Anticancer Drug Development

Unique Aspects of Pharmaceutical Development

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1 Introduction

Around the world, tremendous resources are being invested in prevention, diagnosis, and treatment of cancer. Cancer is the second leading cause of death in Europe and North America. Discovery and development of anticancer agents are the key focus of several pharmaceutical companies as well as non-profit government and non-government organizations, like the National Cancer Institute (NCI) in the United States, the European Organization for Research and Treatment of Cancer (EORTC), and the British Cancer Research Campaign (CRC).

Identification of cytotoxic compounds led the development of anticancer therapeutics for several decades. Advances in cancer treatment, however, continued to be limited by the identification of unique biochemical aspects of malignancies that could be exploited to selectively target tumor cells. Schwartsmann et al. noted in 1988 that of over 600,000 compounds screened by then, less than 40 agents were routinely used in the clinic [1]. The recent growth in molecular sciences and the advances in genomics and proteomics have generated several potential new drug targets, leading to changes in the paradigms of anticancer drug discovery toward molecularly targeted therapeutics. These shifting paradigms have not only resulted in the greater involvement of biological scientists in the drug discovery process but also required changes in the screening and clinical evaluation of drug candidates. Both small and large molecular compounds continue to be investigated as anticancer agents.

The discovery and development of anticancer drugs, especially cytotoxic agents, differ significantly from the drug development process for any other indication. The unique challenges and opportunities in working with these agents are reflected in each stage of the drug development process. This chapter will highlight the unique aspects of anticancer drug discovery and development.

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2 Approaches in Anticancer Drug Therapy

Conventional anticancer drug discovery and development have focused on the cytotoxic agents. The drug discovery paradigms selected agents that had significant cytostatic or cytotoxic activity on tumor cell lines and caused tumor regression in murine tumor allografts or xenografts. The anticancer agents were discovered mainly by serendipity or inhibiting metabolic pathways crucial to cell division. Their exact mechanisms of action were often a subject of retrospective investigation. For example, Farber et al. reported the use of folate analogues for the treatment of acute lymphoblastic leukemia (ALL) in 1948 [2], while its mechanism of action, inhibition of the dihydrofolate reductase, was reported by Osborn et al. in 1958 [3, 4]. Similarly, the nitrogen mustard, mustine, was used as a chemotherapeutic agent long before its mechanism of action was understood [5].

Although this strategy has achieved significant success, the recent developments in molecular biology and an understanding of the pharmacology of cancer at a molecular level have challenged researchers to come up with target-based drugs. These are the agents that are pre-designed to inhibit and/or modify a selected molecular marker deemed important in cancer prognosis, growth, and/or metastasis. Several target-based compounds have emerged in recent years. While most of these compounds are in preclinical testing, several are in clinical trials and a few have been approved in the United States. For example [6],

- Imatinib mesylate (Gleevec®, Novartis) is a small-molecule compound that inhibits a specific tyrosine kinase enzyme, the Bcr–Abl fusion oncoprotein. It is used for gastrointestinal stromal tumor and chronic myeloid leukemia.
- Gefitinib (Iressa®, AstraZeneca & Teva) is a small-molecule inhibitor of the epidermal growth factor receptor's (EGFR, or erbB1) tyrosine kinase domain. It is used for non-small-cell lung cancer.
- Bortezomib (Velcade®, Millenium Pharmaceuticals) is a small-molecule proteasome inhibitor used for the treatment of multiple myeloma refractory to other treatments.
- Rituximab (Rituxan®, Biogen Idec & Genentech) is a monoclonal antibody used in the treatment of B-cell non-Hodgkin's lymphoma and B-cell leukemia. It binds the CD20 antigen on the CD20 + B-cells, causing their apoptosis.
- Trastuzumab (Herceptin®, Genentech) is a monoclonal antibody that binds the cell surface HER2/neu (erbB2) receptor and is used in the therapy of erbB2 + breast cancer.

2.1 Drug Development Paradigms for Molecularly Targeted Agents

Conventional screening models for anticancer agents are geared toward the selection of cytotoxic drugs. The animal screening models predominantly focus

on tumor regression and survival advantage, while the early stage human clinical trials are aimed at determining the limiting dose where high drug-related toxicity is observed. Toxicity and tumor-regression effects of cytotoxic agents are based on the same mechanism (Fig. 1A). Thus, these agents are dosed to the allowable maximum levels where serious toxicity is not observed. The molecularly targeted agents, on the other hand, act by mechanisms that may not result in direct and significant toxicity. These agents act on the extra cellular, transmembrane, or nonnuclear intracellular processes and are exemplified by receptor tyrosine kinase inhibitors, farnesyltransferase inhibitors, matrix metalloproteinase (MMP) inhibitors, and angiogenesis inhibitors. For example, compounds such as 5,6-dimethylxanthenone-4-acetic acid (DMXAA) target developing tumor vasculature and have proven useful in cancer treatment when combined with conventional cytotoxic agents [7]. These agents often cause tumor growth inhibition, rather than regression, in animal models. They have better toxicity profiles than cytotoxic drugs and require prolonged administration [8]. The differences between their dose–response and dose–toxicity curves are illustrated in Fig. 1B.
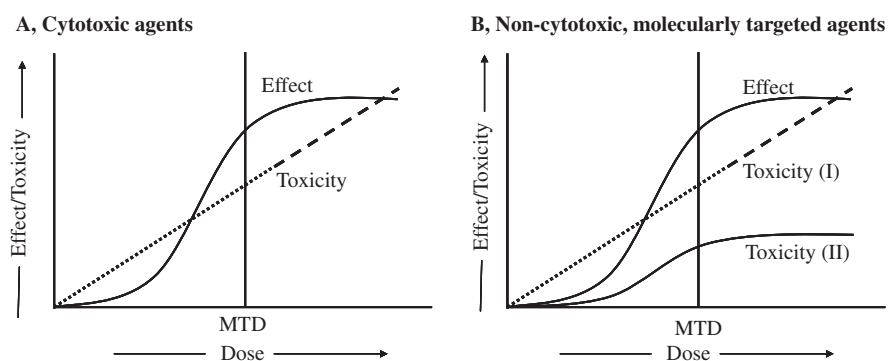

Fig. 1 Hypothetical dose–effect and dose–toxicity curves for cytotoxic (**A**) and non-cytotoxic, molecularly targeted anticancer agents (**B**). The cytotoxic agents are known for their dose-dependent toxicity, which closely follows the dose–effect curve. Non-cytotoxic agents, on the other hand, could have a linear dose–toxicity relationship similar to the cytotoxic agents (I) or a non-linear profile with dose–toxicity curve lower than the dose–effect curve (II). MTD represents the maximum tolerated dose for the cytotoxic agent. Modified from Hoekstra et al. [8]

The discovery and development of molecularly targeted anticancer agents necessitate changes in the anticancer drug preclinical and clinical screening paradigms not only because of the differences in their dose–response and dose–toxicity profiles and mechanisms but also because these agents are discovered with a pre-targeted mechanism of action. Although the development of the molecularly targeted agents is far more complex and demanding, they are being actively pursued with over 1,300 small biotech companies in the United States focusing on molecular targets, of which over half are focusing on cancer

treatment. There are estimated at least 395 agents in clinical trials for cancer treatment, more than in any other therapeutic class of medicine [9].

An important element of preclinical and clinical screening of molecularly targeted agents is the investigation of their effects on the specific molecular targets. Even though the drug effects on its molecular target may not be sufficient to demonstrate clinical benefit, it is necessary to validate this model of drug development and to understand the mechanism of drug action. Furthermore, it may be useful as a surrogate marker to guide dose escalation studies in early stage human clinical development. Evaluation of target effects in clinical trials, however, is not trivial. This requires the development and validation of a target specific molecular assay, and the correlation of the molecular target to the tumor type. Furthermore, physiological levels of molecular markers could have high natural variations, leading to difficulty in proving statistically significant drug effects.

Most molecularly targeted agents, however, do not proceed to advanced stages in human clinical trials due to either efficacy or toxicity concerns. The toxicity profile of an agent includes general toxicity and effects explained by its mechanism of action. While the toxicity remains largely unpredictable and difficult to modify, investigations of efficacy of these agents critically depend upon the selection of appropriate molecular targets and clinical trial designs. The latter includes selection of appropriate drug combinations for clinical studies and end points for the demonstration of efficacy.

Combination therapy is particularly important where the actions of target-based drugs are supplemented or potentiated by other agents and where the target-based drugs may act as sensitizers to the cytotoxic agents, e.g., P-glycoprotein (membrane efflux protein responsible for multi-drug resistance in several cases) inhibitors. The clinical end point for demonstration of efficacy has traditionally been the shrinkage of tumor size, as a surrogate for survival. End points for target-based drugs, such as the levels of surrogate molecular markers, changes in tumor markers, growth rate, time to progression, and the improvement in the quality of life (compared to cytotoxic agents), have been difficult to quantify and correlate with therapeutic benefit to the patient. Accordingly, these have been called "soft end points" [10].

Selection of appropriate molecular targets for inhibition or modification, such that the target is tumor specific, non-redundant, and able to influence the outcome of tumor progression, is a significant challenge given the complexity of molecular signaling pathways in cells. Key molecular mechanisms that have been explored for the development of target-based anticancer agents have been discussed in detail by Baguley and Kerr, for example.

2.1.1 Facilitating Apoptosis

Apoptosis is a physiologic intracellular process involving a well-ordered signaling pathway that leads to cell death and clearance of the dead cells by

neighboring phagocytes, without inflammation. Cytotoxic drug-induced damage to the cells, especially to the DNA, triggers apoptosis through two signaling mechanisms – the activation and release of mitochondrial pro-apoptotic proteins known as caspases under the control of Bcl-2 family of proteins or upregulated expression of pro-apoptotic receptors on cancer cells, whose subsequent interaction with their ligands activates apoptotic signaling pathways. These receptors include the Fas (also called APO-1 or CD95) and the tumor necrosis factor (TNF)-related apoptosis-inducing ligands (TRAIL) receptors. In addition, anticancer drugs can activate lipid-dependent signaling pathways that result in decreased apoptosis threshold or modulate other cytoprotective pathways such as the nuclear factor-κB (NF-κB), heat shock proteins, and cell cycle regulatory pathways.

2.1.2 Inhibiting Metastasis

Metastasis is the spread of the tumor from one organ or part of the body to another and is attributed to the translocation of cancer cells. This process of tumor cell translocation requires cellular movement as well as the remodeling of the extracellular matrix (ECM) that physically entraps cells and defines the shape of a tissue, at both the initial and the metastasized sites of tumor growth. Extracellular enzymes, matrix metalloproteinases (MMPs), proteases, and plasminogen activators (PAs), have been implicated in this remodeling of the ECM, leading to invasion and dissemination of cancer. Thus, drug candidates targeting proteases and MMP inhibitors have been developed for potential anticancer activity.

2.1.3 Inhibiting Angiogenesis

Angiogenesis, the process of formation of new blood vessels from the existing blood supply, is an essential requirement for the growth of tumor mass as well as its metastasis. Thus, prevention of angiogenesis has the potential to block nutrient and oxygen supply to the tumors, resulting in tumor regression. Three key events involved in tumor angiogenesis include the angiogenesis switch that initiates this process, proliferation and migration of endothelial cells to form the lining of new blood vessels, and remodeling of the ECM. Several cellular signal transduction molecules have been identified to play a role in this process including the angiogenic factors such as the vascular endothelial growth factor (VEGF), the integrins, plasminogen activation system, and the MMPs. Drug targets have been identified to inhibit one or more aspects of these pathways, e.g., VEGF receptor antagonists and VEGF antibodies.

2.1.4 Antibodies Against Tumor-Specific Antigens

Induction of antitumor immune responses by using tumor-specific antigens is a cherished goal in cancer therapeutics since it promises to be free of dose-limiting

toxicity. Administration of antibodies against tumor-specific and tumor-associated antigens can be used to target tumors by carrying radioisotopes, toxins, or prodrug converting enzymes. In addition, antibodies per se result in tumor regression by complement fixation or antibody-dependent cellular toxicity (ADCC) through the involvement of natural killer cells, granulocytes, and monocytes. Additional strategies that have been exploited include the expression of target antigens on the antigen-presenting cells (APCs) or dendritic cells to activate body's T-cell immune response.

Thus, preclinical evaluation and clinical development of anticancer agents, especially molecularly targeted therapeutics, present unique challenges – both in the selection of appropriate drug target and in the development of a molecular marker of efficacy. Developing an assay for the surrogate markers and its correlation with antitumor efficacy requires a significant research investment with unpredictable outcome. Also, the molecular understanding of cancer growth and metastasis is still developing and the selection of molecular targets for drug development may not succeed in the clinic. These risks and challenges are inherent in the development of molecularly targeted anticancer agents.

2.2 Pharmacogenetics and Metabolomics

Therapeutic activity and toxicity of cytotoxic drugs are derived from the same molecular mechanisms and usually correlate directly with the dose. To maximize clinical benefits, patients are dosed to the maximum levels that do not result in serious side effects. The resulting narrow therapeutic window of these drugs, along with the serious disease condition of the patients and inter-individual variation in drug response and toxicity, constitutes a significant challenge in their clinical development and use. These considerations, in turn, have generated opportunities for the development of tools for individualization of drug therapy to the patient and monitoring of drug response and toxicity using surrogate markers. Tumor treatment has been individualized for patients based on the tumor type, histology, and the disease state. Pharmacogenetics is an emerging paradigm for individualization of drug therapy using the genetic constitution of the patient.

Pharmacogenetics involves the genotypic and phenotypic imprinting of the individual patient to identify key genes and their proteins that are involved in the pharmacokinetics and/or pharmacodynamics of drug response and/or toxicity. This analysis is expected to reduce the inter-individual variation in drug–response or minimize the side effects by modulating drug doses to adjust for genetic variability in patients. The targets for genotype profiling in patients are usually the drug-metabolizing enzymes or the drug targets. Among the drug-metabolizing enzymes, cytochrome P450 (CYP) superfamily constitutes several isoenzymes that are implicated in the inactivation of anticancer compounds, such as CYP1A2 for flutamide, CYP2A6 for tegafur, CYP2B6 for

cyclophosphamide, CYP2C8 for paclitaxel, and CYP2D6 for tamoxifen [11]. Examples of drug targets whose variation impacts anticancer drug treatment include thymidylate synthase with 5-fluorouracil and the epidermal growth factor receptor with the tyrosine kinase inhibitors gefitinib and erlotinib [12]. Screening of patients for markers of specific metabolizing enzymes or drug targets is important not only in the clinical setting to reduce the probability of drug exposure related toxicities but also in the clinical trials of novel anticancer agents with narrow therapeutic index. This approach can help achieve individualization of drug therapy to optimally balance efficacy with toxicity and, thus, contribute to the success of clinical development of novel drug candidates.

Metabolomics, on the other hand, involve the quantitative analyses of metabolites in a cell, tissue, or organism. It could involve two strategies – target analysis and metabolite profiling [13]. While target analysis is restricted to the quantification of a chosen class of compounds (related to a specific pathway, intersecting pathways, or the investigational drug candidate), metabolite profiling involves analyses of a large number of metabolites with the objective of identifying a specific metabolite profile that characterizes a given sample. The analytical techniques used for metabolomic studies include isotopic (e.g., ^{13}C) labeling of chosen metabolites and monitoring their progress through various pathways and assays for low-level quantification in biological samples such as mass spectroscopy (MS), liquid/gas chromatography – tandem MS (LC-MS/MS or GC-MS/MS), and ion cyclotron resonance (ICR).

Metabolic profiling of a system reflects the net effects of genetic and environmental influences, including disease state and drug therapy. Such profiling can help distinguish between the pre-disease, disease, and normal state of cells and tissues. For example, the metabolic phenotype of cancer cells is characterized by high glucose uptake, increased glycolytic activity, low mitochondrial activity, and increased phospholipid turnover [14]. A metabolic profile indicative of any such characteristics can be utilized as a surrogate marker of disease state. Metabolomic profiling can rapidly detect subtle changes in metabolic pathways and shifts in homeostasis much before phenotypic changes can be detected [15]. Although metabolomics is an emerging science that will require significant developments before its successful clinical application, it has potential in drug discovery in the identification and development of biomarkers and classifying patients as responders or nonresponders to a given therapy. For example, Chung et al. identified that the ratio (phosphomonoesters/phosphodiesters), measured using ^{31}P NMR spectroscopy, could be used as a surrogate marker for the antitumor activity of 17-allylamino-17-demethoxygeldanamycin (17 AAG) in cultured tumor cells and xenografts [16].

Narrow therapeutic index combined with the inter-individual variations in drug pharmacokinetics, response, and toxicity adds uncertainty to the clinical trials and use of novel anticancer agents. Pharmacogenetic and metabolomic profiling of the patients promise to at least partly address these concerns, thus helping in the individualization of medication for patients and improved

therapeutic outcomes. In addition, these techniques can improve patient selection for clinical trials based on molecular features of the tumor and patient response variables, toward more efficient and cost-effective drug development [17]. For example, it has been suggested that mutations in the epidermal growth factor receptor (EGFR) gene can help predict sensitivity to gefitinib in lung cancer patients [18]. However, the data required for generating such correlations are usually obtained much later in the product development and commercialization cycle. Furthermore, these disciplines are still in their infancy and would need significant further developments before their widespread routine use in drug development and clinical application.

2.3 Modulators, Sensitizers, and Supportive Cancer-Care Agents

In addition to the cytotoxic and molecularly targeted anticancer agents, drugs acting through several indirect mechanisms are used in the management of cancer. These include the immunomodulators, chemoprotective agents, multi-drug resistance reversing agents, hormonal drugs, photosensitizers, analgesics, anti-emetics, and bone marrow growth factors.

The prospect of developing therapeutic vaccines using immunomodulators for tumor treatment has attracted considerable research interest. Immunomodulators are the drugs that alter the body's immune response to tumor cells. These are based on generating humoral and/or T-cell responses to the specific tumor antigens being targeted. Several strategies have been applied to produce immune-mediated anticancer activity, e.g., enhancing the activity of antigen-presenting cells, the use of cytokines such as interleukin-12 and interferon-α to enhance immune activation, and inhibition of T-cell inhibitory signals [19]. Very few of these agents, however, demonstrated statistically significant improvement in clinical end points in phase III studies [20].

Multi-drug resistance (MDR) is a phenomenon whereby the tumor cells develop resistance to a variety of drug molecules. MDR could be due to the failure of tumor cells to undergo apoptosis in response to chemotherapy or the upregulation of the membrane protein, P-glycoprotein (P-gp). P-gp acts as an efflux pump for a variety of drugs, leading to reduced intracellular concentration and anticancer efficacy. Drugs that inhibit the P-gp efflux pump, therefore, can improve the efficacy of cytotoxic drug treatment. For example, an amlodipine derivative, CJX1, inhibited P-gp and increased the intracellular concentration of doxorubicin, thus reversing doxorubicin resistance of the human myelogenous leukemia cells [21]. Several highly specific P-gp inhibitors, such as tariquidar, zosuquidar, and laniquidar, have entered early stage clinical trials in combination with cytotoxic anticancer agents [22].

Chemoprotective agents are the drugs that can help mitigate the toxic effects of anticancer drugs. For example, the nitrogen mustard ifosfamide causes nephrotoxicity (hemorrhagic cystitis and hematuria), which was attributed to

its metabolite, chloroacetaldehyde. Co-administration of the sulfhydryl compound sodium-2-sulfanylethanesulfonate (mesna) neutralizes the active metabolite in renal tubules, thus acting as a chemoprotective agent [23]. Another example of a chemoprotectant is amifostine, which reduces the nephrotoxicity of cisplatin. Amifostine is a thiophosphate prodrug that gets dephosphorylated by alkaline phosphatase in the normal endothelium in vivo to the active sulfhydryl compound [24].

Hormonal drugs and photosensitizers are non-cytotoxic agents that can have anticancer effects in target populations. The use of hormonal drugs as anticancer agents is based on the hormone dependence of certain tumor types, such as endometrial, prostate, ovarian, and breast cancers. Thus, antiestrogens, antiandrogens, and antiprogestins are usually not cytotoxic but may prevent the growth of hormone-dependent tumors by changing the endocrine environment. In many cases, these drugs can be administered by a non-parenteral route, e.g., by oral tablets or transdermal patches.

Photosensitizers are the compounds that are therapeutically inactive until irradiated by light. Laser light irradiation of tumor tissues after photosensitizer administration to the patient leads to the generation of free radicals inside and in the vicinity of the tumor tissue, causing tumor destruction. An example of this class of agents includes the porphyrin precursor 5-aminolaevulinic acid, which has been clinically successful [25]. Longer wavelength laser light is preferred over shorter wavelengths because of less direct tissue damage and deeper penetration. Selectivity of tumor damage is achieved by both the concentration of the photosensitizing agent to the tumor and the localized irradiation.

In addition, supportive cancer-care agents include drugs that help alleviate the serious side effects associated with cytotoxic compounds. This class of drugs includes analgesics, anti-emetics, and growth factors. Examples of these compounds include opiates and fentanyl as analgesics; octreotide for diarrhea; and phenothiazines and butyrophenones as anti-emetics. The bone marrow growth factors such as granulocyte colony stimulating growth factor (Filgastrim®) and granulocyte-macrophage (or monocyte) colony stimulating factor (Sargramostim®) help stimulate white cell production and reduce the risk of serious infection due to myelosuppression [26, 27]. These therapies are aimed at improving the quality of life of cancer patients, increase compliance, and reduce hospitalization due to adverse effects [28]. Many of these agents are available through a wide variety of drug delivery options including immediate and sustained release formulations, transdermal products, and depot formulations.

3 Anticancer Drug Development Process

Conventional anticancer drug development efforts focused on cytostatic or cytotoxic compounds that caused tumor regression. These paradigms have been expanded to include target pre-selection for the discovery of molecularly

targeted therapeutic agents. In addition, drug types such as immunomodulators, chemoprotectants, MDR-reversing agents, photosensitizers, and hormonal drugs present an increasing arsenal with unique drug development needs and possibilities of drug combinations to maximize therapeutic outcome. Furthermore, the use of molecular biology technologies such as pharmacogenetics and metabolomics with cytotoxic agents can help control drug toxicity and better predict drug response. Prudent application of these opportunities is significantly influencing the preclinical and clinical development of novel anticancer therapeutics.

The new drug discovery and development process is a systematic approach to identify potential new drug candidates and their evaluation for drug-like properties. Although the discovery and development of anticancer compounds follow the same process as any other new molecular entities (NMEs), they have several unique aspects that impact their development paradigms. The new drug development process typically involves the following stages, not necessarily in a sequential manner:

1. *Acquisition of potential compounds*: This could be achieved by chemical synthesis or by extraction from natural resources. This stage includes the development of analytical methods to confirm identity and purity of the compound, and its stability under real-life and stressed storage conditions. Physicochemical properties of the compound are identified, such as the solid-state form (polymorphism, hydrates, and solvates), melting point, solubility, and stability. Synthesis of the molecule is scaled up as the compound progresses in the development pathway. A formulation suitable for human administration and commercialization is identified and scaled-up.

2. *Drug screening and preclinical pharmacology*: This involves "paper chemistry" whereby the drug structure is compared to those of existing compounds in the databases to identify potential activity, toxicities, degradation pathways, metabolic routes, etc. A preliminary screening in cell culture models is carried out to identify the extent and specificity of its antitumor activity. This is followed by the evaluation of efficacy and toxicity in animal models.

3. *Clinical development*: Clinical development of a drug candidate involves testing in human volunteers to identify the toxicities and the maximum tolerated dose (MTD) in phase I clinical trials. Subsequently, phase II studies are carried out in patients of selected tumor type to quantify efficacy and confirm dosage. Subsequently, larger phase III studies are aimed at head-to-head comparison of the NCE with the then-best-available therapy.

The drug discovery and development process is inherently time and resource consuming with very low success rates, as illustrated in Fig. 2.
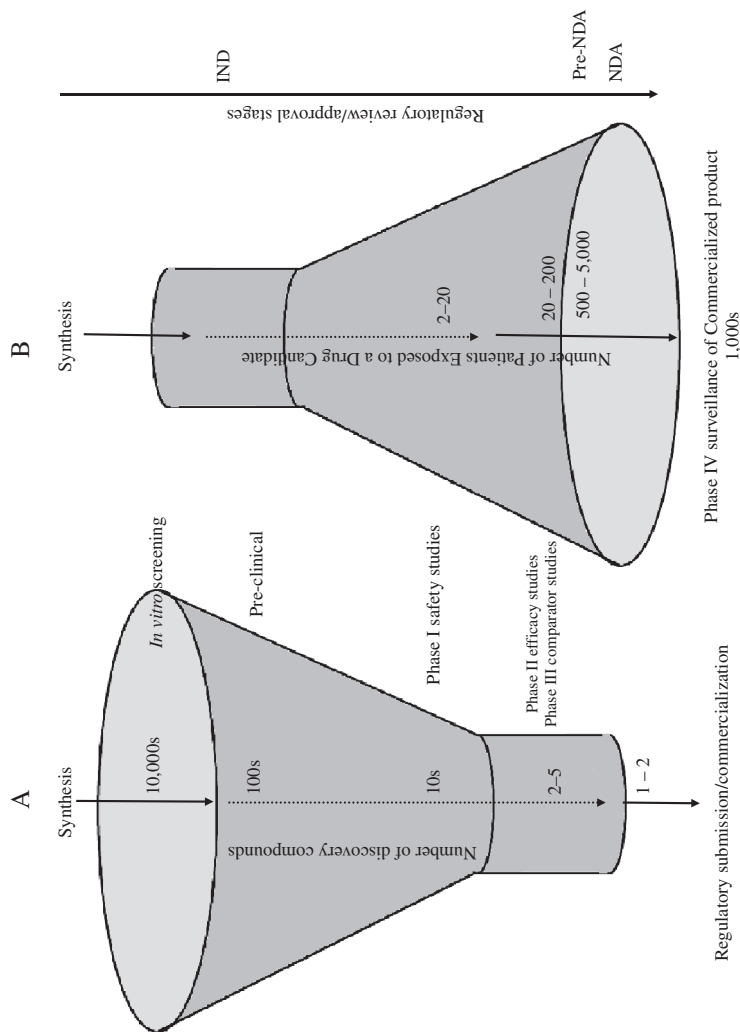
Fig. 2 An example of the funnel of reducing number of compounds progressing through the drug candidate through these phases is accompanied by a progressive increase in the number of patients exposed to the drug (**B**). This figure also illustrates the steps where regulatory review and/or approval is required in the United States, such as the Investigational New Drug (IND) application submission before initiating Phase I studies, a pre-NDA meeting with the FDA after the Phase II studies, and New Drug Application (NDA) submission for drug approval for marketing after the completion of Phase III studies

3.1 Historical Background

The history of cancer chemotherapy has been widely described [9, 29, 30]. Most cytotoxic anticancer compounds were discovered by serendipity or as inhibitors of metabolic pathways involved in cell division. For example, nitrogen mustard was discovered in the 1940s upon investigations by Goodman and Gilman of the lymphoid and myeloid suppression observed in soldiers accidentally exposed to the nitrogen mustard gas during the World War II [5]. The utility of hormone therapy in cancer became evident by the works of George Beatson, who documented shrinkage in breast cancers upon removal of the ovaries [31], and Charles Huggins, who showed that the prostate cancer in dogs can be stalled by castration and by estrogen injection [32]. Similarly, the discovery of anticancer properties of platinum coordination compounds, such as cisplatin, is attributed to Barnett Rosenberg, who was investigating the effect of electric field on the growth of bacteria and observed cessation of cell division due to the contamination of the growth medium with the electrolysis product of the platinum electrode [33, 34]. Mitoxantrone was developed from anthracyclines that were originally developed as stable dyes but had the planar ring structure suitable for intercalation in the DNA double strands [35].

   Cancer was recognized primarily as a disease of uncontrolled cell division. Hence, all efforts were directed toward the identification of antiproliferative compounds. Accordingly, regression of tumor size has been recognized as the primary, objective end point of effectiveness in preclinical and clinical testing. Murine models of cancer were developed that rapidly grew tumors. Screening of new compounds in the drug discovery programs was focused on testing them in these rapidly growing tumor models. Several clinically important anticancer compounds were identified using this screen. Nevertheless, the selective use of rapidly growing tumor models was implicated as the reason that the successes occurred mainly in the rapidly growing malignancies, e.g., lymphomas, childhood leukemia, and germline tumors. Relatively fewer successes were seen for the slow-growing common solid-tumors of the adults, e.g., lung, breast, and colorectal cancers [36]. These criticisms led investigators to modify the pre-screening and screening protocols to include a variety of cell lines and tumor types. These aspects are discussed further in later sections.

3.2 Discovery of Potential Drug Candidates

The compounds selected for evaluation as potential anticancer agents could be of natural or synthetic origin. Compounds of natural origin have often provided new leads in the novelty of structures with anticancer activity. Mans et al. have enlisted several examples of naturally derived anticancer compounds [35]. For example, vincristine derived from the periwinkle plant *Vinca rosea*, etoposide is derived from the mandrake plant *Podophyllum peltatum*, and taxol, which is

derived from the pacific yew, *Taxus brevifolia*. Similarly, doxorubicin and bleomycin are fermentation products of the bacteria *Streptomyces*; L-asparaginase is derived from the broths of *Escherichia coli* or *Erwinia carotovora*; rhizoxin is derived from the fungus *Rhizopus chinensis*; cytarabine was discovered from the marine sponge *Cryptotethya crypta*; and bryostatin from the sea moss *Bugula neritina*.

Analogs of natural compounds have often been synthesized to improve their efficacy or toxicity profiles [35]. For example, carboplatin was developed as an analog of cisplatin with reduced renal toxicity, doxorubicin is an analog of dauno-mycin that reduces its cardiotoxicity, and topotecan is an analog of camptothecin with better toxicity profile. Analogs of existing drugs have also been synthesized to improve drug targeting and the pharmacokinetic profiles of drug candidates [35]. For example, tauromustine is a nitrosourea anticancer agent coupled to the brain targeting peptide taurine for targeting CNS tumors, and 9-alkyl morpholinyl anthra-cyclines are analogs of doxorubicin that have been synthesized to reduce drug affinity to the cellular efflux protein, P-glycoprotein. The use of related analogs has also been used to improve drug supply. For example, taxotere was developed to overcome the supply problems with taxol, a natural compound of plant origin with very low yields.

The synthetic compounds could be the analogs of known compounds or novel structures. The process of identifying and selecting these candidates has under-gone a sea change in the recent decades with the development of solid-state and combinatorial chemistry and computer modeling of drug–receptor interactions. Discovery of new anticancer agents by laboratory synthesis has evolved from analog evaluation and improvement of new leads to rational design based on drug–receptor or drug–enzyme interactions. Examples of synthetic analogs of natural compounds that demonstrated anticancer activity include the folic acid analog methotrexate and the fluorinated pyrimidine base, 5-fluorouracil. Exam-ples of drugs that have been discovered through the rational design approach by the exploration of molecular mechanisms and interactions with drug targets include EO9, which is a mitomycin C-related indoloquinone and is active against hypoxic tumors; and the ether lipid, ET-10-methoxy-1-octadecyl-2-methyl-rac-glycero-3-phosphocholine, which targets the cell membranes [35].

Invariably, the discovery process leads to far more compounds as potential drug candidates than that can be investigated in the clinic, thus necessitating a screening process for short-listing compounds with the highest potential for clinical success. Computer simulation is used to identify novel and potentially active structures. Selected compounds are tested by cell culture and animal assays to quantify efficacy, identify toxicities, and potentially additional pharmacokinetic and phar-macological properties. These aspects are discussed in the following section.

3.3 Preclinical Evaluation

Screening of drug candidates for anticancer activity is done in several stages, which are designed to create a 'funnel' with reducing number of compounds entering the successive stages of development, as exemplified in Fig. 2A. This

screening protocol balances the real-life limitations in the number of drug candidates that can be tested in humans each year with the number of potential new drug candidates that show potential for antitumor activity.

During preclinical development, novel drug candidates are produced in sufficiently large quantities and tested for their physicochemical, biopharmaceutical, and solid-state properties. These include the evaluation of solubility, stability in the solid state and solution, pH solubility and stability studies, identification of degradation pathways, isolation of polymorphic forms and their impact on drug solubility and stability, absorption studies in cell culture models and animals, and the drug-excipient compatibility studies. The anticancer activity is evaluated in vitro in cell culture models by cell growth inhibitory or clonogenic assays, which serves as a pre-screen to identify active compounds. The potential toxicities and early pharmacology of selected compounds are determined in murine allograft or human xenograft mouse models. For example, at the US National Cancer Institute (NCI), new compounds are evaluated for cytostatic or cytotoxic activity against eight cell lines derived from the most common human malignancies. Compounds that show activities in this pre-screen are tested in more detail in a panel of cell lines of the respective tumor type, and subsequently in animal models [37–44].

3.3.1 Preclinical Efficacy Screening

Historically, drug screening in murine models was done in the L1210 mouse leukemia model along with the P388 murine leukemia allograft, and a few other models for special cases such as the sarcoma 180, carcinoma 755, and Lewis lung carcinoma models [45]. The measures of anticancer activity are primarily the (a) reduction of tumor size and (b) increase in the life span of the mice. In addition to the anticancer activity, the in vivo screen provides information on potential toxicities, tolerated doses and dosage regimens, and the spectrum of activity. Drugs that were found effective in this model were evaluated in other rodent models, and, if shown broad activity, were taken up for further development. Several anticancer drugs were identified with activity against lymphomas, leukemias, and some pediatric tumors. However, these models were ineffective to yield drugs against slow-growing adult solid tumors, like the mammary, colon, and lung cancers [37–44, 46].

In 1975, the NCI introduced screening in human tumor xenografts in nude mice, and the P388 model was moved to a pre-screening stage. This approach was further refined in the year 1990 to replace animal testing in the pre-screening stage with a cell culture evaluation in 60 tumor cell lines (called the NCI-60 screen), which are derived from human leukemia, small-cell and non-small-cell lung cancers, and other human carcinomas. These cell lines are continuously being replaced and added and are being characterized for molecular markers and other characteristics relevant to regulation of cell growth, division, and differentiation. This pre-screening stage incorporates a panel of the same cell lines grown as xenograft tumors in nude mice.

The cell culture pre-screen involves inoculation and growth of cells in microtiter plates followed by incubation with different concentrations of the potential

anticancer compounds. At the end of incubation, cell growth is measured by a colorimetric assay and the antitumor potential of the compounds is assessed by their cytostatic or cytotoxic activity. Thus, the NCI-60 screen generates a wealth of information with respect to the dose–response curves of potential compounds in several different cell lines, which represent different cancer types and profiles of molecular markers and biochemical pathways. Collectively, this information on the pattern of cell inhibition can be utilized to generate a 'fingerprint' of the compound. Comparing the fingerprint of the novel compound with the library database of compounds with known mechanisms of action can help generate the hypothesis on the mechanism of action of the novel compound.

Kohlhagen et al. provide an example of the application of NCI-60 screen to generate a hypothesis for the mechanism of action of a novel compound, designated NSC-314622 [47]. This process involves generation of dose–response curves for all the NCI-60 cell lines, usually involving four-log dilution range of the drug [48]. A typical dose–response curve is exemplified in Fig. 3. The compound's concentration that inhibits growth by 50% (GI_{50}) for each cell line can be plotted on the x-axis relative to the mean GI_{50} of the panel of 60 cell lines, with bar to the right indicating higher than the mean concentration and the bar to the left indicating lower than the mean [48]. Using these plots to
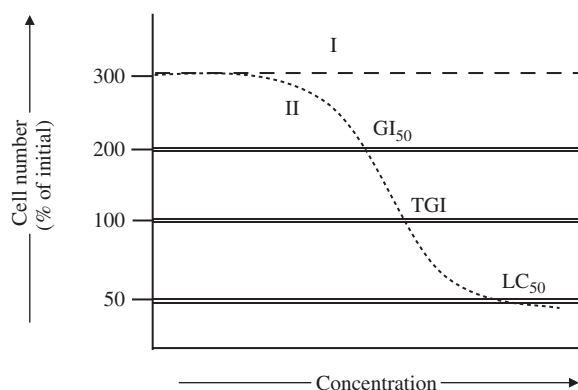

Fig. 3 An illustration of the dose–response curves generated during cell culture pre-screening. This example illustrates the dose-dependent cytotoxic effect of drugs on cells cultured in vitro in cell culture dishes. Cell cultures that are not exposed to the drug (I) grow to a hypothetical three-fold, or 300%, of their initial numbers upon culturing in a growth promoting media for a fixed amount of time. Thus, these cells show 200% growth, or 200% increase in viable cell count. However, the cells exposed to the drug (II) have less number of viable cells upon culture under similar conditions for the same amount of time. The number of viable cells in the drug-exposed culture dish depends upon the drug concentration in a manner illustrated by curve II. The drug concentration at which the viable cell count after culture remains the same as the initial, i.e., at 100%, is defined as the total growth inhibitory (TGI) concentration. Drug concentration that halves the growth of cells in culture, i.e., increase in cell numbers to half of the levels seen without drug (which was 200%), is defined as the GI_{50} (growth inhibition to 50% level). Similarly, the concentration of drug that halves the viable cell count from its initial level (which is 100%) is defined as LC_{50} (lethal concentration to 50% level). Modified from Shoemaker [44]

define a fingerprint of the compound, Kohlhagen et al. observed similarity in the cytotoxicity profiles of NSC-314622 with that of topotecan, camptothecin, and camptothecin derivatives with a Pearson correlation coefficient of 0.74, 0.63, and (0.78–0.84), respectively (Fig. 4). This information helped define the initial hypothesis that NSC-314622 was a topoisomerase I poison, which was then developed further using a battery of tests [47].
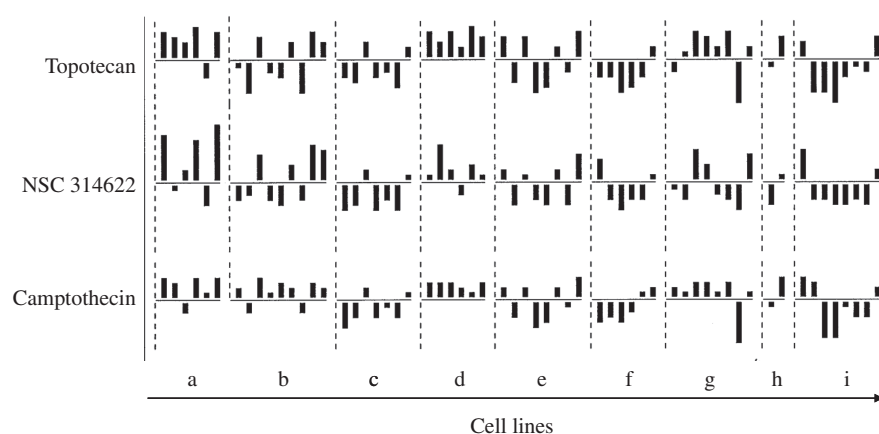

Fig. 4 An example of the use of the NCI-60 screen fingerprint to indicate the mechanism of drug action. This graph displays the drug concentration that inhibits cell growth by 50% (GI_{50}) in the 60 cell lines of the NCI Anticancer Drug Screen, representing different tumor types: (a) leukemia, (b) non-small cell lung cancer, (c) colon cancer, (d) CNS cancer, (e) melanoma, (f) ovarian cancer, (g) renal cancer, (h) prostate cancer, and (i) breast cancer. The y-axis represents the mean GI_{50} in milligram of drug dose as a positive (upwards) or negative (downwards) deviation from the mean. Similarity between different cell lines in their pattern of antitumor efficacy is compared by the Pearson correlation coefficient analyses. In this example, the Pearson correlation coefficient of NSC 314622 with Topotecan was 0.74 and with Camptothecin was 0.63. Modified from Kohlhagen et al [47].

Although the use of cell culture screens has the advantage of cost-effectiveness, high throughput, and minimizing the use of animals; they inherently lack the pharmacological advantages of in vivo assays. These are relevant not only in cases where prodrug activation is required in vivo but also in several cases where cell culture activity may not be a good indicator of in vivo activity. Furthermore, the changing paradigms of anticancer drug development toward molecularly targeted therapeutics sometimes necessitate the utilization of animal models to validate their mechanism of action. For example, drug candidates that act by such specific mechanisms as inhibition of angiogenesis, prevention of metastasis, and induction of differentiation require specialized approaches that are often developed on a case-by-case basis. Thus, several pharmaceutical companies and other research organizations continue to rely on murine allograft and xenograft models for anticancer drug screening.

3.3.2 Preclinical Toxicity Studies

The animal toxicological studies of anticancer agents are aimed at predicting (a) a safe starting dose and dosage regimen for human clinical trials, (b) the toxicities of the compound, and (c) the likely severity and reversibility of drug toxicities. For most cytotoxic anticancer agents, toxicity is expected at standard, therapeutically active, doses. This is because the therapeutic effect and toxicity are attributed to the same mechanism. Therefore, cytotoxic compounds are dosed to the maximum tolerable levels to maximize their anticancer efficacy. Hence, their clinical dosages are determined by their anticipated tolerance.

Toxicological testing is mainly done in small animals under the precept that the common toxicities of cytotoxic agents, such as bone marrow suppression, can be observed in rodent species. The presence and severity of acute toxicities is ascertained in the test organs by histopathology, biochemistry, and hematology investigations shortly after dosing, while the chronic or long-term toxicities are identified by sacrificing and examining the animals several weeks after dosing. Higher animals, such as primates, are avoided in routine animal toxicological studies due to cost and ethical considerations. Drug–dose correlation between different species is usually derived on the basis of body surface area, although other parameters such as age and body weight have also been used.

To determine the phase I entry dose of a cytotoxic anticancer agent, the dose levels that are lethal to 10, 50, and 90% of mice (LD_{10}, LD_{50}, and LD_{90}, respectively) is determined by the same route of administration. Instead of measuring death as an end point, these dose limits could also be defined in terms of doses that cause severe, life-threatening toxicity (severe toxic dose, STD). The projected phase I entry dose is usually $^1/_{10}$th of the LD_{10} or STD_{10}. To minimize the risk associated with human administration of a novel cytotoxic compound, the projected phase I entry dose is first tested in another species, usually rats or dogs, to ascertain lack of significant toxicity. Thus, the preclinical animal toxicology protocol usually involves single- and multiple-dose lethality or severe-toxicity studies in mice, followed by single- and multiple-dose confirmatory toxicity studies in rats or dogs. If serious, irreversible toxicities are exhibited in the non-rodent species at the projected starting dose, then the human starting dose is reduced to the $^1/_6$th of the highest dose tested in the non-rodent species that did not cause any severe, irreversible toxicity.

Evaluation of toxicities of anticancer drugs in animals has several limitations, since anticancer agents are inherently toxic with usually a dose-dependent manifestation of symptoms. The estimation of dose levels in animal toxicology studies that would correspond to the human clinical doses within the therapeutic window is difficult, leading to the possibility of underestimation or overestimation of the drug's toxicological profile. This could be due to species differences in the pharmacokinetics and pharmacodynamic responses of drugs, e.g., due to differences in the metabolic and elimination pathways, protein binding, and the sensitivity of target cells. Furthermore, rare toxicities, e.g., those of cardiovascular or neuromuscular origin, are difficult to detect in

animal models. A retrospective study observed 0.5% of toxic deaths in the phase I trials of anticancer agents among ∼6,000 participants [49].

An over prediction is highly undesirable for safety reasons. Under prediction, on the other hand, could increase the duration and steps in the dose escalation studies, thus increasing the development costs and time and the unethical administration of ineffective doses to a large number of seriously ill patients [50]. For these reasons, both under and over predictions could result in the dropping out of a molecule from further development. An example of over prediction was seen with the anticancer drug fludarabine. It caused significant bone marrow suppression in phase I studies at the $^{1}/_{10}$th of mice LD_{10} dose, while dose levels up to 20 times higher than this dose did not cause significant bone marrow suppression in dogs. This difference was explained in terms of species differences in drug pharmacodynamics – higher efficiency of phosphorylation of the drug in human bone marrow cells than those of the dog [51].

Increase in clinical study time due to underestimation of dose is exemplified by brequinar sodium, which needed 19 dose escalation steps over a period of more than 3 years to reach the MTD, since the MTD was 40 times higher than the mouse LD_{10} [52]. Another example is flavone acetic acid, for which the single-dose LD_{10} in mice was similar to that in rats, but dogs and humans tolerated up to four times higher doses. This was attributed to faster drug clearance in the higher species, thus resulting in under prediction of the clinical entry dose [53].

These examples illustrate the need to better estimate drug toxicities in humans to avoid lengthy phase I trials as well as severe drug toxicities. Toward this end, pharmacokinetic analyses are frequently being included in the toxicology protocols. Recently, drug microdosing have been proposed in humans to understand pharmacokinetic properties before projected doses are administered. This aspect is discussed further in Section 3.4.2.

3.4 Clinical Testing

Clinical trials of drug candidates are carried out in three distinct phases: phase I studies to identify safe dose levels and schedules, phase II studies to identify the spectrum of anticancer activity, and phase III studies to compare the NCE with the up-to-then best-available treatment. In addition, post-marketing surveillance phase IV studies continue to monitor drug safety as it is then administered to a significantly greater number of patients. Regulatory involvement is critical at all stages of clinical drug development. As illustrated in Fig. 2, an Investigational New Drug (IND) application is filed with the U.S. Food and Drug Administration (FDA) before the initiation of phase I studies. At the end of phase II studies, usually a pre-NDA meeting is held with the FDA to discuss the results and the plans for the phase III clinical trials. Upon completion of the phase III studies, a New Drug Application (NDA) is filed with the FDA for the grant of marketing authorization.

In the case of anticancer drug development, frequently, drug combinations are evaluated instead of a single compound monotherapy. Phase I studies of anticancer agents are usually conducted in patient, rather than healthy, volunteers. Frequently, this aspect adds to the challenges of developing anticancer compounds since (1) recruitment of tumor-specific patient volunteers becomes difficult and (2) the recruited volunteers are usually in the advanced stages of the disease and refractory to the currently available standard-of-care treatment options. These factors also escalate the clinical costs of drug development.

Phase I clinical trials are carried out at progressively escalating doses to identify the dose-limiting toxicities for cytotoxic compounds and are concluded when the maximum tolerated dose (MTD) is reached. Increments in drug doses in these trials are based on the type, severity, and duration of observed toxicities and their correlation to the expected profile of the given structural class of drugs. Phase I trial concludes when the MTD is reached and the necessary information on the clinical toxicity, pharmacokinetics, and preliminary anti-tumor activity has been gathered.

3.4.1 Dose Escalation Studies

Dose escalation refers to increasing the dose of the drug in phase I clinical trials to identify the maximum tolerated dose. The dose could be increased periodically within the same clinical trial or in each new trial arm. The choice of starting dose and dose escalation steps determine the duration of phase I studies, the number of patients who may be treated with subtherapeutic doses, and the precision of the recommended phase II dose. In the special case of cytotoxic anticancer agents, the likelihood of efficacy is dose dependent. In addition, these agents present a clear dose–toxicity relationship. Therefore, dose-related toxicity is regarded, in general, as a surrogate for efficacy [54]. Thus, the dose escalation process is a careful balance between a conservative approach to ensure safety and a guided approach to ensure early detection of the MTD.

Historically, the most frequently used scheme for phase I dose escalation of cytotoxic agents has been the 'modified Fibonacci search.' This scheme involves dosage increment steps with *increasing decreases* over the previous dose, e.g., (2, 3.3, 5, 7, 9, 12, 16d) as multiples of initial dose (d), or (100, 65, 52, 40, 29, 33, 33%) increases over the previous dose [55]. In contrast to this empirical approach, pharmacologically guided dose escalation (PGDE) scheme proposed by Collins et al. [56] is based on using the preclinical toxicology data to rapidly escalate doses to a target area under the curve (AUC) value obtained from murine pharmacokinetic data.

The PGDE scheme is based on the key assumptions that the drug concentration in the plasma can be used as a predictor for dose-limiting toxicity (DLT) and that the quantitative relationship between toxicity and drug exposure (AUC) is similar across species [56]. Practical limitations of this scheme include the difficulty in obtaining real-time pharmacokinetic data at each dose level, extrapolation of preclinical pharmacokinetic data especially when the dosing

schedules were different, and because of the inter-patient variability. In a retrospective evaluation of this dose escalation design, Fuse et al. found that the log AUC for LD_{10} in mice correlated well with the log AUC for MTD in humans for cytotoxic agents whose mechanism of action does not depend upon the cell cycle phase, but not for cell cycle phase-specific agents [57]. Furthermore, accounting for protein binding showed better correlation between the mouse and the human AUC for the unbound drug.

In addition, non-pharmacokinetic statistical modeling approaches have been recommended to guide the dose escalation. These statistical approaches model the dose–toxicity relationship as a sigmoidal curve to predict the MTD. The predicted value of the MTD is adjusted as data on the occurrence or the absence of toxicity accumulate from the clinical trial. Thus, the statistical prediction of the MTD is higher when low toxicity is observed, allowing rapid dose escalation, and the predicted MTD is low when dose-related toxicity is observed, calling for conservative dose escalation steps. This approach of dose escalation has been termed the continual reassessment method (CRM) [54].

3.4.2 Inter-patient Variability and Dose Normalization

Cytotoxic anticancer compounds are inherently toxic and have low therapeutic window. Nevertheless, they are dosed to very high levels, close to but lower than the MTD, to maximize their therapeutic benefit to the patient. Therefore, inter-patient variability in drug exposure has serious implications on drug effectiveness and toxicity to the patients. The variation in drug exposure arises from differences in drug metabolism and elimination. For example, the total body clearance of carboplatin can range from 20 to 200 mL/min due to inter-patient differences in renal function, since most of the drug is eliminated by glomerular filtration through the kidneys [58]. Similarly, topotecan clearance correlates with renal function [59]. On the other hand, clinical drug exposure and toxicity of 6-mercaptopurine are significantly related to the polymorphic phenotype of its metabolizing enzyme, thiopurine methyltransferase [60].

For drugs with clinically established exposure-physiological parameter correlations, dosage adjustment for an individual patient can be done a priori, based on the patient's physiological parameters, such as genotype and/or phenotype of the metabolizing enzymes (pharmacogenetics, see Section 2.2), renal clearance, serum protein, or hepatic function. In addition, for drugs that are dosed repeatedly or continuously, dosage modification can be based on the measurement of drug blood levels and toxicities in the patient. This strategy has been used for continuous intravenous infusions of etoposide and fluorouracil [61]. Another dosage individualization strategy involves administration of a low test dose of the compound to determine the exact pharmacokinetic parameters for an individual patient (micro-dosing, see Section 3.4.3), followed by modifying the dose to achieve a target drug exposure. Such strategies, however, can only be applied to drugs for which the pharmacokinetic–pharmacodynamic relationships, or relationships between physiological parameters and drug exposure, have been clearly established.

However, many drugs present complex pharmacokinetic relationships, hindering the establishment of such correlations. Furthermore, the clinical experience is usually very limited with new drugs under development. In such situations, clinical oncologists frequently use body surface area (BSA) for drug dose scaling between individuals. Other physiological scaling parameters, such as age, gender, weight, or body mass index, may have also been used in specific circumstances [62].

The use of BSA as a dose-scaling parameter is credited to its early use showing a correlation of BSA with MTD between species [63, 64]. While the use of BSA in preclinical research for scaling between species is well accepted, its use as a scaling parameter has been widely debated and challenged recently [62, 65–69]. For example, BSA correlates well with the total blood volume and the basal metabolic rate, but not with liver function or the glomerular filtration rate [67, 70–71]. Furthermore, BSA varies significantly more among pediatric (0.4–2.0 m^2) than adult (1.6–2.2 m^2) patient populations [62]. The limitations inherent in use of BSA for inter-patient drug scaling are also reflected in the drug–dosage modifications from BSA predicted values. For example, the relative dose per square meter is usually increased in children compared to the adult dose [72], ideal body weight is often used in BSA calculation rather than the actual weight [73], BSA is usually capped at 2 m^2, and dose reduction is undertaken in patients with compromised renal or hepatic function [67]. Nevertheless, the use of BSA as a scaling parameter has shown reduced inter-patient variability in drug exposure in several cases [62, 74] and remains an established clinical practice that usually gives way to or complements the use of more direct correlations as they get established in clinical practice.

Clinical oncologists undertaking new drug development, therefore, need to carefully evaluate the requirement for and the merits and demerits of each modality of inter-patient dose scaling. New drug development programs frequently incorporate measurement of physiological variables, such as the phenotype of key drug-metabolizing enzymes, to establish scaling parameters, where feasible.

3.4.3 Microdosing in Human Clinical Trials

The first-in-human clinical trials of novel cytotoxic compounds constitute a significant safety risk for the patient volunteers. A microdosing strategy has been proposed to mitigate this risk, gather pharmacokinetic data in earlier in clinical development, and to increase the efficiency of drug development. The microdosing concept is based on using extremely low doses of a drug, which are pharmacologically inactive but are able to delineate the pharmacokinetic profile of the drug in humans [75, 76]. This strategy is also expected to reduce the number of participants required for preclinical safety studies and to more accurately predict the first-in-human doses.

The microdose of a small-molecule drug has been defined by the US and European regulatory authorities as "less than $1/_{100}$th of the dose calculated to

yield a pharmacological effect of the test substance to a maximum dose of less than 100 μg." For a protein drug, 30 nmol is considered the maximum dose [77–79]. One key consideration of microdosing studies is the requirement of highly sensitive analytical methods. Such analytical methods include liquid chromatography with tandem mass spectroscopy (LC/MS/MS), positron emission tomography (PET), and accelerated mass spectroscopy (AMS). The use of AMS, however, requires the use of ^{14}C radiolabeled drug, making it less popular.

The American College of Clinical Pharmacology recently issued a position statement on the use of microdosing in the drug development process [80]. In this chapter, Bertino et al. discussed the key considerations for the predictive success and validation of utility of the microdosing protocol. They noted that the success of microdosing strategy depends upon its ability to accurately predict the key pharmacokinetic parameter estimates, e.g., bioavailability, clearance, and the elimination rate, of a drug at much higher therapeutic doses of the drug. The authors noted that only a few studies have reported the comparison of the therapeutic with the microdose data. These studies, however, have used currently marketed drugs and suffer from the limitation of 'prior knowledge', which helps clinical study design in aspects such as the sampling intervals.

A significant limitation of microdosing studies is their inability to predict PK parameters where drugs exhibit nonlinear pharmacokinetics. Nevertheless, this new paradigm of anticancer drug development can complement the existing animal-to-human dose-scaling strategies to improve the safety and the success of early clinical trials.

3.4.4 Drug Combinations and Dosing Strategies

New anticancer agents are categorized in different classes based upon their chemistry, bioactivity profile, and mechanism of action. Furthermore, their clinical use is usually proposed in combination with current therapy, utilizing the established principles and advantages of combination drug therapy to achieve clinical outcomes better than the then-best-available treatment. This section briefly reviews the basis of clinical anticancer drug combinations to understand the drug combinations and dosing strategies utilized during new drug development.

Currently established anticancer drugs include the cytotoxic agents, that damage or kill cells by inhibiting cell division, and hormonal agents, which antagonize hormone action or inhibit its secretion. Hormonal drugs include the glucocorticoids, estrogens, antiestrogens, androgens, and antiandrogens. Cytotoxic agents act as antimetabolites such as pentostatin, 6-mercaptopurine, methotrexate, and 5-fluorouracil; DNA polymerase inhibitors, such as cytarabine; alkylating agents such as cisplatin and mitomycin; RNA synthesis inhibitors, such as doxorubicin, etoposide, and amsacrine; microtubule function inhibitors, such as vinca alkaloids, vincristine and vinblastine; or protein synthesis inhibitors, such as crisantaspase. Based on their action during the cell cycle, these drugs could be classified as being cell cycle active, with or without

phase specificity (e.g., G0, G1, M, G2, or S phase of the cell cycle), or non-cell cycle active. Thus, antimetabolites such as 5-fluorouracil and 6-mercaptopurine, and the dihydrofolate reductase inhibitor, methotrexate, are S-phase specific; bleomycin and vinca alkaloids are G2/M-phase specific; alkylating agents (e.g., nitrogen mustard, cyclophosphamide) and doxorubicin are non-phase specific; and corticosteroids such as prednisone and dexamethasone are non-cell cycle active.

Combining drugs in clinical use is a purview of the clinical oncologist and is an ever evolving discipline. Over a hundred clinically used chemotherapy combinations are recognized [81], a detailed discussion of which is beyond the scope of this chapter. Nevertheless, there are a limited set of principles that underlie drug combinations in anticancer treatment [82]. Briefly, the drugs used in combination should possess one or more of the following features:

1. Act by different mechanisms
2. Have some efficacy by themselves
3. Have a different spectrum and/or cell cycle phase specificity of cell kill
4. Have different toxicity profiles
5. Have different mechanisms of resistance development.

Synergistic or additive cell kill, without increasing toxicity, is a frequent goal of drug combinations [83]. The need for higher cell kill is indicated by the first-order nature of this phenomenon, whereby chemotherapy cycles reduce tumor cell number by a given percentage irrespective of the starting cell count. For example, if a drug leads to 99.99% cell kill, it would reduce the tumor cell load of a usually detectable 2 cm solid tumor mass from $\sim 10^9$ to $\sim 10^5$ cells (the cell kill principle) [84]. The cell kill efficiency of cytotoxic drugs is expressed by the negative log of the fraction of tumor cell population killed by a single course of treatment. Thus, a drug that results in 99.99% cell kill is a 4-log drug, while another drug with 99.9% cell kill would be called a 3-log drug. Additive combination of these drugs, for example, would be expected to result in 7-log cell kill per treatment cycle. Thus, different drugs are given at full doses to increase the percent cell kill toward improved overall clinical outcome and patient survival, while reducing the number of chemotherapy cycles and the emergence of drug resistant cancers.

The principles of chemotherapeutic drug combinations resulting in better clinical outcomes can be exemplified by the use of MOPP combination in Hodgkin's disease and M-BACOP in diffuse lymphoma [85]. The MOPP combination uses nitrogen mustard with vincristine, prednisone, and procarbazine with significantly improved antitumor efficacy and remission rate than any drug alone. It further exemplifies the principles of dose combination, i.e., it uses full dose of drugs with different toxicity profiles (neuropathy with vincristine and typical steroid toxicity with prednisone) and reduced dose of drugs with similar toxicity profiles (bone marrow toxicity of procarbazine and nitrogen mustard). The M-BACOP combination uses methotrexate with bleomycin, adriamycin, cyclophosphamide, vincristine, and prednisone with the same principles of reducing the dose of drugs with overlapping toxicities

(bone marrow suppression with adriamycin and cyclophosphamide), but not for different target toxicities (lung toxicity with bleomycin, neuropathy with vincristine, and steroidal toxicity with prednisone).

In addition to drug dosing based on individual and overlapping toxicities of anticancer agents, cytotoxic drugs are dosed in short-duration high-dose cycles rather than a continuous low-dose administration. This is designed to achieve the most cell kill with a high drug dose, while allowing the body to recover from the side effects of chemotherapy between different cycles of treatment. The cell kill efficiency of cytotoxic drugs is the most evident in a solid tumor model (e.g., lung, uterus, and stomach cancer) whereby the tumor consists of actively dividing surface cells overlaying resting cells in the middle, and non-dividing, often non-viable, cells in the core [84].

While short-duration chemotherapy to aggressively kill actively dividing cancer cells is the most common practice, the introduction of novel target-based anticancer agents has allowed changes in the regimens to include continuous, low-dose administration of targeted drugs. For example, Klement et al. report a low-dose anti-angiogenesis regimen utilizing vinblastine combined with an antibody against the VEGF receptor-2 [86]. The authors reported tumor remission without undue toxicity of drug treatment. This approach is particularly applicable to the use of antiangiogenic drugs and has been called low-dose metronomic (LDM) chemotherapy [87].

The clinical development of new anticancer agents builds on the knowledgebase and current practices with existing therapies. Thus, an understanding of drug combinations and dosing relevant to specific disease conditions allows the clinical oncologists to appropriately place new chemical entities in a clinical program to maximize the probability of its beneficial outcome to the patient.

3.4.5 Adverse Effects and Toxicities of Anticancer Drugs

An understanding of toxicities, adverse effects, and special dosing considerations of existing anticancer compounds is important to the design of effective drug combinations and to the interpretation of the toxicological profile of new chemical entities. Most cytotoxic anticancer agents are dosed to maximum tolerated levels to achieve maximum cell kill. The toxicities incumbent with these compounds are often a manifestation of their mechanism of action and killing of the rapidly growing, normal cells such as hair follicle cells, gastrointestinal surface epithelial cells, and stem cells.

The common toxicities of cytotoxic anticancer drugs include the following:

- Bone marrow depression due to damage to the growing stem cells causes reduction in the blood white cell, platelet, and red cell counts. These, in turn, could cause susceptibility to infections, excessive bleeding, and anemia. In addition, certain drugs cause unique and serious bone damage, such as the osteonecrosis of the jaw associated with bisphosphonates [88].

- Damage to growing cells may cause temporary loss of hair (alopecia), skin rashes, changes in the color and texture, or loss of fingernails and toenails. These toxicities are usually reversible.
- Surface epithelial damage to the gastrointestinal tract may result in ulcers, stomatitis, difficulty swallowing (dysphagia), vulnerability to oral infections such as candidiasis, and changes in saliva secretion. In addition, nausea, vomiting, diarrhea, or constipation occur commonly.
- Some drugs may cause kidney damage due to extensive cell destruction, purine catabolism, and deposition of urates in the renal tubules. In addition, liver damage may occur it receives large blood supply. Metabolic conditions of the liver and the kidney are usually monitored for possible correlation to drug blood levels and dosage adjustment, since these are the major drug elimination sites.
- Certain symptoms and side effects associated with cancer could be secondary to disease progression. For example, cancer metastases to the bones could cause chronic pain due to proliferation of cancer cells in the bones and the associated bone remodeling and destruction [89]. Also, tumors that compress veins, the use of central vein catheter [90], and relative immobility of the patient could lead to deep vein thrombosis with potential pulmonary embolism [91].
- Certain drugs, such as paclitaxel and vincristine, could cause peripheral neuropathy [92]. Similarly, anthracyclines are known for rare but serious cardiotoxicity [93, 94].

Thus, adverse drug effects and dose-limiting toxicities of anticancer compounds could be a manifestation of either their mechanisms of action or unrelated toxicities common to a given chemical class of compounds, such as anthracyclines. A close attention to monitor for the emergence of known side effects of anticancer drugs as well as those observed in the preclinical animal toxicology studies ensures patient safety in early oncology drug clinical trials.

3.4.6 Special Patient Populations

Clinical trials in special populations, such as pediatric and geriatrics, nursing and pregnant women, and patients with reduced renal function, are routinely carried out to define the subtleties of clinical application of all drug candidates. These usually involve delineation of a drug's metabolic and elimination pathways, identification of biochemical markers to define the metabolic status of the patient with respect to drug's elimination, genotypic and phenotypic profiling of the patient, defining pharmacokinetic – pharmacodynamic relationships, and dosage adjustment. These principles are practiced with greater vigor for anticancer drugs due to their dose-limiting toxicities, dosing to maximum tolerated levels, and other serious adverse effects.

In addition, pediatric testing of anticancer agents is necessitated by childhood prevalence of fast growing cancers, such as lymphomas, leukemias, and

myelomas. Furthermore, regulatory agencies are increasingly encouraging pediatric clinical trials to establish safe and effective doses for pediatric labeling [95, 96]. Phase I clinical trials in children are usually multi-institutional, due to the number of patients available. Ethical considerations further limit the number of levels of dose escalation in children, since treatment with ineffective doses is undesirable. In addition, these trials also enlist patients with intensive prior therapy, which has implications on the maximum tolerated dose determination. Heavily pretreated patients tend to have lower MTDs, especially when DLT involves myelosuppression; which is not the case for patients with minimum prior therapy – thus complicating the determination of MTDs [97].

Pediatric testing of anticancer agents is carried out after the efficacy of these drugs has been established in the adults. A common practice in pediatric oncology is to administer 80% of the MTD determined in adult patients with significant prior therapy and to conduct dose escalation in 30% increments. Further, dose escalation is carried out in successive cohorts of patients since intra-patient dose escalation is usually not permitted and the number of dose escalation steps is sought to be minimized [97]. A retrospective investigation of 69 pediatric oncology trials found that the pediatric MTD strongly correlated with adult MTD and differed by not more than 30% of the dose. They further found that not more than four dose levels were studies in the escalation schemes in over 80% of the trials [98].

3.4.7 Phase II and III Clinical Trials

As a drug candidate progresses through the development stages after the initial proof-of-concept and phase I studies in humans, a reverse funnel of increasing patient exposure to the drug becomes evident (Fig. 2B).

Phase II studies are carried out in a small group of patients with a specific tumor type to determine anticancer efficacy and to define the therapeutic window of the compound. To avoid exposing patients to inactive compounds, these clinical trials use statistical tools to interrupt studies where the in-process data indicate low probability of success. Phase III trials are conducted in a much greater number of patient volunteers of the selected tumor type with prospective and randomized evaluation against the then-available best-possible therapy for the disease, regarded as the standard-of-care in the specific cancer setting. Phase II studies act as a screen of antitumor efficacy to select the most promising agents to enter the pivotal phase III clinical trials. The demonstration of statistically significant improvement in tumor response in large phase III clinical studies against the currently best-available treatment in a tumor type-specific patient population is the ultimate benchmark for regulatory approval and marketing of a novel anticancer agent.

Phase III cancer clinical trials are usually conducted by certain cooperative groups that were founded in the 1960s and later years and include several member institutions participating in a multitude of trials that are actively ongoing at any given time [99]. Examples of these groups include the Children's

Oncology Group, the Eastern Cooperative Oncology Group, and the Cancer and Leukemia Group B [100–102]. Several of these groups are associated with academic institutions. A phase III cancer clinical trial, therefore, is a complex interaction among the cooperative groups involved, their associated academic institutions, the commercial sponsors, and the regulatory agencies.

There are certain key elements of any clinical trial that are incorporated in the study protocol. These include a clear definition of the objectives, end points, inclusion and exclusion criteria for the selection of patient volunteers (study population), treatment plan, clinical assessments, laboratory tests, trial design (including randomization), statistical considerations, data monitoring protocols, and informed consent. Conduct of cancer clinical trials adds unique perspectives and limitations on several of these elements. For example, blinding is often not utilized. This is because of distinct dosing schedules, routes of administration, and toxicity profiles that makes blinding difficult [99]. In addition, often non-inferiority trials are conducted with the goal to prove that the therapeutic benefit of a drug is not lost with a new regimen or treatment approach, such as drug combination or change in the route of administration.

3.4.8 Trial Design

Phase II clinical cancer trials are traditionally designed as single-arm trials utilizing historical controls on the currently best-available treatment, while phase III studies usually use a parallel-arm design. These designs are in contrast to the preference for crossover randomized designs for both phase II and phase III studies in other drug classes. Crossover designs are not preferred for cancer clinical trials to avoid carryover of the treatment effect of the first trial period into the second. The end points used in the cancer clinical trials require that the patients be in the similar overall clinical state at the beginning of both treatment periods. For example, the end point of survival benefit cannot be used in a crossover design. Also, patient tolerance to toxicities may change for the second treatment cycle in the crossover design [99].

Single-arm designs for phase II clinical trials use the proportion of patients who achieve a complete or partial response to the treatment as the primary efficacy measure. This design eliminates truly ineffective therapy and is based on the 'historical control' that only a limited number of tested drugs had any activity [103]. Although this design has served well for cytotoxic drugs, recent high attrition rates in phase III oncology trials has led to its criticism for inability to predict comparative performance vis-à-vis the then-available best-possible, standard-of-care therapeutic option. Furthermore, the molecularly targeted agents, e.g., gefitinib, bevacizumab, and cituximab, may not achieve consistent, high-level tumor regression. These aspects have prompted the consideration of randomized, parallel-arm designs controls and alternative end points [104].

3.4.9 End Points of Cancer Clinical Trials

End point for determining the efficacy in clinical trials of anticancer drugs is an evolving subject. Phase III cancer clinical trials focus on one primary end point to provide evidence of clinical efficacy and one or more secondary end points to delineate biological activity or benefits to the patient, e.g., reduced side effects. Three kinds of end points have been used: (1) objective tumor response, e.g., size regression; (2) time to event end points; and (3) patient-reported outcomes, e.g., palliation of side effects [99].

Tumor regression as an end point is quantified by unidimensional or bidimensional measurement of the size of lesions by clinical examination or imaging-based methods, such as X-ray, computer tomography (CT) and magnetic resonance imaging (MRI) scans, ultrasound, endoscopy, and laparoscopy. The determination of overall tumor response (as complete response, partial response, stable disease, or progressive disease) is based on the observed responses in target and non-target lesions and the appearance of new lesions after treatment [105]. This approach is limited in its inability to account for stable disease and minor response, which could be the only observable direct tumor responses for molecularly targeted agents. In addition, it requires the consideration of inherent variations in biological responses, subjectivity in measurement, and measurement techniques.

Time to event end points measure either of the following [99]:

- Overall survival (OS) is defined as the time from randomization to time of death from any cause. It is often considered an optimal efficacy end point for phase III cancer clinical trials.
- Disease-free survival (DFS) is defined as the time from randomization to disease recurrence or death owing to disease progression. It is frequently used as a primary end point in phase III trials.
- Time to progression (TTP) is defined as the time from randomization to time of progressive disease or death.
- Time to treatment failure (TTF) is defined as the time from randomization to documentation of progressive disease, death, patient discontinuation of study.
- Progression free survival (PFS) is defined as the time from randomization to objective tumor progression or death. It is a preferred regulatory end point since it includes death and may correlate better with overall survival [106].

These studies increasingly also include the quality of life analyses to determine whether the improvements in PFS or survival outweigh the disadvantages of toxicity and inconvenience [107]. The development of newer molecularly targeted anticancer agents is further influencing the paradigms of anticancer efficacy evaluation [8]. Determination of clinical end points for these drugs could be based on the quantifiable pharmacodynamic characteristics such as the target inhibition or the levels of a tumor-specific biochemical marker in the plasma. The use of target markers for determining drug response is exemplified by the

measurement of farnesyltransferase activity in buccal scrapings for farnesyltransferase inhibitors [108] and plasma vascular endothelial growth factor (VEGF) concentration for the angiogenesis inhibitor anti-VEGF receptor-2 monoclonal antibody [109]. The use of this strategy, however, requires marker validation and correlation with anticancer response, which is not trivial. For example, while a biologically effective dose of marimastat was defined based on tumor marker levels in plasma in phase I–II clinical studies, the phase III studies did not show substantial benefit [8].

4 Potentials and Practices in Anticancer Drug Delivery

Initial screening of drug candidates in cell culture and animal toxicology studies is usually carried out in the solution form utilizing relatively small quantities. Early stage drug development requires physicochemical characterization of the drug candidate for its solubility and stability characteristics in addition to the chemistry, i.e., proof of structure and control of impurities during synthesis. This stage involves the development of stability indicating analytical methods for the assay of potency and impurity content, and the selection of a solvate or hydrate and the crystal form of the compound. As a compound is funneled down to successively higher stages of drug development, the compound is synthesized in larger quantities with much higher purity and a parallel formulation development effort is undertaken to prepare a dosage form for clinical testing.

Although formulation development of anticancer drugs follows the same precepts as for any drug candidate, special considerations are applicable to the formulation of anticancer compounds for early clinical screening. Formulation choices for anticancer drugs depend upon the physicochemical and biopharmaceutical properties of the drug candidate, its intended dose and route of administration, and the patient and disease factors. An important paradigm for anticancer drug delivery is the preference of the intravenous (IV) route of administration, especially for cytotoxic compounds. The IV route is preferred to avoid any bioavailability issues and problems with oral administration, especially since nausea and vomiting are common side effects of most cytotoxic agents. This also allows accurate dosing, flexibility of dose and dosing schedule, and rapid withdrawal of the drug if undue toxicity is observed. Another important consideration is to minimize the possibility of compromising the therapeutic efficacy of the drug. Thus, preservatives are avoided and excipients are minimized to reduce the possibilities of potential incompatibities, such as physical adsorption or chemical complexation.

A historical review of formulations most commonly used for anticancer drug delivery indicates that parenteral, especially IV, injection is the first choice, followed by oral tablets or capsules, with only a handful of formulations appearing as gel, implant, or aerosol [110]. Some examples of parenteral formulations and the basis of their selection are included in Table 1 [111].

Table 1 Examples of parenteral formulations of cytotoxic anticancer agents

S. No.	Example of drug	Formulation details	Remarks
Simple aqueous solutions for drugs with high solubility and stability in water			
1	Tetraplatin	Solution in normal saline	Platinum analog
2	CHIP, *cis*-dichloro, *trans*-dihydroxybis-iso-propylamine platinum IV	Solution in normal saline	Platinum analog
3	Topotecan	5 mg/mL base solution in 0.1 M gluconate buffer at pH 3.0	Topoisomerase I inhibitor. Acidic pH of the solution prevents hydrolysis of the lactone ring
Solubility improvement using cosolvent and surfactant			
1	Etoposide (Vepesid®)	Drug formulated with polysorbate 80, PEG 300, and ethanol along with benzyl alcohol as preservative and citric acid for pH adjustment	Large doses of IV ethanol can cause phlebitis. The amount of ethanol that can be administered per hour depends on its rate of metabolism, which is up to 10 g/h
2	Teniposide (Vumon®)	Drug formulation contains N,N-dimethylacetamide, Cremophor EL, and ethanol for solubilization in addition to maleic acid for pH adjustment	High dose teniposide could lead to ethanol intoxication and toxicity due to Cremophor EL
3	Paclitaxel (Taxol®)	Solution in 1:1 mixture of Cremophor EL and ethanol.	IV Cremophor EL can cause hypersensitivity reactions
4	Carzelesin Adozelesin Bizelesin	Uses PEG 400, ethanol, and Tween 80 for solubilization	Must be diluted in the IV infusion fluid before administration
Solubility improvement using cosolvents			
1	Busulfan	Aqueous solutions of 40% PEG 400 in normal saline	
2	2-Amino-5-bromo-6-phenyl-4(3)-pyrimidone (ABPP)	Aqueous solution in sodium carbonate buffer containing *N,N*-dimethylacetamide (DMA)	

Table 1 (continued)

S. No.	Example of drug	Formulation details	Remarks
3	2-Chloro-2',3'-dideoxyadenosine (2-CIDDA)	Phosphate-buffered solution containing 60% propylene glycol and 10% ethanol	Propylene glycol is hemolytic in vitro and should be administered at less than 40% concentration
4	Melphalan	Aqueous solution containing 60% propylene glycol and 5% ethanol	It is diluted with normal saline before administration
Complexation to improve aqueous solubility and stability			
1	N-nitrosourea-based anticancer agents	Form complex with Tris buffer (Tris(hydroxyethyl)amino ethane)	Rate of degradation of drug in the complex is slower than free drug
2	5-Fluorouracil	Formulated in Tris buffer	Cardiotoxicity observed upon IV administration. Attributed to the presence of adducts of two degradation products of the drug with Tris
3	Erbuzole Benzaldehyde	Complexation with cyclodextrins	
Hydrotropic solubilizing agents			
1	Etoposide	Formulated in sodium salicylate solution. Planar orientation of both the drug and the salicylate salt tend to improve solubility in aqueous solution	
2	Doxorubicin Epirubicin	Use parabens in the lyophilized formulation	Drug has a tendency to form dimeric and polymeric self-aggregates, increasing the time required to dissolve the lyophilized vial. Incorporating parabens facilitates drug-paraben complexation, reduces drug self-aggregation, and facilitates rapid dissolution of the drug

Table 1 (continued)

S. No.	Example of drug	Formulation details	Remarks
Liposomes for improving PK profile, drug activity, and drug targeting			
1	Doxorubicin	Commercially available as a stable, lyophilized liposomal formulation	IV administered liposomes concentrate in fenestrated capillaries such as liver, spleen, and the bone marrow. IV doxorubicin liposomes have been shown to reduce its cardiotoxicity
2	Camptothecin (CPT) 9-Amino CPT (9-ACPT)	Formulated as liposomes of cholesterol, phosphatidyl serine (PS), and phosphatidyl choline (PC)	Freebase of CPT has ~10-fold higher activity than the sodium salt. Therefore, formulation in liposomes provided higher activity
3	Tin protoporphyrin (SnPP)	Formulated as liposomes	IV administration increased drug accumulation in spleen due to its high concentration of reticuloendothelial cells
Microencapsulation for improving toxicity profile, controlled release			
1	Merbarone	Microdispersion of nanoparticles at neutra pH	IV administration of the N-methyl glucamine salt solution at pH 10 caused injection site vasculitis, which was overcome with the nanoparticle formulation
2	Methotrexate	Methotrexate was conjugated with gelatin and incorporated in gelatin microspheres	Reduced renal toxicity compared to the free drug
Parenteral emulsion formulations for improvement in solubility, stability, local irritation or toxicity, and/or compatibility issues			
1	Hexamethyl melamine (HMM)	Ethanol or DMA-solubilized drug to be diluted in Intralipid parenteral emulsion before administration	Overcomes drug solubility problems
2	Perrilla ketone	Drug formulated in propylene glycol, ethanol, and water; to be diluted in a parenteral emulsion before IV administration.	IV administration in 5% dextrose led to loss of 20–60% drug by adsorption to the polyvinylchloride (PVC) of the infusion tubing. This problem was overcome in IV emulsion formulation

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Table 1 (continued)

S. No.	Example of drug	Formulation details	Remarks
Lipoproteins for tumor targeting			
1	Prednimustine	Drug microemulsion complexed with the apo **B** receptor of the low-density lipoprotein (LDL) particle.	Its cytotoxic activity against breast cancer cells was higher than the free drug. This was attributed to the upregulation of LDL receptors on tumor cells
2	Vincristine	LDL-associated vincristine compared with free drug.	Reduced neurotoxicity with the LDL formulation
Prodrug approaches to increase drug activity and aqueous solubility			
1	1-β-D-arabinofuranosylcytosine (ara-C)	Lipophilic prodrug prepared by conjugation with phosphatidic acid	Significant increase in the life span of mice with L1210 and P388 leukemia
2	Chlorambucil	Drug conjugation to α, β-poly(N-hydroxyethyl-DL-aspartamide) by ester linkage.	Increased water solubility
Lyophilization to improve drug stability			
1	Bryostatin I	Bryostatin lyophilized from butanolic solution with povidone; to be dissolved in PEG 400, ethanol, and Tween 80 mixture (PET diluent) followed by dilution in normal saline immediately before administration	Improved drug solubility with reduced requirement of cosolvents for administration and improved shelf-life of the lyophilized formulation
2	Tumor necrosis factor-α (TNF-α)	Lyophilized solution with mannitol and the sugar based amorphous protectant dextran, sucrose, or cyclodextrin in citrate buffer	Stabilization of solution from tendency for dimeric and polymeric self-aggregation, leading to the formation of particulates in solution

One of the blessings of having anticancer drugs in the pipeline is the fact that these drugs allow the exploration of sophisticated and unconventional formulation approaches due to their urgent need in the clinic and the special circumstances of the care of cancer patients. For example, the water-insoluble and unstable nitrogen mustard, carmustine, is supplied in lyophilized vials with sterile drug. Separately, vials with sterile, dried ethanol and sterile water for injection are provided. At the time of use, the drug is dissolved in ethanol and further diluted with water before injection. Another example of a water-insoluble and water-unstable drug administered unconventionally is spiromustine. It is supplied as a lyophilized drug in vials, which is first dissolved in sterile ethanol and then dispersed in a sterile emulsion for intravenous administration. Commercially available IV nutrition emulsions, such as those of soybean oil, e.g., Intralipid®, or safflower oil, e.g., Liposyn®, are used for this purpose.

The investigational drug carzelesin offers another example of the unique drug delivery possibilities with anticancer therapeutics. Carzelesin is highly insoluble and is available as a solution in polyethylene glycol 400, ethanol, and polysorbate 80 for dilution in the IV infusion fluid immediately before administration. However, due to its tendency for rapid crystallization, it is administered to patients with a two-pump infusion system such that the drug solution and the infusion solution come in contact with each other for a very brief period before entering the bloodstream.

The use of unconventional drug delivery systems often presents unique drug development challenges. For example, paclitaxel is formulated in a 1:1 mixture with the surfactant cremophor and ethanol (Taxol®). Intravenous administration of this agent resulted in local toxicity and systemic hypersensitivity reactions when the drug was infused over a 3 hour period [112]. This resulted in prolongation of the infusion rate of taxol to 6 hours or longer [113]. Further clinical studies to define the appropriate rate and amount of drug administration to minimize systemic toxicity resulted in a clinical protocol that identified a low-dose, low-duration (135–175 mg/m^2 infused in less than 6 hours) administration regimen with superior hematologic toxicity and neurotoxicity profile than a similar or higher dose, longer duration (170 mg/m^2 or more infused over 24 hours) administration [114]. Thus, sophisticated formulations can potentially lead to toxicity to the patients, resulting in increased clinical testing, delays, and possibly the drug development program.

A significant requirement of anticancer drug development is the extraordinarily high amount of safety precautions necessary in the handling of these drug substances from the first discovery stages through commercial production. These safety precautions often slow down the pace of drug development and necessitate infrastructural investments to explore technologies that minimize potential exposure and hazard to the employees. Pharmaceutical companies actively engaged in anticancer drug development commonly have special containment areas and ventilation hoods for the handling of these substances. An example of investment in technologies for employee safety reasons is the adoption of single-pot processors for wet granulation, which enables granulation

followed by drying in the same mixer [115]. Several of these equipments are now commercially available [116].

Increasingly, oral drug formulations of anticancer agents are being developed. The incentives for oral drug formulation of anticancer agents include improved safety, efficacy, quality of life, reduced cost, and the ability to deliver chemotherapy at home and to apply drug schedules that maximize an agent's efficacy [117]. The development of oral drug formulations is constrained by restrictions in dose size, bioavailability concerns, and patient compliance – especially for drugs that cause nausea and vomiting. The preference for oral route of administration is reflected in the increasing number of drugs being formulated as tablets or capsules. Examples of anticancer compounds that have been marketed as oral solid dosage forms include anastrozole, dasatinib, gefitinib, tamoxifen, mercaptopurine, 6-mercaptopurine, estramustine, cyclophosphamide, levamisole, toremifene, letrozole, capecitabine, and exemestane [110].

5 Regulatory Considerations

Anticancer drug development brings forth unique perspectives and their regulation has evolved to accommodate and address those unique aspects. One key driving force for anticancer drugs is the urgent patient need for the development of new agents and the need to rapidly move the promising agents into clinical trials. Another is the recognition that these agents are dosed to toxic levels, close to the maximum tolerated dose, MTD, with the precept that the side effects of drug therapy would be less threatening to the patient than their disease. Control of clinical toxicity is sought by careful dosing, monitoring, and prompt treatment of toxicity, or drug withdrawal.

The regulatory requirements for anticancer compounds focus on drug safety evaluation in preclinical toxicology studies, based on the intended use and mechanism of action of the drug, and the target patient population. As DeGeorge et al. point out, in situations where the potential benefits of therapy are the greatest, e.g., advanced, life-threatening disease, the greater risks of treatment toxicity can be accepted and the requirements for preclinical testing can be minimal [118]. Nevertheless, in cases where the patient population is free of known disease, e.g., adjuvant therapy, chemoprevention, or healthy volunteers, the acceptable risks are much less and preclinical evaluation is more extensive.

As discussed before, two acute preclinical toxicity studies are required. The first is in a rodent species to identify doses that result in lethality or life-threatening toxicities to derive the clinical phase I entry dose. The second study is conducted in a non-rodent species to confirm that the selected dose is not lethal and does not cause serious or irreversible toxicity. It is highly desirable that these preclinical toxicology studies be conducted with the same schedule, duration, formulation, and route of administration of the drug as

proposed in the clinical trials. The requirements for preclinical studies depend upon the nature of the drug being developed.

Cytotoxic anticancer agents are administered in short-term phases and thus need acute preclinical toxicity studies (generally, less than 28 days). On the other hand, non-cytotoxic agents, such as immunomodulators or hormonal drugs, are intended for long-term use with continuous daily administration. Thus, the preclinical toxicology study requirements for non-cytotoxic drugs are equivalent to the duration of intended therapeutic use in patients, up to 6 months in rodent and 12 months in a non-rodent species. In addition, genotoxicity, carcinogenicity, and reproductive toxicity studies are required for the new drug application (NDA) submission. Special toxicity studies may be needed in cases where compound or drug-class-specific toxicities are known. For example, anthracyclines are known for their cardiotoxic potential and platinum-based drugs are likely to exhibit ototoxicity [119]. The dosing of non-cytotoxic agents, such as immunomodulators, is aimed to a pharmacodynamically active range, usually much lower than the MTD.

In addition, pharmacokinetic and pharmacodynamic studies are recommended to support the safety profile of the drug, which may help in deciding the starting dose, route, schedule, the dose escalation steps, and optimum plasma concentrations for the phase I clinical trials. Combinations of cytotoxic agents generally do not need preclinical toxicology testing if the agents have individually been used in humans and have an established safety profile, unless there is a reason to believe there could be synergistic interactions that might lead to increased toxicity [118].

Preclinical toxicological evaluation of non-cytotoxic agents depends on the kind of agents and therapeutic options being investigated. For example, photosensitizers require special testing protocols because of their unique modes of action and toxicity. Photosensitizers form free radicals upon absorption of light energy, which are then responsible for site-specific tumor destruction. Exposure of the patients to sunlight could cause retinal damage or phototoxicity similar to sunburn [120]. Therefore, toxicological evaluation of photosensitizers involves photosensitivity assessment as a function of the dose of light (total energy of irradiant light) in relation to that of the drug, and the correlation of photosensitivity to the plasma levels of the photosensitizer. Also, knowledge of the elimination half-life of the compound may be used to determine the duration of time a patient needs to take precautions against exposure to intense light.

Regulatory preclinical testing requirements for specialized drug delivery systems such as antibody-drug conjugates, liposomes, and depot formulations include the proof-of-concept studies that the claimed advantage of these systems is indeed being derived without additional toxicity burden. For example, safety concerns for antibody-drug conjugates include the potential for toxicity from abrupt release of the drug and the potential for unexpected specific toxicity in normal human tissues [118]. Thus, in addition to the standard toxicity testing, investigations of the stability of the conjugate as a function of the release mechanism and the reactivity of the conjugate with a complete panel

of human tissues (with and without the target antigen expression) are recommended. In addition, pharmacokinetic studies that distinguish between the conjugate, free antibody, and the free drug are desirable [118].

Toxicology studies for hormonal drugs, e.g., antiestrogens, antiprogestins, antiandrogens, aromatase inhibitors, and gonadotropin releasing hormone agonists, are recommended using the same route, formulation, schedule, and duration of treatment. In addition, preclinical evaluation of both sexes is recommended, even though these drugs are usually prescribed for sex-specific indications, to delineate the toxicities unrelated to the primary hormonal action of the drug. In addition, genotoxicity, reproductive toxicity, and carcinogenicity studies are indicated [118].

Agents that target the multi-drug resistance (MDR) of the tumors to anti cancer drugs may lead to increased toxicity of the combination. Thus, preclinical toxicity evaluation of new MDR-reversing agents is recommended in combination with the cytotoxic drug at both minimally and significantly toxic doses, in addition to the toxicological evaluation of the agent alone. Similar approach is applied for chemotherapy sensitizers [118]. In brief, the preclinical toxicology evaluations of novel agents are based on the mechanism of action and the potential additional toxicities that may emanate from the modalities of drug administration.

6 Conclusions

The clinical application of anticancer drugs brings forth unique perspectives that are evident in their discovery and development. Historical development of cytotoxic compounds, with significant contributions from serendipity, and the currently shifting focus on target-based drug discovery is evident in the evolving paradigms of preclinical and clinical evaluation of new drug candidates. Current challenges of anticancer drug development include the significant time and cost involvement, and the low success rates. These have led to increasing efforts of the pharmaceutical industry toward increasing the effectiveness of the drug discovery and development process and to minimize failure of drug candidates at later stages of development. These efforts include development of high throughput preclinical screening methods and biological assays with greater specificity and predictability. Increasing emphasis is being placed on developing a mechanistic understanding of the physicochemical and biological phenomena involved in drug development such as chemical and polymorph stability, and pharmacokinetics. The use of mathematical models to explain the mechanisms of drug degradation and predict the outcomes of formulation and process changes and scale-up is increasingly being adopted. The paradigm of continuous improvement is now incorporating a risk-based approach, where the risk to the patient is continuously evaluated through the course of drug development. The level of risk is mitigated or minimized by appropriate

measures. The critical product quality attributes (CQAs) are defined and a design space is created around all the formulation and process variables with demonstrated, reproducible achievement of the product CQAs.

This chapter has attempted to highlight the unique aspects of anticancer drugs from a pharmaceutical development viewpoint, some of which are highlighted in Table 2. The evolving paradigms of anticancer drug development demonstrate the increasing influence of scientific advancements in diverse fields and increased understanding of the disease process. These trends are expected to continue with the hope for more effective and less toxic therapeutic options.

Table 2 Blessings and liabilities of anticancer drugs in the pipeline from a pharmaceutical development viewpoint

Development aspect	Blessings	Liabilities
Drug discovery	Well-established objective screens for cytotoxic drug evaluation in both cell cultures and animal models are available	Animal and cell culture models for drug discovery screening are not representative of all tumor types and are constantly evolving
Material handling during the lifecycle of the product		Extraordinarily high safety precautions for the protection of the employees, patients, and the general population
		Cost of the active pharmaceutical ingredient (API) is usually high
		Availability of the API for development use is usually very limited
Pharmaceutical development	Sophisticated and unusual formulation choices can be made depending on the potential of the drug candidate and the disease condition	Most cytotoxic agents have low solubility, dissolution rate, stability, and bioavailability
	Most cytotoxic compounds are formulated as IV parenterals, thus obviating bioavailability issues	Usually the amount of material available for development use is very limited and the development timelines accelerated for promising candidates
		Safety considerations require specialized manufacturing processes and facilities to be used
Clinical trials	Patient willingness to participate in the clinical trials may be higher depending upon the severity of the disease condition and availability of alternative therapies	For cytotoxic compounds, clinical trials usually need to be done in patients rather than healthy volunteers
		This increases the cost and time involved in clinical testing

Table 2 (continued)

Development aspect	Blessings	Liabilities
	Potential for making the greatest contribution to the most needy patients. These drugs help 'extend and enhance human life' (Bristol-Myers Squibb, Co.'s mission statement, http://www.bms.com).	Safety of the clinical trial participants is a significant concern for cytotoxic compounds since toxicity and efficacy are usually closely dose related
Regulatory considerations	Relatively rapid regulatory review times because of the urgent need of these therapies for the patients Regulatory tolerance of the side effects of cytotoxic agents depending upon indication and the current patient need for the drug	Higher regulatory proof-of-concept and preclinical toxicology requirements, especially for target-based anticancer agents and specialized drug delivery systems
Patient and marketing considerations		The drug development programs are expensive and the drugs have high costs to the patient, often with marginal benefit over pre-existing drugs in terms of extending human life and/or improving the quality of life

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