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Innovative Strategies, Statistical Solutions and Simulations for Modern Clinical Trials

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Preface

The pharmaceutical industry’s approach to drug discovery and development has rapidly transformed in the last decade from the more traditional Research and Development (R & D) approach (e.g., “sequential” progression from phase 1 to phase 2 to phase 3) to a more innovative approach in which strategies are employed to compress and optimize the clinical development plan and associated timelines. To date, this paradigm shift, although real, has been mostly limited within a small scope of the development spectrum. For example, adaptive trial designs are increasingly being used to achieve higher power and mitigate the risk at interim analyses. However, these strategies are generally being considered on an individual trial basis and not as part of a fully integrated overall development program. Such optimization at the trial level is somewhat near-sighted and does not ensure cost, time, or development efficiency of the overall program. For this reason, the purposes of this book are set to establish a statistical framework for overall/global clinical development optimization and provide tactics or techniques to support such optimization, including clinical trial simulations. Under such a framework, depending on the unique circumstances of each development program, a mix of “classical” and “adaptive” trial designs can be implemented to achieve such an overall optimization.

It has been reported that among the reasons for failure of a clinical development program between 2013 to 2015, efficacy issues account for 52% of all failures, whereas the figures for safety, strategy, commercial and operational failures are 24%, 15%, 6%, and 3%, respectively (Harrison, 2016). Innovative statistical methodologies, employed wisely, can mitigate these risks and have the potential to significantly reduce the risk of a development program failing. Optimization tools, such as adaptive designs and other innovative approaches require strong collaborations among and between different stakeholders.

Statisticians are key members of the team tasked with assembling the clinical development strategy and plan and need to work very closely with these different stakeholders who represent different perspectives, all of which need to be considered in order to meet all program goals. As such, it is important for statisticians to possess a deep knowledge of the drug development process beyond statistical considerations. For these reasons, this book was written to incorporating both statistical and “clinical/medical” input.

The book is structured as follows:

Chapter 1, Overview of Drug Development, introduces the key elements of the “sequence” of the drug development process from inception through
approval, including drug discovery, preclinical development, and clinical development. Chapter 2, Formulating Clinical Development Plan and Trial Design, describes the role and the key components of a clinical development program, followed by a review of critical concepts of clinical trial designs. Chapter 3, Clinical Development Program (CDP) Optimization, analyzes the benchmarks in clinical development, including determination of the net present value (NPV), Clinical Program Success Rates and Reasons for Failure, deconstructing costs associated with clinical trials, timelines associated with advancing through each phase of development, regulatory review time after a file is submitted, and competitive landscape as well. In the second part of the chapter, Stochastic Decision Process as a statistical model is introduced as a method to incorporate into CDP optimization. Chapter 4, Global-Optimal Adaptive Trial Designs, provides different examples of how to design an adaptive trial that achieves global optimization. These first four chapters serve as the framework or foundation that is necessary to achieve global optimization in drug development. The rest of chapters describe specific techniques to support such optimization. Specifically, Chapter 5, Designing Trials for Precision Medicine, discusses marker-adaptive designs, basket, and population-adaptive designs. Chapter 6, Clinical Trial with Survival Endpoint, given the complexity and richness of survival endpoint trials, discusses a variety of challenging issues and how best to mitigate or resolve them, including delayed drug effect, treatment switching, and competing risks. Chapter 7, Practical Multiple Testing Methods in Clinical Trials, discusses different multiplicity issues that often arise in clinical trials, including dose-finding, coprimary, multiple-endpoint and mixed-endpoint trials. Many different testing procedures are compared, including some new powerful testing procedures. Chapter 8: Missing Data Handling in Clinical Trials, discusses practical approaches to missing data handling in SAS. Chapter 9: Special Issues and Resolutions is a collection of statistical issues and resolutions in clinical trials, including the drop-loser design with efficacy and safety, Estimation of Treatment Effect with Interim Blinded Data, and the relative advantages and disadvantages associated with choosing either the Fisher or the Barnard exact tests. Chapter 10, Controversies in Statistical Science and Applications, addresses key controversial issues in statistics from the perspectives of both “general science” and “statistical science”, and discusses how these various perspectives can be applied to resolving some of these controversies for both statistical considerations as well as a decisions or choices we make in our daily lives.

The book includes a large amount of SAS code. To reduce the burden of retyping, relevant programming code is available at www.statisticians.org

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**John Balser**, PhD, co-founder and President of Veristat, has developed the company as industry leaders in areas of clinical monitoring, data management, biostatistics and programming, medical writing, and project management. John is actively involved with clinical projects in his role as one of Veristat’s principal statistical consultants. In this role, he assists clients with clinical study design and program development based on his many years of experience in the statistical aspects of clinical research. He is often called upon to assist clients on a variety of statistical issues at meetings with regulatory agencies. Prior to founding Veristat in 1994, John served as Vice President, Biostatistics, and Data Management at Medical & Technical Research Associates, Inc. He has held positions of increasing responsibility in the biostatistics departments at various pharmaceutical companies including E.R. Squibb, Biogen, and Miles. John received his MS and PhD in Biometrics from Cornell University, and has been actively engaged in clinical biostatistics for over 25 years. John is an avid runner and has competed in the Boston Marathon.

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**Robin Bliss**, PhD joined Veristat in October, 2011 and has served as Director, Biostatistics since October, 2017. Through her experience at Veristat, Dr. Bliss has implemented complex adaptive designs across clinical trials in Phases I, II, and III as well as seamless Phase I/II and II/III trials. She has also provided strategic advice to sponsor companies, including representation of such companies at regulatory agencies, participation with scientific advisory committees, performance of simulation studies, and other consulting services. Dr. Bliss has taught conference short courses in adaptive design as well as statistical courses as a university adjunct faculty member. Prior to Veristat, Dr. Bliss held a post-doctoral fellowship position at Brigham and Women’s Hospital (Boston) in the Orthopedic and Arthritis Center for Outcomes Research. Dr. Bliss earned her PhD in Biostatistics from Boston University where her research focused on spatial and environmental statistics.
1

Overview of Drug Development

1.1 Introduction

Pharmaceutical research and biotechnology companies are “devoted to inventing medicines that allow patients to live longer, healthier, and more productive lives”.


A pharmaceutical or biopharmaceutical company is a commercial business licensed to research, develop, market and/or distribute drugs, most commonly in the context of healthcare. They are subject to a variety of laws and regulations regarding the patenting, testing and marketing of drugs, particularly prescription drugs. From its beginnings at the start of the 19th Century, the pharmaceutical industry is now one of the most successful and influential, attracting both praise and controversy. Most of today’s major pharmaceutical companies were founded in the late 19th and early 20th centuries. The origins of the Food and Drug Administration (FDA) as a federal consumer protection agency can be traced back to the passage of the Pure Food and Drug Act, which was signed into law by President Theodore Roosevelt in 1906. This law was prompted by egregious marketing practices within the industry, and prohibited the introduction of adulterated or misbranded drugs. At this time, however, there was no mandate to require evidence of efficacy or safety of drugs prior to marketing. Key discoveries of the 1920s and 1930s, such as insulin and penicillin, became mass-manufactured and distributed. Switzerland, Germany and Italy had particularly strong industries, with the UK and US following suit.

Over time, attempts were increasingly made to increase regulation and to limit financial links between pharmaceutical companies and prescribing physicians, including by the relatively new US FDA (which became known “formally” as FDA in 1930). In 1938, the Federal Food, Drug and Cosmetic Act (FDCA) was enacted, which required manufacturers to demonstrate evidence of safety prior to obtaining approval by FDA. Such calls increased in the 1960s after the thalidomide tragedy came to light, in which the use of a new tranquilizer in pregnant women caused severe birth defects. The Kefauver-Harris amendments were implemented in 1962, further strengthening the provisions of the FDCA and requiring pharmaceutical manufacturers to also demonstrate
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Evidence of efficacy to FDA prior to approval, and in 1964, the World Medical Association issued its Declaration of Helsinki, which set standards for clinical research and demanded that subjects give their informed consent before enrolling in an experiment.

The industry remained relatively small scale until the 1970s when it began to expand at a greater rate. Legislation allowing for strong patents, to cover both the process of manufacture and the specific composition of matter of the products themselves, came in to force in most first world countries. Different from traditional pharmaceuticals, most of which are “small molecule drugs” that are relatively easy to manufacture, biopharmaceuticals are “large molecule drugs” produced using biotechnology. They are usually proteins (including antibodies) or nucleic acids (DNA, RNA or antisense oligonucleotides) used for therapeutic or in vivo diagnostic purposes, and are produced by means other than direct extraction from a native (non-engineered) biological source.

By the mid-1980s, small biotechnology firms started to form, and since that time the growth of biotechnology companies has been explosive. Based on market capitalization, many biotechnology companies are now represented in the list of the top 20 most successful firms. Additionally, the majority of drugs now approved by FDA originated in biotechnology companies. Many biotech companies have also forged mutually beneficial partnerships with large pharmaceutical companies and/or have been acquired. Since the early 1990s, costs of drug development have increased dramatically, and the productivity from internal Research and Development (R&D) efforts has not followed suit. In 2004 FDA released the Drug Modernization Act – Innovation/Stagnation: Challenge and Opportunity on the Critical Path to New Medical Products. Pharmaceutical industries are clearly seeking new and more efficient ways to develop drugs such as genomic & biomarker utilization, adaptive design, targeted molecular design, and computer simulations.

In the new millennium, drug development is increasingly characterized by Globalization. According to ASPE’s estimates in 2016, prescription drug spending in the United States was about $457 billion in 2015, or 16.7 percent of overall personal health care services. Of that $457 billion, $328 billion (71.9 percent) was for retail drugs and $128 billion (28.1 percent) was for non-retail drugs.

Bringing a drug to market requires extensive and lengthy collaborations among people from dozens of disciplines. The entire development process includes multiple stages or phases: from Discovery, Preclinical, Clinical Trials, through Phase IV commitments and Marketing (Figure 1.1). Interactions with regulatory authorities usually start before starting phase I clinical trials. All clinical trial protocols (see later in this chapter) have to be approved by an Institutional Review Board (IRB) as well as the relevant competent regulatory authorities before conducting clinical trials. It is estimated that, on average, a drug takes 10 to 12 years from initial research to reach the commercialization stage. The cost of this process is estimated to be more than US $2.5 billion.
1.2 Drug Discovery

Drug discovery involves: (1) identifying and defining medical needs, i.e., an effective prophylactic or therapeutic intervention, (2) researching on disease mechanism, i.e., identifying and validating target(s) (receptors) involved in disease processes, (3) searching for lead compounds that interact with the target, and (4) optimizing the properties of the lead compounds to generate potential drug molecules.

1.2.1 Target Identification and Validation

For a drug to work, it has to interact with a disease target in the human body. In most situations, it is the proteins or receptors that drug molecules are developed to interact with to provide the therapeutic benefit. The exceptions are in cases such as antisense drugs and gene therapy, where the nucleotides and genes are targeted, respectively. When presented to the target, drug molecules can elicit reactions to switch on or switch off certain biochemical reactions. The main drug targets in the human body can be classified into three categories: enzymes, intracellular receptors, and cell surface receptors. Enzymes are biomolecules that catalyze (i.e. increase the rates of) chemical reactions. Drugs can interact with enzymes to modulate their enzymatic activities. Intracellular receptors are in the cytoplasm or nucleus. Drugs or endogenous
ligand molecules have to pass through the cell membrane (a lipid bilayer) to interact with these receptors. The molecules must be hydrophobic or coupled to a hydrophobic carrier to cross the cell membrane. Cell surface receptors are on the cell surface and have an affinity for hydrophilic binding molecules. Signals are transduced from external stimuli to the cytoplasm, and affect cellular pathways via these surface receptors. There are three main superfamilies (groups) of cell surface receptors: G-protein coupled receptors (GPCRs), ion channel receptors, and catalytic receptors using enzymatic activities.

When the action of the drug is to activate or switch on a reaction, the drug is called an ‘agonist’. On the other hand, if the drug switches off the reaction, or inhibits or blocks the binding of other agonist components onto the receptor, it is called an ‘antagonist’. When the interaction is with an enzyme, the terms ‘inducer’ and ‘inhibitor’ are used to denote drugs that activate or deactivate the enzyme. The interactions between drug molecules and targets are desired to be binding-specific: binding occurs at particular sites in the target molecule, and binding is most often reversible.

Cells communicate to coordinate the biochemical functions within the human body. If the communication system is interrupted or messages are not conveyed fully, our bodily functions can go haywire. For example, if the p53 protein is mutated, cell growth is unchecked and cancer can form. G-protein coupled receptors (GPCRs) represent possibly the most important class of target proteins for drug discovery. They are always involved in signaling from outside to inside the cell. The number of diseases that are caused by a GPCR malfunction is enormous, and therefore it is not surprising that most commonly prescribed medicines act on a CPCE. It is estimated that more than 30% of drugs target this receptor superfamily.

For most diseases, genetic makeup and variations determine a person’s individuality and susceptibility to diseases, trauma, pathogens and drug responses. The current method of drug discovery is to break down the disease process into the cellular and molecular levels such that more specific (fewer side effects) and effective (high therapeutic index) drugs can be discovered and manufactured to intervene or restore the cellular or molecular dysfunction. Among approximately three billion base pairs that make up the DNA module, about 30,000 – 40,000 genes (DNA segments) encode proteins. Based these genes, many thousands of proteins are produced. Common drug targets are protein or glycoprotein molecules because proteins are the ingredients for enzymes and receptors, with which drugs interact.

After a potential disease-causing target has been identified, validation is necessary to confirm the functions and effects of the target. Validations are carried out in two ways: in vitro laboratory tests, and in vivo disease models using animals. Typically, in vitro tests are cell- or tissue-based experiments. The aim is to study the biochemical functions of the target as a result of binding to potential drug ligands. Parameters such as ionic concentrations, enzyme activities, and expression profiles are studied.
For in vivo studies, animal models are set up and how the target is involved in the disease is analyzed. One such model is the mouse knockout model. It should be borne in mind, however, that there are differences between humans and animals in terms of gene expression functional characteristics and biochemical reactions. Nevertheless, in addition to in vitro tests, animal models can provide the results to further inform the biology and pathophysiology associated with human disease.

1.2.2 Irrational Approach

There are two main approaches to discovering small molecule drugs (molecular weights < 500 Da): the irrational approach, or the rational approach.

The steps in the traditional irrational approach include: (1) target identification, (2) target purification, and (3) modification of lead compound.

To find lead compounds or potential drug molecules that bind with receptors and modulate disease pathways, thousands of compounds (through natural product collection or lab-produced) are screened using high throughput screening (HTS) or ultra-HTS (UHTS?). When an interaction happens, it is referred to as 'a hit'. The so-called lead compounds are those that have shown some desired biological activities when tested against the assays. However, these activities are not optimized. Modifications to the lead compounds are necessary to improve the physicochemical, pharmacokinetic, and toxicological properties for clinical applications.

Following 'hits', the lead compounds are purified using chromatographic techniques and their chemical compositions identified via spectroscopic and chemical means. Structures may be elucidated using X-ray or nuclear magnetic resonance (NMR) methods. Protein purification is a series of processes intended to isolate a single type of protein from a complex mixture. Protein purification is an important step in the characterization of the function, structure and interactions of the protein of interest.

Further tests are carried out to evaluate the potency and specificity of the lead compounds isolated. This is usually followed up with modifications of the compounds to improve properties through synthesis of variations to the compounds via chemical processes in the laboratory and frequently with modifications to the functional groups.

The lead compounds go through processes with many iterations to keep improving and optimizing the drug interaction properties to achieve improved potency and efficacy.

Potency is an important consideration and is a measure of drug activity expressed in terms of the amount required to produce an effect of given intensity. A highly potent drug evokes a larger response at low concentrations. It is proportional to Affinity and Efficacy, as well as on-target toxicity. Affinity is the ability of the drug to bind to a receptor. Efficacy is the relationship between receptor occupancy and the ability to initiate a response at the molecular, cellular, tissue or system level. On-target toxicity, which is also referred
to as mechanism-based toxicity, relates to adverse effects that occur because of interactions of the drug with its intended target.

After all these exhaustive tests, a few candidates are selected for preclinical in vivo studies using animal disease models. Many tests based on tissue cultures or cell-based assays, as they are less costly and provide results more readily. At the end of this long process is the identification of selected drug candidates with sufficient efficacy and safety to support the initiation of human clinical trials.

1.2.3 Rational Approach

The rational approach is based on an understanding of the geometric structure of molecules/proteins to predict and define structure-activity relationships (SARs) and the knowledge to identify the genes that are involved in disease pathogenesis, or nanotechnology.

Computer aided molecular design and modeling is the central part of computational chemistry, which use 3-D structures of compounds in virtual chemical compound libraries to determine the SARs of ligand-protein receptor binding. The aim of computational chemistry is to perform virtual screening using computer-generated ligands. Libraries of virtual ligands are generated on computer based on certain building blocks or framework (scaffolds) of chemical compounds. Methods such as genetic algorithm and genetic programming can be used, which simulates the genetic evolutionary process to produce ‘generations’ of virtual compounds with new structures that have improved ability to bind the receptor protein, similar to the concept of ‘survival for the fittest’ in the biological process. See Chapter 13 for more discussion.

Combinatorial chemistry is a laboratory chemistry technique to synthesize a diverse range of compounds through methodical combinations of building block components.

The aim of antisense therapy is to identify the genes that are involved in disease pathogenesis. A strategy for antisense therapy is based on the binding of oligodeoxyribonucleotides to the double helix DNA. This stops gene expression either by restricting the unwinding of the DNA or by preventing the binding of transcription factor complexes to the gene promoter. Another strategy centers on the messenger RNA (mRNA). Oligoribonucleotides form a hybrid with the mRNA. Such a duplex formation ties up the mRNA, preventing the encode translation message from being processed to form the protein.

Although all these seem like elegant ways to stop the disease at the source, at the DNA or mRNA level, there are practical problems. First, the antisense drug has to be delivered to the cell interior, and the polar groups of oligonucleotides have problems crossing the cell membrane to enter the cytoplasm and nucleus; secondly, the oligonucleotides have to bind to the intended gene sequence through hydrogen bonding; and, thirdly, the drug should not exert toxicities or side effects as a result of the interaction. For these reasons, there have been difficulties in bringing antisense drugs to the market.
1.2.4 Biologics

Unlike the small molecule drugs (pharmaceuticals), large molecule drugs (biopharmaceuticals) are mainly protein-based and similar to natural biological compounds found in the human body or they are fragments that mimic the active part of the natural compounds. Today biopharmaceuticals discovery is largely based on examining the compounds within human body, for example, hormones or other biological response modifiers, and determining how they affect the biological process.

Pharmaceuticals are new chemical entities (NCEs) and they are produced (synthesized) in manufacturing plants using techniques based on chemical reactions of reactants. Biopharmaceuticals are made using totally different methods. These protein-based drugs are manufactured in biological systems such as living cells, producing the desired protein molecules in large reaction vessels or by extraction from animal serum.

Biopharmaceuticals are products which are derived using living organisms to produce or modify the structure and/or functioning of plants or animals with a medical or diagnostic use. Biopharmaceuticals are becoming increasingly important because they are more potent and specific, as they are similar to the proteins within the body, and hence are more effective in treating our diseases. There are three major areas in which biopharmaceuticals are used: as prophylactic (preventive, as in the case of vaccines), therapeutic (antibodies) and replacement (hormones, growth factors) therapy. Another term that is used for protein-based drugs is biologics.

Vaccines

The basis of vaccination is that administering a small quantity of a vaccine (antigen that has been treated) stimulates the immune system and causes antibodies to be secreted to react against the foreign antigen. Later in life, when we are exposed to the same antigen again, the immune system will evoke a 'memory' response and activate the defense mechanisms by generating antibodies to combat the invading antigen.

In cancer, the immune system does not recognize the changes in cancer cells. Cancer vaccines seek to mimic cancer-specific changes by using synthetic peptides to challenge the immune system. When these peptides are taken up by T cells, the immune system is activated. The T cells search for cancer cells with specific markers and proceed to kill them.

Antibodies

The human immune system is a remarkable system for combating against foreign substances that invade the body. It protects us from infections by pathogens such as viruses, bacteria, parasite and fungi. An important aspect of the immune system is the self-non-self recognition function, by means of markers present on a protein called the major histocompatibility complex (MHC). Substances without such markers are discerned and targeted for destruction (Ng, 2005).
When this aspect of the immune system is not regulated properly, this gives rise to autoimmune diseases such as rheumatoid arthritis, diabetes, and multiple sclerosis. However, mistakes can happen occasionally when the immune system responds to the environment, leading to allergies, as in the case of asthma and hay fever.

B cells are produced by the bone marrow. In response to activation of CD4$^+$ T helper cells, B cells proliferate and produce antibodies. The antibodies produced by B cells circulate in the bloodstream and bind to antigens. When this happens, other cells are in turn activated to destroy the antigens.

T-cells are lymphocytes produced by the thymus gland. CD4$^+$ (CD positive, helper cells) and CD8$^+$ (CD positive also called T killer, or suppressor cells) are the two types of T cells involved in immune response. When the antigen-presenting cells (APCs) present the antigens to CD4$^+$ helper T cells, the secretory function is activated and growth factors such as cytokines are secreted to signal the proliferation of CD8$^+$ killer T cells and B cells. When the CD8$^+$ cells are activated by the APCs, the CD8$^+$ killer T cells directly kill those cells expressing the antigens. Activated B cells produce antibodies, as described above (Ng, 2005).

**Cytokines and Hormones Therapies**

Cytokines are produced mainly by leukocytes (white blood cells). They are potent polypeptide molecules that regulate the immune and inflammation functions, as well as hemopoiesis (production of blood cells) and wound healing.

Hormones are intercellular messengers. Hormones maintain homeostasis — the balance of biological activities in the body; for example, insulin controls blood glucose level, epinephrine and norepinephrine mediate response to external environment, and growth hormone promotes normal healthy growth and development.

Diabetes mellitus occurs when the human body does not produce enough insulin. Production of insulin is triggered when there is a rise in blood sugar, for example after a meal. Most of our body cells have insulin receptors which bind to the insulin secreted. When the insulin binds to the receptor other receptors on the cell are activated to absorb sugar (glucose) from the bloodstream into the cell.

When there is insufficient insulin to bind to receptors the cells are starved because sugar cannot reach the interior to provide energy for vital biological processes. Patients with insulin-dependent diabetes mellitus (IDDM) become unwell when this happens. They depend on insulin injection for survival.

**Gene Therapies**

Gene therapy is the technology involves the transfer of normal functional genes to replace genetically faulty ones so that proper control of protein expression and biochemical processes can take place. However, it is challenging to get the normal genes to the intended location using delivery tools or vehicles, called vectors (gene carriers). Whether using the in vitro or in situ method,
Drug Discovery

genes are first loaded onto the vectors, which usually are viruses. Retroviruses are the preferred candidates, as they are efficient vectors for entering humans and replicating their genes within human cells. The hurdle of gene therapy is to overcome toxicities associated with immune and inflammatory response.

**Stem Cell Therapies**

Stem cell treatment is a cell therapy that introduce new cells into damaged tissue in order to treat a disease or injury. The ability of stem cells to self-renew and give rise to subsequent generations that can differentiate offers a large potential to culture tissues that can replace diseased and damaged tissues in the body, without the risk of rejection. However, cell rejection due to the host’s immune system recognizing the cells as foreign has to be overcome to ensure stem cell therapy as a viable treatment.

Bone-marrow is the spongy tissue inside the cavities of bones. Bone marrow stem cells grow and divide into the various types of blood cells: white-blood cells (leukocytes) that fight infection, red blood cells (erythrocytes) that transport oxygen, and platelets that are the agents for clotting.

**1.2.5 NanoMedicine**

Nanomedicine is an application of nanotechnology in medical science. Nanotechnologies study features of materials on the scale of nanometers or billionths of a meter. In biology the scale of a single human hair is about 80,000 nanometers wide and a red blood cell is about 7,000 nanometers wide. Nanoscale materials often have novel properties related to their high ratio of surface area and quantum effects. The current research and development efforts on Nanomedicine are concentrated in six primary categories (The Royal Society, 2004, Tegart, 2003):

1. **Antimicrobial Properties.** Investigating nanomaterials with strong antimicrobial properties. Nanocrystalline silver, for example, is already being used for wound treatment.
2. **Biopharmaceutics.** Applying nanotechnology to drug delivery system, e.g., using nanomaterial coatings to encapsulate drugs and to serve as functional carriers. Nanomaterial encapsulation could improve the diffusion, degradation, and targeting of a drug.
3. **Implantable Materials.** Using nanomaterials to repair and replace damaged or diseased tissues. Nanomaterial implant coatings could increase the adhesion, durability, and lifespan of implants, and nanostructure scaffolds could provide a framework for improved tissue regeneration. Nanomaterial implants could be engineered for biocompatibility with the host environment to minimize side effects and the risk of rejection.
4. **Implantable Devices.** Implanting small devices to serve as sensors, fluid injection systems, drug dispensers, pumps and reservoirs, and aids to restore vision and hearing functions. Devices with nanoscale components could monitor environmental conditions, detect specific properties, and deliver appropriate physical, chemical, or pharmaceutical responses.

5. **Diagnostic Tools.** Utilizing lab-on-a-chip devices to perform DNA analysis and drug discovery research by reducing the required sample sizes and accelerating the chemical reaction process. Moreover, imaging technologies such as nanoparticle probes and miniature imaging devices as well as IV imaging agent could promote early detection and diagnosis of disease.

6. **Understanding Basic Life Processes.** Using nanoscale devices and materials to learn more about how biological systems self-assemble, self-regulate, and self-destroy at the molecular level. Insights into basic life processes will overlap multiple disciplines and could yield scientific breakthroughs.

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**1.3 Preclinical Development**

**1.3.1 Objectives of Preclinical Development**

Pre-clinical development is a stage of research that bridges between Discovery and Clinical Trials (trials in human subjects/patients). After a lead compound has been identified, it is subjected to a development process to optimize its properties. The development process includes pharmacological studies of the lead compound to influence and optimize the therapeutic index. Pre-clinical research includes in vitro (in tubes), ex vivo (in cells/tissues but outside an organism), and in vivo (in animals) tests. Preclinical research includes pharmacology and toxicology studies as well as pharmacodynamics and pharmacokinetics studies. Many iterations are carried out and at the end of this process, an optimized compound will hopefully be selected to move forward into clinical studies.

Two different animal species are typically required for toxicology studies. The most commonly used species are murine and canine, although primate and porcine are also used. The choice of species is based on which are anticipated to be most predictive of response in humans. Differences in the gut, enzyme activity, circulatory system, or other considerations make certain models more appropriate in terms of the dosage form, site of activity, or noxious metabolites. For example, rodents cannot act as models for antibiotic drugs because the resulting alteration to their intestinal flora causes significant adverse effects. Studies are sometimes performed in larger species such as dogs,
pigs and sheep which allow for testing in a similar sized model as that of a human. Some species are used for similarity in specific organs or organ system physiology. Such examples are: swine for dermatological and coronary stent studies; goats for mammary implant studies; dogs for gastric studies.

Drug development also extends to formulation and delivery. Most drugs that are administered to patients contain more than just the active pharmaceutical ingredients (the drug molecules that interact with the receptors or enzymes). Other chemical components are often added to improve manufacturing processing, or the stability and bioavailability of drugs. Effective delivery of drugs to target sites is an important factor to optimize efficacy and reduce side effects.

An ideal drug is potent, efficacious and specific, that is, it must have strong effects on a specific targeted biological pathway and minimal effects on all other pathways, to reduce side effects. Potency is the dose required to generate an effect. A potent drug elicits an effect at a low dose. An important concept is so-called therapeutic Index (window). The index is defined by the ratio of $TD_{50}/ED_{50}$, where $TD_{50}$ is the toxic dose for 50% of the population, and $ED_{50}$ is the effective dose for 50% of the population. A high value of the index is preferable. The lower this index, the less likely that the compound could be considered as a viable drug candidate. Another commonly used team is the so-called standard safety margin (SSM) defined as

$$SSM = \frac{LD_{1} - ED_{99}}{ED_{99}}100\%$$

where $LD_{1}$ is the lethal dose for 1% of the population, and $ED_{99}$ is the effective dose for 99% of the population. Again, a high SSM is desirable.

As stated in European Pharmaceutical Review (EPR, 2009), the journey from molecular target and early drug lead to the clinic is an arduous one with many hurdles to cross prior to developing a successful clinical candidate. The high rate of attrition of drug molecules has forced drug researchers to pay greater attention to drug metabolism and pharmacokinetics (DMPK) of lead molecules at even the earliest stages of drug discovery. Throughout the development of a successful molecule the researcher must bear in mind three important questions: will enough drug reach the target (pharmacokinetics)? What form will it arrive in (metabolism)? And what will it do when it gets there (pharmacodynamics)? These are the main questions that the DMPK scientist attempts to answer.

### 1.3.2 Pharmacokinetics

Pharmacokinetics is often studied in conjunction with pharmacodynamics. Pharmacodynamics explores what a drug does to the body, whereas pharmacokinetics explores what the body does to the drug. Specifically, pharmacokinetics is the study of drug Absorption, Distribution, Metabolism, and Excretion (ADME). Absorption is the process of a substance entering the body. Distribution is the dispersion or dissemination of substances throughout the
fluids and tissues of the body. Metabolism is the irreversible transformation of parent compounds into daughter metabolites. Excretion is the elimination of the substances from the body. In rare cases, some drugs irreversibly accumulate in a tissue in the body.

**Drug administration (Absorption)**

Pharmacokinetic properties of drugs may be affected by elements such as the site of administration and the rate of drug administration. There are several ways to administer a drug such as oral and intravenous. With intravenous administration, a drug is injected directly into the bloodstream, oral administration requires the drug to be absorbed through the gastrointestinal tract before it can enter the bloodstream for distribution to target sites and metabolism may precede the distribution to the site of action.

The oral route is the most common way of administering a drug. For a drug to be absorbed into the bloodstream, it has to be soluble in the fluids of our gastrointestinal tract. Drugs are often formulated with excipients (components other than the active drug) to improve manufacturing and dissolution processes. The gastrointestinal tract is lined with epithelial cells, and drugs have to cross the cell membrane. In the stomach with low pH, drugs that are weak acids are absorbed faster. In the intestine, where pH is high, weak basic drugs are absorbed preferentially.

When a drug is injected (intravenous administration), the entire dose can be considered as being available in the bloodstream to be distributed to the target site. Hence, the dosage can be controlled, unlike with other routes of administration where the bioavailability of the drug is difficult to predict because of complex diffusion processes. Intravenous injection is the normal route for administration of protein-based drugs, as they are likely to be destroyed when taken orally because of the pH conditions in the gastrointestinal tract and/or too large to be effectively absorbed. The onset of drug action with intravenous injection is quick, therefore it is especially useful for emergency cases, but also potentially the most dangerous. Once a drug is injected, it is almost impossible to remove it.

**Distribution**

The distribution patterns of a drug from the bloodstream to various tissues depends on a number of factors such as (1) vascularity of the tissue, (2) binding of the drug to protein molecules in blood plasma, and (3) drug substance transportation types: perfusion or diffusion of the drug.

Drugs absorbed through the gastrointestinal tract pass into the hepatic portal vein, which drains into the liver. The liver metabolizes the drug, thus potentially reducing the availability of the drug for interaction with receptors. At a certain time after administration when the rate of drug absorption equals the rate of clearance, it reaches an equilibrium condition called 'steady state'. The area under the concentration curve represents the total amount of drug in the blood, which measures the bioavailability of the drug. Comparison of drug
concentrations in the bloodstream administered via intravenous injection and oral route provides information for the bioavailability of the oral drug.

Drug molecules in the blood are transported to the tissue until equilibrium is reached. The transporting speed depends on the transportation types: perfusion (fast) and diffusion (slow). Acid drugs usually bind to albumins and basic drugs to glycoproteins. When the drug bind to albumin and proteins in the blood, it becomes less available for distribution to tissues. Lastly, lipid-soluble drugs can cross the cell membrane more readily than polar drugs and move into the tissues to interact with receptors.

**Metabolism**

Most drugs are metabolized in the body, though different in extent. Metabolism changes the chemical structures and generally reduces pharmacological activity of a new molecular entity (NME) (although sometimes metabolites are active as well). The liver is the major organ for metabolizing drugs, followed by the kidneys. Some drugs also are metabolized in tissue systems.

Two types of biochemical metabolism reactions take place in the liver: (1) Phase I reactions include oxidation, reduction and hydrolysis, which transform the drugs into metabolites by means of the family enzymes, cytochrome P-450. They convert lipid-soluble drugs to more water-soluble metabolites. (2) Phase II reactions involve the addition or conjugation of subgroups, such as -OH, -NH and -SH to the drug molecules. Enzymes other than P-450 are responsible for these reactions. These reactions give rise to less lipid-soluble or more polar molecules and are excreted from the body (Ng, 2005).

**Excretion**

Drug excretion is the process of discharging medical waste matter from the blood, tissues, or organs. The common routes for drugs to excrete from the body are: kidneys, lungs, intestine and colon, and skin. The kidneys are the primary organs for clearing drugs from the body. Water-soluble drugs are usually cleared more quickly than lipid-soluble drugs. Some drugs may be re-absorbed into the intestine and colon and later passed out as solid waste.

Clearance is a measure of drug elimination from the body without identifying the mechanism or process. Clearance considers the entire body as a drug-eliminating system from which many elimination processes may occur. The clearance of a drug is given by the following expression:

\[
CL = \frac{\text{Rate of drug elimination}}{\text{Drug concentration in blood}}
\]

In general, drugs that are highly bound to plasma protein have reduced overall drug clearance. Drug elimination is governed mainly by renal and other metabolic processes in the body. When a drug is tightly bound to a protein, only the unbound drug is assumed to be metabolized — restrictively eliminated. In contrast, some drugs may be eliminated even when they are protein bound — nonrestrictively eliminated.
Albumin is a protein (molecular weight about 70k Da) synthesized in the liver and is the major component of plasma proteins responsible for reversible drug binding. In the body, albumin is distributed in the plasma and in the extracellular fluids of skin, muscle, and various other tissues. The elimination half-life of albumin is about 18 days. Albumin is responsible for maintaining the osmotic pressure of the blood and for the transport of endogenous and exogenous substances (Shargel, et al., 2005).

**PK Analysis Method**

Pharmacokinetic analysis is traditionally performed by noncompartmental or compartmental methods. Noncompartmental methods estimate the exposure to a drug by estimating the area under the curve of a concentration-time curve, whereas compartmental methods estimate the concentration-time curve using kinetic models.

Noncompartmental PK analysis is highly dependent on estimation of total drug exposure. Total drug exposure is most often estimated by Area Under the Curve (AUC) methods using the trapezoidal rule. In this method, the area estimation is highly dependent on the blood/plasma sampling schedule and the closer the time points are, the closer the trapezoids are to the actual shape of the concentration-time curve. Other important PK parameters include $C_{\text{max}}$ (maximum concentration), $T_{\text{max}}$ (the time to $C_{\text{max}}$), and the half-time $T_{1/2}$ (time to $C_{\text{max}}/2$).

Compartmental PK analysis uses kinetic models to describe and predict the concentration-time curve. The advantage of compartmental to noncompartmental analysis is the ability to predict the concentration at any time. The disadvantage is that the results are model-dependent and it is often difficult to validate these models. The simplest PK compartmental model is the one-compartmental PK model with oral dose administration and first-order elimination (Figure 1.2). The most complex PK models are based on physiological information, which hopefully can more precisely model each organ PK compartment. Some typical PK parameters of interest are presented in Table 1.1.

<table>
<thead>
<tr>
<th>TABLE 1.1</th>
<th>Example of Preclinical PK Parameters.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (mg/kg)</td>
<td>20</td>
</tr>
<tr>
<td>$C_{\text{max}}$ ($\mu g/ml$)</td>
<td>99.2 ± 22.3</td>
</tr>
<tr>
<td>$T_{1/2}$ (hr)</td>
<td>0.86</td>
</tr>
<tr>
<td>AUC ($\mu g/hr/ml$)</td>
<td>14.2</td>
</tr>
<tr>
<td>Clearance (l h/kg)</td>
<td>1.42</td>
</tr>
</tbody>
</table>
1.3.3 Pharmacodynamics

Pharmacodynamics is the study of the biochemical and physiological effects of drugs on the body. Mechanisms of most drugs either mimic or inhibit normal physiological processes or inhibit pathological processes in animals. The drug actions can be classified into five main categories: depressing, stimulating, destroying cells (cytotoxic), irritation, and replacing substances.

Many drugs interact with proteins or other macromolecules (e.g., melanin and DNA) to form a so-called drug-protein complex. Most drug-protein binding is reversible. Unlike free or unbound drug, the protein-bound drug can’t easily transverse cell or possibly even capillary membranes. The drug in the form of “drug-complex” is usually pharmacologically inactive. Studies that critically evaluate drug-protein binding are usually performed in vitro using a purified protein such as albumin. The commonly used methods to determine the protein-binding are equilibrium dialysis and ultrafiltration, each of which

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**FIGURE 1.2**
Concentration Curve of Oral Administration.

---
uses a semipermeable membrane to separate the protein and protein-bound drug from the free drug.

**Kinetics of Protein Binding**

According to the occupancy theory in pharmacology, the drug effect depends on (1) binding of drug to the receptor and drug-induced activation of the receptor, and (2) propagation of this initial receptor activation into the observed pharmacological effect that is proportional to the number of receptor sites occupied by the drug.

The kinetics of reversible drug-protein binding for a protein with one simple binding site can be modeled by the law of mass action, as follows:

\[ [P] + [D] = [PD], \]  
where \([P] = \text{protein}, [D] = \text{drug}, \) and \([PD] = \text{drug-protein-complex}.\)

From (1.1), the ratio of the molar concentration of the products and the molar concentration of the reactants is a constant expressed by (assume one-binding site per protein molecule).

\[ K_a = \frac{[PD]}{[P][D]} \]  
(1.2)

The magnitude of \(K_a\) indicates the degree of drug-protein binding. To study the binding behavior of drugs, another ratio \(r\) is used, defined as:

\[ r = \frac{[PD]}{[PD] + [P]} \]  
where \([PD] + [P]\) is the total moles of protein and \([PD]\) is the moles of drug bound.

\[ r = \frac{K_a[D]}{1 + K_a[D]} \]  
(1.4)

**Pharmacodynamic Drug Interactions**

Pharmacodynamic interactions can occur when two or more drugs have mechanisms of action that result in the same physiological outcome. Most drugs are metabolized to inactive or less active metabolites by enzymes in the liver and intestine. Inhibition of this metabolism can increase the effect of the object drug and increase the chance of drug toxicity.

Pharmacodynamic interactions can be characterized into: (1) synergistic when the effect of two drugs is greater than the sum of their individual effects, (2) antagonistic when the effect of two drugs is less than the sum of their individual effects, (3) additive when the effect of two drugs is merely the sum of the effects of each, and (4) sequence-dependent when the order in which two drugs are given governs their effects.

**1.3.4 Toxicology**

Study of the toxicology of a potential drug is critical to determine the anticipated safety profile of a drug before it is given to humans in clinical trials.
Toxicological studies show the functional and morphological effects of the drug, including the mode, site and degree of action, dose relationship, sex differences, latency and progression, and reversibility of these effects.

To study the toxicity of a drug, the maximum tolerable dose and area under curve are generally established in rodents and non-rodents. There are two types of toxicity studies: single dose and repeated dose. Single dose acute toxicity testing is conducted for several purposes, including the determination of the doses selected for repeated dose studies, identification of target organs subjected to toxicity, and provision of data to be incorporated into the selection of a starting dose in human clinical trials.

The experiments are carried out on animals, usually on two mammalian species: a rodent (mouse or rat) and a non-rodent (rabbit). Two different routes of administration are generally studied; one is the intended route for human clinical trials, and the other is intravenous injection. Various characteristics of the animals are monitored, including weights, clinical signs, organ functions, biochemical parameters, and mortality. At the completion of the study, autopsies are performed to analyze the organs histopathologically, especially the targeted organ for the drug.

Repeated dose chronic toxicity studies are also generally performed on two species of animals, a rodent and non-rodent. The aim is to evaluate the longer-term effects of the drug in animals. Plasma drug concentrations are measured and pharmacokinetics analyses are performed. Vital functions studied include cardiovascular, respiratory and nervous systems. Animals are retained at the end of the study to check toxicity recovery.

Carcinogenicity studies are performed to identify the tumor-causing potential of a drug. Drugs are administered to rates or rodent continuously for months. Data for hormone levels, growth factors and tissue enzymatic activities are analyzed after the experiments.

Genotoxicity studies are to determine if the drug compound can induce mutations to genes, including: assessment of genotoxicity in a bacterial reverse mutation test, detection of chromosomal damage using in vitro method, and detection of chromosomal damage using rodent hematopoietic cells.

The aim of reproductive toxicology studies is to assess the effect of the potential drug on mammalian reproduction. All the stages, from pre-mating through conception, pregnancy and birth, to growth of the offspring, are studied on rats and/or rabbit.

The outcomes of all of the toxicity studies provide the basis for the selection of the starting dose for clinical trials in humans. The FDA Guidance — Estimating the Safe Starting Dose in Clinical Trials for Therapeutics in Adult Healthy Volunteers outlines the derivation of the maximum recommended starting dose (MRSD) for a drug to be used in humans for the first time. This MRSD is based on (1) no observed adverse effect level (NOAEL) in animals—the highest dose level that does not produce a significant increase in adverse effects, and (2) conversion of the NOAEL to human equivalent dose (HED)
using the following formula:

\[ \text{HED} = (\text{animal dose in mg/kg}) \times (\text{animal weight/human weight})^{0.33} \]

### 1.3.5 Intraspecies and Interspecies Scaling

DMPK study concerns the animal subjects, while clinical PKPD study concerns human subjects. However, the principles are the same for the two studies. Interspecies scaling is a method used in toxicokinetics and the extrapolation of therapeutic drug doses in humans from nonclinical animal drug studies. Toxicokinetics is the application of pharmacokinetics to toxicology and pharmacokinetics for interpolation and extrapolation based on anatomic, physiologic, and biochemical similarities (Shargel, et al., 2005; Mordenti and Chappell, 1989; Bonate and Howard 2000; Mahmood, 2000).

The basic assumption in interspecies scaling is that physiologic variables, such as drug concentration, clearance, heart rate, organ weight, and biochemical processes, are related to the weight or body surface area of the animal species. It is commonly assumed that all mammals use the same energy source (oxygen) and energy transport systems are similar across animal species (Hu and Hayton, 2001). The general allometric equation obtained can be written as (Shargel, et al., 2005)

\[ \theta = \beta \omega^a, \quad (1.5) \]

where \( \theta \) is the pharmacokinetic or physiologic property of interest, \( \beta \) is an allometric coefficient, \( \omega \) is the weight or surface area of the animal species, and constant \( a \) is the allometric exponent.

The allometric equation (1.5) can also be derived based on assumption about fractal structures of animals. As we discussed earlier the fractal structure leads to a power-law for a geometric or physiologic property. A list of allometric relationships for interspecies parameters is presented in Table 1.2.

### 1.4 Clinical Development

#### 1.4.1 Overview of Clinical Development

After a lead compound has successfully progressed through the preclinical testing phase, the next step is clinical trials. Clinical trials are trials conducted on human subjects in accordance with Good Clinical Practice (GCP) regulations (in the US, these regulations are governed by the FDA). Clinical development is a joined effort by different stakeholders including but not limited to clinical research scientists, study coordinators, clinical and medical monitors, clinical investigators, physicians, medical liaisons, statisticians, data management professionals, CMC professionals, regulatory affairs professionals, project managers, financial managers, sales managers, and strategic
### TABLE 1.2
Allometric Relationship for Interspecies Parameters.

<table>
<thead>
<tr>
<th>Physiologic or Pharmacokinetic Property</th>
<th>Allometric Exponent $a$</th>
<th>Allometric Coefficient $b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal O$_2$ consumption (mL/hr)</td>
<td>0.734</td>
<td>3.8</td>
</tr>
<tr>
<td>Endogenous N output (g/hr)</td>
<td>0.72</td>
<td>0.000042</td>
</tr>
<tr>
<td>O$_2$ consumption by liver slices (mL/hr)</td>
<td>0.77</td>
<td>3.3</td>
</tr>
<tr>
<td>Creatinine Clearance (mL/hr)</td>
<td>0.69</td>
<td>8.72</td>
</tr>
<tr>
<td>Methotrexate apparent volume (L/kg)</td>
<td>0.92</td>
<td>0.859</td>
</tr>
<tr>
<td>Kidney weight (g)</td>
<td>0.85</td>
<td>0.0212</td>
</tr>
<tr>
<td>Uter weight (g)</td>
<td>0.87</td>
<td>0.082</td>
</tr>
<tr>
<td>Heart weight (g)</td>
<td>0.93</td>
<td>0.0066</td>
</tr>
<tr>
<td>Stomach and intestines weight (g)</td>
<td>0.94</td>
<td>0.112</td>
</tr>
<tr>
<td>Blood weight (g)</td>
<td>0.99</td>
<td>0.055</td>
</tr>
<tr>
<td>Total volume (mL)</td>
<td>1.0</td>
<td>1.0062</td>
</tr>
<tr>
<td>Methotrexate half-life (min)</td>
<td>0.23</td>
<td>54.6</td>
</tr>
</tbody>
</table>

Source: adapted from Ritschel and Banerjee, 1986

The traditional approach is to divide the development process into stages, from phase I to phase IV trials (Figure 1.3).

However, before we design clinical trials, we have some important up-front work to do, including the clinical development plan (CDP), which is a integrated document to describe the master plan outlining the development of a compound from phase I to phase IV. It is a bird eye’s view of the plan for all the sequential trials regarding this compound, starting with verification of medical needs, which is usually done through literature review, and consulting with KOLs (key opinion leaders) in the field. The key issues to consider while developing a CDP may include the size of the target population, the feasibility of running such trials, the key inclusion/exclusion criteria to identify the target population, and major competitors/challenges. Meanwhile, the commercial and marketing groups of the company also will gather information regarding the size of the target population to inform different CDP options and return on investment (ROI) through a net present value (NPV) analysis. The company has to evaluate their core competence against their goals and will also develop risk mitigation plans. After several iterations of this process, the team has to make the decision on market position and deliver a sound CDP.

#### 1.4.2 Classical Clinical Trial Paradigm

As mentioned early, clinical trials are divided into phases chronologically. The size of the trial generally increases gradually from phase I to phase III to maximize patients safety and to advance development in a cost-effective manner.
FIGURE 1.3
Clinical Development Process.

Phase I Trial
Traditionally, Phase I trials are the first stage of testing in human subjects. Normally, a small (6-60) group of healthy volunteers will be selected with the exception of some special diseases such as oncology and HIV in which these studies may be conducted in patients with the disease under evaluation. The objectives of the phase trials are to assess/explore the safety, tolerability, pharmacokinetics, and pharmacodynamics of a drug. These trials are often conducted in an inpatient clinic, where the subject can be closely monitored. The subject who receives the drug is usually observed until a minimum of five half-lives of the drug have passed. A Phase I trial is often a dose escalation study to determine the appropriate dose for therapeutic use. If the drug is intended to be used for multiple dose, then phase I trials will usually include a single ascending dose (SAD) study followed by a multiple ascending dose (MAD) trial.

SAD studies are those in which small groups of subjects are given a single dose of the study drug while they are observed for a period of time. If there are no sufficient adverse side effects observed, and the pharmacokinetic data is in line with predicted safe values, the dose is escalated to treat a new group of subjects. This is continued until pre-calculated pharmacokinetic safety levels are reached, and/or intolerable side effects start showing up. MAD are conducted to better understand the pharmacokinetics & pharmacodynamics
associated with multiple doses of the drug. In these studies, a group of patients receives multiple low doses of the drug, whilst samples (of blood, and other fluids) are collected at various time points and analyzed to understand how the drug is processed within the body. In addition to SAD and MAD, a study for food effect may also be conducted, which is designed to investigate any differences in absorption of the drug by the body, caused by eating before the drug is given. These studies are usually run as a crossover study, with volunteers being given two identical doses of the drug on different occasions following a washout period; one while fasted, and one after being fed.

Phase II Trial

Once the initial safety of the study drug has been confirmed in Phase I trials, Phase II trials are performed on larger groups (20-300) and are designed to assess how well the drug works, as well as to continue to assess safety in a larger group of patients. In phase I, the dose range that will produce some biological effects with tolerable side-effects has been estimated. In phase II, the dose range will be further explored to identify/confirm the dose(s) that can be expected to be clinically effective with an acceptable safety profile. Therefore, Phase 2 endpoints are usually clinical endpoints instead of biomarker or PD markers.

Phase II studies are sometimes divided into Phase IIA and Phase IIB. Phase IIA is specifically designed to assess dosing requirements (how much drug should be given), whereas Phase IIB is specifically designed to study efficacy (how well the drug works at the prescribed dose(s)).

Many drug programs are discontinued in phase II if the data generated is not compelling because the next phase (phase III) is the most expensive phase of drug development in terms of both cost and time. Unless Phase II has clearly demonstrated that the drug is likely to prove to be both safe and effective in the confirmatory Phase III studies, sponsors will often choose not to make further investment in the program.

Phase III

A Phase III study is usually a randomized controlled multicenter trial including a large number of patients (300–10,000) and are aimed at being the definitive assessment of how effective and safe the drug is, in comparison with current ‘gold standard’ treatment. Because of their size and comparatively long duration, Phase III trials are the most expensive, time-consuming and difficult trials to design and run (the cost for a typical oncology trial is over $50,000 per patient). The results from Phase III trials (noting that usually two “adequate and well-controlled trials” are required) are the basis for drug approval for marketing.

Upon the completion of Phase III trials, the efficacy and safety results are presented in a so-called integrated efficacy summary (IES or ISE), which includes the analysis of all the efficacy data from all of the relevant trials conducted, and also integrated safety summary (ISS), which includes all the relevant safety data regarding the NME. These integrated results as well as
other documents are organized according to ICH guidance and submitted to
the regulatory agency for approval. In the United States, generally after a 10-
month review process, the sponsor will receive a response from FDA regarding
their NDA. The FDA response letter can be three possible types: (1) Approval
for marketing the drug, (2) Application denied, and (3) Complete response -
request for more information.

**Phase IV Trial**

After drug approval, a Phase IV trial may sometimes be required by regu-
latory agencies. A Phase IV trial is sometimes called a Post Marketing Surveil-
rance Trial. Phase IV trials often mandate additional safety surveillance (phar-
macovigilance) and ongoing technical support of a drug after it receives per-
mission to be sold. Phase IV studies may be required by regulatory authorities
because of potential long-term safety concerns or for label expansion (e.g., ex-
tend the use for pediatric population). The safety surveillance is designed to
detect any rare or long-term adverse effects over a much larger patient popula-
tion and longer period than was possible during the Phase I-III clinical trials.
Harmful effects discovered by Phase IV trials may result in a drug being no
longer sold, or restricted to certain uses.

1.4.3 **Adaptive Trial Design Paradigm**

In recent years, the cost for drug development has increased dramati-
cally, but the success rate of new drug applications (NDAs) remains low.
The Pharmaceutical industry has devoted a significant amount of effort in
innovative approaches to clinical development, including adaptive design.
An adaptive design is a clinical trial design that allows adaptations or
modifications to aspects of the trial after its initiation without undermin-
ing the validity and integrity of the trial (Chang 2007). The adaptation can
be based internal or external information to the trial.

The purposes of adaptive design trials are to increase the probability of
success, reduce the cost and the time to market, and deliver the right drug to
the right patient.

1.4.4 **New Drug Application**

According to the FDA, the regulation and control of new drugs in the United
States has been based on the New Drug Application (NDA). Since 1938, every
new drug has been the subject of an approved NDA before U.S. commercial-
ization. The NDA application is the vehicle through which drug sponsors
formally propose that the FDA approve a new pharmaceutical for sale and
marketing in the U.S. The data gathered during the animal studies and hu-
man clinical trials of an Investigational New Drug (IND) become part of the
NDA.

The goals of the NDA are to provide enough information to permit the
FDA review team to reach the following key decisions:
Clinical Development

FIGURE 1.4
A Simplified View of the NDA.

- Whether the drug is safe and effective in its proposed use(s), and whether the benefits of the drug outweigh the risks.
- Whether the drug’s proposed labeling (package insert) is appropriate, and what it should contain.
- Whether the methods used in manufacturing the drug and the controls used to maintain the drug’s quality are adequate to preserve the drug’s identity, strength, quality, and purity.

The documentation required in an NDA is supposed to tell the drug’s whole story, including what happened during the clinical tests, what the ingredients of the drug are, the results of the animal studies, how the drug behaves in the body, and how it is manufactured, processed and packaged. The following resources (Figure 1.4) provide summaries on NDA content, format, and classification, plus the NDA review process:

![Diagram of NDA Components]

- Package Insert
- Overall Summary
- Technical Section Summaries
  - Integrated Efficacy Summary
  - Integrated Safety Summary
- Technical Section
  - Chemistry, Manufacture, and Control (CMC)
  - Preclinical Pharmacology Toxicology
  - Microbiology (if appropriate)
  - Human Pharmacokinetics and Bioavailability
- Row Data
- Case Report Form
- Case Report Form Tabulation

FIGURE 1.4
A Simplified View of the NDA.
1.5 Summary

Drug development processes are divided into Drug Discovery, Preclinical, and Clinical Development. A successful development program will lead to drug approval and commercialization.

The objective of Drug Discovery is to identify and optimize new molecular entities. There are the traditional irrational approaches or the rational approaches. Pharmaceutical and biotech companies increasingly are transitioning away from irrational approaches into rational approaches thanks to the advancement in genomics, molecular and system biology, computational chemistry, and bioinformatics in general.

When a leading compound is identified and confirmed, further in vitro, ex-vivo, and in vivo tests of the NCE will be conducted in the Preclinical phase to optimize the properties. Preclinical research includes pharmacology, toxicology, pharmacokinetics, and pharmacodynamics studies. Pharmacokinetics is the study of drug absorption, distribution, metabolism, and excretion (AMDE). Pharmacodynamics is the study of the biochemical and physiological effects of drugs on the body. In layman's terms, pharmacokinetics is the study of what the body does to the drug, whereas pharmacodynamics is the study of what a drug does to the body. Toxicological studies explore the adverse functional and morphological effects of the drug, including the mode, site and degree of action, dose relationship, sex differences, latency and progression, and reversibility of these effects.

Clinical Development traditionally includes phase I to Phase IV clinical trials. Clinical trials are experiments of the test drug conducted on human subjects in accordance with Good Clinical Practice (GCP) guidances and regulations. Phase I trials are usually conducted on a small group healthy volunteers. The objectives of a phase I trial are typically to assess the safety, tolerability, pharmacokinetics, and pharmacodynamics of a drug in humans. Successful Phase I trials will lead to a further test of the NCE in a Phase II trial with an increased sample-size to further define the safety profile, preliminary efficacy, and optimal dose range, and to mitigate risks of investing an ineffective NCE in a large scale. If the Phase II results show the test drug is safe and efficacious enough to warrant for further study. Phase III trials are launched with the objective of providing definitive trial data regarding the safety and efficacy for the target indication. The size of the populations in the trials or sample-size should be sufficiently large so that there are adequate probabilities (power) to demonstrate statistical significance if the test drug in fact is effective. Successful Phase III trials lead ultimately to approval and commercialization of the drug. However, a Phase IV trial may sometimes be conducted as a requirement for the conditional regulatory approval.
Bibliography

FDA, 2005. Clinical Trial Endpoints for the Approval of NonSmall Cell Lung Cancer Drugs and Biologics, April 2005
FDA, 2017b. Multiple Endpoints in Clinical Trials Guidance for Industry (draft), Jan, 2017


Carini, C., Menon, S.M., Chang (Eds). (2014). Clinical and Statistical Considerations in Precision Medicine. CRC


FDA, Oct 2013. *Paving the Way for Personalized Medicine*, FDA’s Role in a New Era of Medical Product Development


