkaloids are cell cycle non-specific drugs as they inhibit spindle formation and alignment of chromosomes
   D. Vinca alkaloids are most active during the S phase of cell cycle because they exert their cytotoxic activity by inhibiting DNA synthesis
   E. Vinca alkaloids prevent transcription and replication of DNA and are most active during G1-phase and G2 phase

3. **A signaling protein inside the cell is mutated and hence constitutively active driving cell proliferation, and resulting in the formation of a tumor. What type of targeted therapy might be effective?**

   A. Monoclonal antibody that prevents growth factors from interacting with the receptor

B. Monoclonal antibody that holds the growth factor receptor in the "OFF" position
C. Small molecule that selectively binds to the mutated protein
D. Monoclonal antibody that selectively binds to the mutated protein

4. **What is meant by a lead compound in medicinal chemistry?**

A. A drug containing the element lead
B. A leading drug in a particular area of medicine
C. A compound that acts as a starting point for drug development
D. A drug which is normally the first to be described for a particular disease/aliment

5. **Which of the following statements is one of the Lipinski's rules (Rule of Five)?**

A. An orally active drug has a molecular weight equal to 500
B. An orally active drug has no more than five hydrogen bond acceptor groups
C. An orally active drug has no more than 10 hydrogen bond donor groups
D. An orally active drug has a calculated logP value less than +5

6. **Which of the following objectives in drug development is not related to pharmacodynamics?**

A. The reduction of side effects
B. The optimization of activity
C. The reduction of toxicity
D. The maximization of oral bioavailability

7. **Pharmacokinetics is defined as**

A. The study of biological and therapeutic effects of drugs
B. The study of absorption, distribution, metabolism and excretion of drugs
C. The study of mechanisms of drug action
D. The study of methods of new drug development

8. **Which of the following types of clinical trials determines whether a targeted therapy works against cancer?**

A. Phase I
B. Phase II
C. Phase III
D. Phase II and Phase III
E. Phase I, Phase II, and Phase III

Answers

1. **Which of the following drugs is an alkylating agent?**

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B. 5-Fluorouracil
C. **Dacarbazine**
D. Doxorubicin
E. Topotecan

Paclitaxel, 5-Fluorouracil, Doxorubicin, Topotecan are not alkylating agent, they act through different modes of action. Paclitaxel prevent microtubule disassembly, 5-Fluorouracil is an anti-metabolite, Topotecan and Doxorubicin are topoisomerase II inhibitors

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   D. Vinca alkaloids are most active during the S phase of cell cycle because they exert their cytotoxic activity by inhibiting DNA synthesis
   E. Vinca alkaloids prevent transcription and replication of DNA and are most active during G1-phase and G2 phase

Vinca alkaloids are cell cycle specific. They inhibit spindle formation and alignment of chromosomes most important during M-phase of the cell cycle.

3. **A signaling protein inside the cell is mutated and hence constitutively active driving cell proliferation, and resulting in the formation of a tumor. What type of targeted therapy might be effective?**

   A. Monoclonal antibody that prevents growth factors from interacting with the receptor
   B. Monoclonal antibody that holds the growth factor receptor in the "OFF" position
   C. **Small molecule that selectively binds to the mutated protein**
   D. Monoclonal antibody that selectively binds to the mutated protein

Most small molecule can pass the plasma membrane and act inside the cell

4. **What is meant by a lead compound in medicinal chemistry?**

   A. A drug containing the element lead
   B. A leading drug in a particular area of medicine
   C. **A compound that acts as a starting point for drug development**
   D. A drug which is normally the first to be described for a particular disease/aliment

Lead compound is a biologically active, drug like molecule which suitable for the lead optimization process in drug development

5. **Which of the following statements is one of the Lipinski's rules (Rule of Five)?**

   A. An orally active drug has a molecular weight equal to 500
   B. An orally active drug has no more than five hydrogen bond acceptor groups
   C. An orally active drug has no more than 10 hydrogen bond donor groups
   D. **An orally active drug has a calculated logP value less than +5**

Lipinski's rues are:

1. <5 hydrogen bond donors (nitrogen or oxygen atoms with one or more hydrogen atoms)
2. <10 hydrogen bond acceptors (all nitrogen or oxygen atoms)
3. A molecular mass < 500 daltons
4. log P (octanol-water partition coefficient) < 5

6. **Which of the following objectives in drug development is not related to pharmacodynamics?**

   A. The reduction of side effects
   B. The optimization of activity
   C. The reduction of toxicity
   D. **The maximization of oral bioavailability**

Oral bioavailability is part of Pharmacokinetics

7. **Pharmacokinetics is defined as**

   A. The study of biological and therapeutic effects of drugs
   B. **The study of absorption, distribution, metabolism and excretion of drugs**
   C. The study of mechanisms of drug action
   D. The study of methods of new drug development

Pharmacokinetics is related to the impact of the body on the drug, in other words how the drug is absorbed, distributed, metabolized and excreted

8. **Which of the following types of clinical trials determines whether a targeted therapy works against cancer?**

   A. Phase I
   B. Phase II
   C. Phase III
   D. **Phase II and Phase III**
   E. Phase I, Phase II, and Phase III

Clinical phase I trials focus on drug safety, Phase II on efficacy and Phase III on efficacy and safety

Acknowledgements This work was supported by Fundação para a Ciência e a Tecnologia (FCT) Research Center Grant UID/BIM/04773/2013 Centre for Biomedical Research 1334. The laboratory of WL was funded by a research grant from Liga Portuguesa Contra o Cancro – Núcleo Regional do Sul (LPCC/NRS), Terry Fox.

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