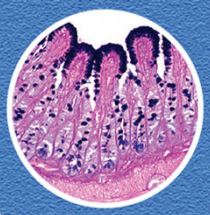
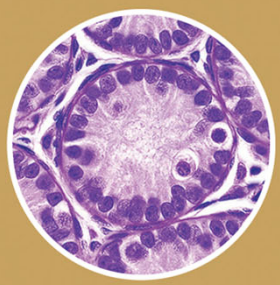
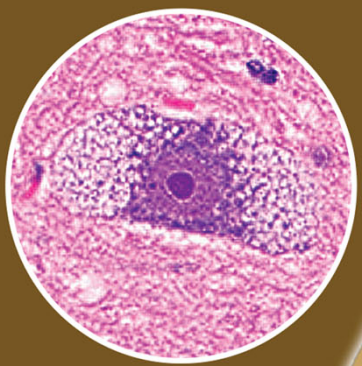
# TOXICOLOGIC PATHOLOGY

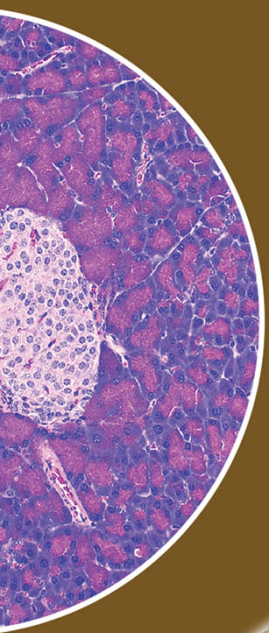
## NONCLINICAL SAFETY ASSESSMENT

Second Edition









edited by
Pritam S. Sahota
James A. Popp
Page R. Bouchard
Jerry F. Hardisty
Chirukandath Gopinath

CRC Press
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# Preface

The second edition of *Toxicologic Pathology: Nonclinical Safety Assessment* is designed to improve on the first edition published 5 years previously. Since the first edition was extremely well received and met the objective of being a functional and ready reference to the bench pathologist as well as to toxicologists, the basic structure of the book has been maintained. Specifically, the second edition includes concept chapters to provide background information important to toxicologic pathologists beyond specific diagnostic and interpretative information. However, the second edition has included two new concept chapters. The first of the new chapters addresses approaches for the evaluation of unique therapeutic modalities such as cell therapies, gene therapies, and gene expression knockdown therapies. While these still represent new developing therapeutic approaches, there has been significant experience with the therapeutic modalities in the last 5 years. The second new chapter addresses the nonclinical safety assessment of medical devices, a topic of increasing importance that was not addressed in a unique chapter in the first edition. The other concept chapters have been updated and cover important topics including overview of drug development, principles of nonclinical safety assessment, introduction to toxicologic pathology, techniques used in toxicologic pathology, clinical pathology, toxicokinetics and drug development toxicogenomics, and spontaneous lesions. The 13 organ system chapters provide the specifics related to pathologic characteristics, differential diagnosis, and interpretation of toxic responses in each organ system. These chapters are specifically important for the bench pathologist but also for the toxicologist who interacts with pathologists and function as study toxicologists and project team representatives in the drug development arena.

As in the original edition, the editors continue to understand that the quality of the book is primarily dependent on the expertise, diligence, and commitment of the authors of the respective chapters. While maintaining the basic knowledge presented in the first edition, the additions and updates contributed by the authors clearly enhance the value of the second edition. These additions and refinements will be particularly useful to readers of the book as therapeutic drug classes have continued to evolve and, in some cases, result in toxicity issues not previously appreciated. The identification and understanding of these newer toxicity issues and mechanisms may in some cases be published but in other cases are presented based on the firsthand experience of the authors.

The editors and authors are committed to continual improvement of the book for the benefit of future readers and the advancement of drug development in general. Therefore, the editors and authors solicit comments and suggestions that will result in improvement in future editions of *Toxicologic Pathology: Nonclinical Safety Assessment.*

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The editors wish to acknowledge and thank the many individuals who made valuable contributions toward the completion of this book. Their efforts not only ensured the quality of the text and photographs but also contributed to its timely completion.

We would like to thank Gregory Argentieri for his outstanding contribution as Editors' Assistant, Dropbox Administrator, and Content Liaison to CRC Press/Taylor & Francis Group, as well as his skillful contribution to the postprocessing of images, photocomposition, and photo layout. Special thanks goes to Diane Gunson and Oliver Turner for their review of the chapter figures and legends. The successful completion of this project would not have been possible without the sincere dedication of Gregory, Diane, and Oliver, and their willingness to devote considerable time and significant expertise to this book. In addition, we would like to thank Robert Stull and Mary Arnella-Venezia for spot verification of literature references as a quality control check.

The editors also wish to acknowledge those individuals who provided additional scientific review of selected chapters: Philip Bentley, Kristin Henson, William Kluwe, and Karen S. Regan. Lastly, the editors wish to acknowledge the excellent working relationship with the Taylor & Francis staff, especially Sylvester O'Gilvie, Danielle Zarfati, Hector Mojena, and Stephen Zollo, as well as Jonathan Achorn at Manila Typesetting Company, which resulted in expert advice and timely responses to their many inquiries.

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# Editors

**Pritam S. Sahota,** Global ToxPath LLC, Kennewick, Washington, has extensive experience in toxicologic pathology and drug development within the framework of nonclinical safety assessment of pharmaceuticals. He was previously Executive Director and Head of Pathology, Preclinical Safety, at Novartis Pharmaceuticals, East Hanover, New Jersey. Dr. Sahota obtained his veterinary medicine (BVSc) and veterinary pathology degrees (MSc and PhD) from Punjab Agricultural University, India. He is a Diplomate of the American Board of Toxicology.

After receiving his PhD in 1976, Dr. Sahota started working as a toxicologic pathologist at Dawson Research Corporation (DRC), Orlando, Florida, a contract research organization involved in the preclinical safety evaluation of drugs and chemicals. At DRC, he received increasing responsibility over the next 10 years. As a Scientific Director, he was responsible for the scientific aspects of pathology and toxicology at DRC. While working briefly for Dynamac Corporation, Research Triangle Park, North Carolina, Dr. Sahota conducted retrospective scientific audits of over 20 rodent carcinogenicity studies of the National Toxicology Program (NTP) and participated in discussions with the representatives of NTP, FDA, and EPA to review the results of scientific audits of over 200 NTP carcinogenicity studies. In 1987, Dr. Sahota joined Ciba-Geigy Pharmaceuticals in New Jersey as Head/Manager of pathologists in preclinical safety and was responsible for establishing pathology peer review, scheduling, and quality control systems. He continued to work primarily in this position with increasing responsibilities at Ciba-Geigy and then Novartis (after Ciba-Sandoz merger in 1997) to become director and eventually Executive Director/Head of Pathology. During this time, he also served as an international project team representative for a number of successfully marketed CNS, immunosuppression, diabetes, and cardiovascular drugs, including Diovan, which eventually became one of the 15 all-time, best-selling prescription drugs.

Dr. Sahota also held an adjunct academic appointment at the University of Medicine and Dentistry, New Jersey, for 8 years. He successfully led several global preclinical safety initiatives at Novartis, including patient centricity (patient in the lab), review of best practices in cardiotoxicity and ocular toxicity as well as evaluation of rodent carcinogenicity potential based on non-carcinogenicity data to minimize future delays in regulatory submissions.

**James A. Popp,** Stratoxon LLC, Morgantown, Pennsylvania, is widely recognized for his research and leadership contributions in toxicologic pathology and toxicology with special emphasis on nonclinical safety assessment of pharmaceuticals. He is an independent consultant at Stratoxon LLC. Dr. Popp received a doctor of veterinary medicine followed by a PhD in comparative pathology and is a Diplomate of the American College of Veterinary Pathologists. Following postdoctoral training in biochemical pathology and chemical carcinogenesis, he served on the faculty of the Division of Comparative Pathology in the College of Veterinary Medicine and Department of Pathology in the College of Medicine at the University of Florida before joining the Chemical Industry Institute of Toxicology (CIIT) shortly after the institute was founded. Over the ensuing 15 years, Dr. Popp developed and directed a productive research program in hepatotoxicity and hepatocarcinogenesis with emphasis on liver tumor promotion using stereologic approaches for assessing morphological development of tumors. During part of his tenure at CIIT, he served as a head of the Department of Experimental Pathology and Toxicology and vice president of the institute. He held several vice president positions overseeing safety assessment programs in the pharmaceutical industry for 11 years before initiating consulting activities in safety assessment at Stratoxon LLC.

Dr. Popp has served in the leadership of several professional societies including the positions of president of the Society of Toxicologic Pathology, president of the Academy of Toxicological Sciences, and president of the Society of Toxicology. Dr. Popp has been a frequent contributor to governmental toxicologic pathology and toxicology efforts including participation in numerous

pathology working groups at the National Toxicology Program (NTP). He has completed a 3-year term on the NTP Board of Scientific Counselors and the report on carcinogens subcommittee. Dr. Popp has also served as chair of NTP special workshops and served as chair of the Science Advisory Board for the FDA National Center for Toxicological Research.

**Jerry F. Hardisty,** Experimental Pathology Laboratories, Sterling, Virginia, has extensive expertise in nonclinical safety assessment of pharmaceuticals through his direct microscopic evaluation of tissues and contribution to resolution of toxicologic pathology issues related to drug development. He is a Director and Senior Staff Pathologist at Experimental Pathology Laboratories, Inc. (EPL). He graduated from Iowa State University College of Veterinary Medicine and received his pathology training in the US Army Preceptorship Program. He has been a Diplomate of the American College of Veterinary Pathologists since 1976.

Dr. Hardisty is an adjunct assistant professor with the North Carolina State University College of Veterinary Medicine. He has worked closely with the NCI/NTP Carcinogenesis Testing Program for over 25 years. He has participated in the publication and presentation of significant results of the NCI/NTP Pathology Quality Assessment Program and of several specific carcinogenesis bioassay tests. He has coauthored several publications in experimental pathology, pathology quality assessment, and pathology peer review.

Dr. Hardisty has served on the editorial board for Toxicologic Sciences, Toxicologic Pathology, and Experimental and Toxicologic Pathology. He specializes in the conduct of Pathology Peer Review of subchronic and carcinogenicity nonclinical toxicology studies. Dr. Hardisty also organizes and chairs pathology working groups and scientific advisory panels in the United States, Japan, and Europe. He is active in the Society of Toxicologic Pathologists (STP) as a member of the Executive Committee, Standard Systematized Nomenclature and Diagnostic Criteria Committee (SSNDC), liaison with the American College of Toxicology, and as president (2001–2002). He has also served as the chair of the STP nominating and fundraising committees. He is a member of the International Academy of Toxicologic Pathologists (IATP) and served as the North American director of the IATP.

**Chirukandath Gopinath,** Alconbury, Cambridgeshire, UK, has expertise in toxicologic pathology related to safety assessment of pharmaceuticals based on a distinguished career as a bench pathologist, supervisor of other toxicologic pathologists, and author of publications relevant to drug development. He is an independent consultant in toxicological pathology in the United Kingdom. He has worked as director of pathology at Huntingdon Research Center, Cambridgeshire, UK. His other work positions include head of pathology at Organon International, the Netherlands; lecturer at the Department of Veterinary Pathology, University of Liverpool, UK; veterinary officer, British Guyana; lecturer of veterinary pathology, University of Kerala, India; and veterinary surgeon, Kerala, India.

Dr. Gopinath received his veterinary degree from the University of Kerala, India, and did his postgraduate training at the University of Liverpool, UK, where he obtained his master's and PhD. He gained his membership with the Royal College of Pathologists, London, in 1977 and was awarded an honorary fellowship of the International Academy of Toxicological Pathologists in 2004. Dr. Gopinath has held several positions in various professional societies including past president of BSTP and IFSTP. He has published extensively in scientific journals and many books on toxicological pathology. Dr. Gopinath has organized and operated several educational modules on the topics of toxicological pathology in different countries including India, China, and Brazil.

**Page R. Bouchard,** Novartis Institutes for Biomedical Research, Cambridge, Massachusetts, is Vice President and Global Head of Preclinical Safety for Novartis Pharmaceuticals. Dr. Bouchard received his DVM from Tufts Veterinary School, residency training in anatomic pathology at Cornell School of Veterinary Medicine, and ACVP board certification in 1993. Dr. Bouchard has

worked as a toxicologic pathologist and project team toxicologist at Searle Pharmaceuticals, Head of Preclinical Safety at Genetics Institute, Head of Pathology and Investigative Safety at Wyeth Pharmaceuticals, Head of Preclinical Safety at Millennium Pharmaceuticals, Head of Preclinical R&D at Archemix Pharmaceuticals, and is currently Global Head of Preclinical Safety at Novartis. Dr. Bouchard has worked across all phases of drug discovery and development, and virtually all therapeutic modalities and disease areas. He has had the privilege of working with many companies and regulatory authorities across all regions of the world. Although the focus of his career has been managing R&D strategy and project portfolio, toxicologic pathology is the scientific lens through which he has viewed the process.

Dr. Bouchard has been an active member of the ACVP and STP and has served in a variety of volunteer roles for both organizations. He is passionate about continually advancing and modernizing the science and practice of anatomic and clinical pathology and challenging pathologists to adopt new tools and fully appreciate the use, context, and impact of their work.

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# Section I

## Concepts in Drug Development

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# 1 Overview of Drug Development

*James A. Popp and Jeffery A. Engelhardt*

## CONTENTS



## 1.1 SCIENTIFIC HISTORY

### 1.1.1 Origin of Modern Therapeutic Agents

As with all other endeavors in human progress, the identification and use of therapeutic agents to treat disease and alleviate pain and suffering have changed dramatically over time (Rubin 2007; Scheindlin 2001; Tsinopoulos and McCarthy 2002). The origin of the use of potential therapeutic agents is lost in antiquity but certainly dates back several millennia. The use of presumed therapeutic agents was described in written records from ancient Greece and Egypt, as well as other areas of the world. While a detailed history of drug discovery of pharmacologic agents is available (Sneader 2005), only a brief overview is provided here.

As might be expected, the origin of the use of various agents for therapy apparently began through trial and error, although it was probably influenced by significant levels of superstition. From ancient

times until the nineteenth century, agents of known therapeutic value were primarily, although not exclusively, "botanicals" but also included selected metals and, in some cases, various animal parts. The collection of various plant materials, including leaves and roots, provided the primary resources of the "pharmacy" for several millennia. To enhance the possibility of therapeutic success, concoctions made from several dozen sources were sometimes prepared, providing an early approach to "polypharmacy." While some materials had varying therapeutic value, the specter of toxicity stalked the use of these agents. In the highly developed world of today, the use of relatively crude botanical products in native, dried, or extracted form has been largely supplanted by much purer products made by synthetic processes. While we may at first think of botanical products as being associated with less-developed cultures, it is important to recognize that the use of botanicals has continued to this day for marketed drugs, an example being the senna-based laxatives that are currently on the market. Indeed, in the last several decades, we have seen a resurgence of the use of many crude plant-based agents with reputed therapeutic effects, which have been collectively referred to as herbal products or "nutraceuticals." It is important to note that these products do not fall under the review of the US Food and Drug Administration (FDA) in the United States as long as no therapeutic claim is made. However, anyone can visit the local drugstore or "natural products" store and find innumerable products that appear to be making therapeutic claims. These agents have generally not been subjected to modern toxicologic evaluation or controlled clinical trials and, in most cases, have not been subjected to even rudimentary toxicity testing. Toxicologic pathologists rarely see the results of these products unless they participate in government programs such as the National Toxicology Program.

The identification of the action of naturally derived agents, such as curare, which was used in poison arrows, and the subsequent study of the action of chloroform in the latter half of the nineteenth century, set the basis for the future of pharmacology. In the later part of the nineteenth century and the early decades of the twentieth century, the population of the Western world became more health conscious and interested in disease remediation. This led to the rather bleak period of "patent medicines," where numerous manufacturers produced a wide assortment of products for sale with broad disease prevention and disease curative claims. It should be noted that patent medicines during this era do not suggest that they were legally patented as occurs under current legal processes. Indeed, "patent medicines" in the earlier era were not legally patented. These products were widely marketed through extensive advertising campaigns using a print medium. Claims for cures ranged from the improvement of normal bodily functions to a cure for cancer; most impressively, or perhaps unimpressively, diverse curative capacities were claimed for a single product. During this period, there was no regulatory control over claims of either efficacy or toxicity, with the United States lagging behind several other Western countries in developing a modicum of control. As one can well imagine, the efficacy claims could not be substantiated. Based on knowledge of the ingredients, it is apparent today that these substances would have most likely not had any therapeutic value. While the use of these products undoubtedly prevented or delayed the patient's efforts to seek medical attention for real medical conditions, an equal if not greater issue was the fact that a number of these products were toxic. Multiple incidences of life-threatening toxicity occurred in adults as well as in children, either through the administration of toxic "medicines" of the day or through adulterated foods. The attention to these issues through the effort of government officials, such as Harvey Wiley, and a newly interested press resulted in the first laws addressing the safety of foods and drugs, which occurred in the first decade of the twentieth century. This effort provided a basis for a very nascent activity to evaluate safety, and later efficacy, although progress on this front was relatively slow.

Giant strides toward the scientific development of therapeutic agents occurred in the middle of the twentieth century with the advent of what some have referred to as the antibiotic era (Tsinopoulos and McCarthy 2002). Along with the identification of the first sulfa drugs, the identification of penicillin in 1928 was a landmark event resulting from an interesting combination of serendipity, careful scientific observation, and pursuit of the scientific process. The use of these new antibiotics, after the development of production techniques, resulted in a dramatic change in survival of battlefield combatants in World War II, setting the basis for wider acceptance and use

throughout the general population in the postwar period. The value of these early antibiotics stimulated the scientific quest for additional antibiotic drugs, resulting in substantial success. It should be noted that penicillin is a "natural product," that is, produced by a living organism and adding emphasis for the search for new drugs via the collection of biota from around the world. Indeed, this effort resulted in the identification of a number of useful therapeutic agents, particularly during the middle and later half of the twentieth century. While the identification of the therapeutic agent may have been derived from natural sources, many of the resultant drugs were soon being produced through chemical synthesis of the identified active agent, resulting in marketed products that were cheaper and of higher purity.

Fortunately, the development of the scientific process with the resultant increase in scientific knowledge led to the modern era of drug discovery and drug development. In addition to continuing progress in combating infectious diseases, scientific expertise was increasingly devoted to developing pharmaceuticals for noninfectious conditions. With regard to drug discovery, "targets" known or believed to be associated with specific diseases were identified as potential sites for therapeutic intervention as the result of progress in basic medical research. The attempts (in many cases, highly successful) in chemically developing molecules to directly interact with specific targets have been referred to as "rational design" of drugs in contrast to screening or serendipitous identification of drugs (Scheindlin 2001). Success in rational drug design was substantial beginning in the 1970s, one excellent example being the antihypertensive drugs. In this case, a molecule was developed to fit into the active site of the angiotensin-converting enzyme, resulting in the blockage of formation of angiotensin, thereby preventing its hypertensive effects. A second area of rational drug design was related to the specific targeting of drugs against cellular receptors in an attempt to block key steps in the pathogenic process of a specific or related set of diseases. Early progress in the development of receptor-blocking agents (receptor antagonists) occurred with the development of adrenergic receptor active agents (Rubin 2007). While receptor biology is complex owing in part to the plethora of receptor types, this approach opened the opportunity for development of numerous receptor active agents that continue to be the basis for extensive drug development efforts today. In the last several decades, increasing numbers of biologically derived (as opposed to chemically derived) compounds have been developed as therapeutic agents, again based on advanced understanding of disease processes provided through basic medical research. Despite this progress in the understanding of the basic biology of disease, it is increasingly clear that drug development is often stymied by the lack of an adequate understanding of disease pathogenesis at the cellular and molecular levels. The 1990s were recognized as the decade of neuroscience, with official designation by the US Congress as the "Decade of the Brain." Efforts during this and the subsequent decade resulted in astounding progress in neurobiology, with the results pursued in attempts to develop new therapies. While new therapies have been identified and marketed in the last several years, numerous pharmaceutical companies have had great difficulty in utilizing this knowledge to advance the treatment of neurologic-based diseases, particularly neurodegenerative diseases. Indeed, toward the end of the first decade of the twenty-first century, multiple pharmaceutical companies retrenched by reducing efforts to develop therapies for dreaded neurologic diseases such as Alzheimer's that will become more prevalent with an aging population.

Despite the enhancement of the scientific basis for drug development that has been important in the last several decades, everyone in drug development should be cognizant that serendipity still plays an important role in drug identification and development. It is not uncommon for a potential therapeutic agent to be under development for a specified therapeutic use, but for it to be finally marketed for a different indication based on observations noted during development. Several specific examples include the marketing of minoxidil to treat male baldness of specified types when the drug was originally being developed as an antihypertensive. Likewise, the development of sildenafil for erectile dysfunction resulted from observations made during development of this drug for hypertension and angina. Such coincidental observations are likely to occur in the future. Therefore, it is important that everyone in drug development, including the toxicologic pathologist, make careful

observations and give full consideration to the potential mechanisms that may be related to the observations noted during toxicity studies. Such attention may result in the serendipitous finding of a potential therapeutic use that was not previously considered.

Until the last several decades, nearly all drugs (or potential drugs) were chemicals, whether created through natural synthesis by a living object or through the expertise of a synthetic chemist. The well-known exceptions to this generalization include the early isolation and subsequent therapeutic use of insulin and several of the steroids. The last several decades have seen a dramatic increase in the successful development and production of peptides and proteins of natural origin as effective therapeutic agents (biologics). More recently, there is an increasing effort in the development of the oligodeoxynucleotide, gene- and cell-based therapy products. It is important for a toxicologic pathologist to note that the evaluation and development of these more complex agents have resulted in new and different issues to be addressed during safety evaluation of potential therapeutic agents.

The toxicologic pathologist's role in the scientific development of drugs has slowly but progressively evolved, such that the pathologist now plays a central role in drug development efforts. While pathology may or may not have been included in the rudimentary evaluation of toxicity in the early part of the twentieth century, the mandated evaluation of safety in 1938 (see discussion on page 7) set the stage for the development of modern toxicologic pathology. In the middle of the twentieth century, pathology was included in toxicity studies on a sporadic basis, generally with the evaluation of a very restricted tissue list compared with today. The advent of the National Cancer Institute's Bioassay Program in the United States and, very importantly, its successor, the National Toxicology Program, resulted in great advancements in the standardization of pathology evaluation in toxicity studies that have affected all aspects of diagnostic toxicologic pathology, including the safety evaluation of drugs. Just as importantly, the inclusion of more modern technologies into the evaluation of drug effects on organisms, tissues, and cells continues to provide the basis for current and future scientific contributions of the toxicologic pathologist.

## 1.2 REGULATORY HISTORY

### 1.2.1 Regulatory Aspects of Drug Development

The development of new medicines around the globe is highly regulated by a wide variety of governmental agencies. But three major regions set the tone for much of what follows in the rest of the world, namely, the United States, the European Union (EU), and Japan. Legislation and guidelines are shaped continuously by emerging adverse events and the evolution of science. To understand global drug development and the role that the toxicologic pathologist must play in the development of new medicinal and biopharmaceutical agents, a basic understanding of the history of the genesis of regulatory drug laws in the different regions and the basic framework of their pharmaceutical legislation is necessary.

### 1.2.2 US Food and Drug Law

The US FDA had its beginnings in 1906 with the passage of the Pure Food and Drugs Act (also see the FDA website: http://www.fda.gov). Until that time, the only federal controls on drugs in place involved the inspection of imported drugs, which started in 1848, and the production of the reliable smallpox vaccine (the Vaccine Act) in 1813. Around 1848, the United States Patent Office established a unit to conduct analyses on agricultural products, which was passed on to the Department of Agriculture in 1862 as the Bureau of Chemistry. The Chief Chemist, Dr. Harvey Washington Wiley, arrived at the Bureau of Chemistry in 1883 and changed the course of how the government handled adulteration and misbranding of food and drugs. In 1927, the Bureau of Chemistry was divided and the Food, Drug, and Insecticide Administration was established to oversee regulatory

functions. In 1930, the name was shortened to what we know today, the FDA. In 1940, the FDA was transferred from the Department of Agriculture to the Federal Security Agency, which became the Department of Health, Education, and Welfare in 1953. Though the function has passed from department to department over the years, the core public health mission of the agency has never changed.

Dr Wiley's concern regarding chemical preservatives as adulterants led to the formation of his much publicized "poison squad" lunches where volunteers consumed different quantities of food additives of questionable value to determine any ill effects. As Dr. Wiley continued to pursue the enactment of a law to protect consumers, the publication of *The Jungle* by Upton Sinclair caused a stirring public outcry for action. Finally, on June 30, 1906, President Theodore Roosevelt signed the Pure Food and Drugs Act, known simply as the Wiley Act. The act prohibited the interstate shipment of unlawful food and drugs and enforced truth in product labeling. After Dr. Wiley resigned in 1912, the bureau continued the campaign for drug regulation. It was not for another two decades that the issue regarding false claims for products would come to a head.

A new bill intended to replace the 1906 Act wandered aimlessly through Congress for 5 years until a major therapeutic disaster occurred, the result of which was to increase momentum. In 1937, a production batch of elixir sulfanilamide containing an untested solvent, propylene glycol, was released. Over 100 people died, many of them children, after consumption of the drug. The incident prompted Congress to move quickly and President Franklin Roosevelt signed the Food, Drug, and Cosmetic Act on June 25, 1938. The new law added cosmetics and medical devices to the regulatory listing and required that drugs be labeled with adequate information for safe use. Importantly, the act mandated premarketing approval for all new drugs where the manufacturer was obligated to demonstrate the safety of the drug before it could be sold. Amendments to the law occurred over the years to address regulatory issues as they arose. One of the most important amendments, the Kefauver–Harris Amendment, came about as a result of a near-therapeutic catastrophe in the United States in 1962 after the introduction of thalidomide. It is notable that the approval of thalidomide was delayed by the FDA in the early 1960s by Frances Kelsey, who had concern for the drug's safety. Thalidomide, though, was approved and marketed in approximately 20 countries and resulted in serious malformations in children. The response to crisis again changed the oversight of drug development. The new law mandated demonstration of efficacy as well as safety before a drug could be sold and instituted the concept of informed consent to be part of all clinical studies. It also went further in mandating that clinical studies must be based on animal investigations to ensure safety. Other amendments addressed the presence of pesticide residues in food, food additives, and color additives culminating in 1958 with the Delaney Clause, which banned any carcinogenic additive in foods, but did not apply to drugs. The Delaney Clause did, however, permit the use of possible carcinogens in food-producing animals as long as the residues of the product did not remain in any edible tissues. This allowed diethylstilbestrol to continue to be used in beef cattle as a growth-promoting agent. In 1962, the Good Manufacturing Practice regulations went into effect. The Good Laboratory Practice regulations were established in 1978.

The regulation of biologics has followed a similar route of maturation. The Biologics Control Act was passed in 1902 to ensure the purity and safety of vaccines and serums to prevent or treat diseases in humans after administration of tetanus-contaminated diphtheria vaccine derived from horses. The Hygienic Laboratory of the Public Health and Marine Hospital Service was the home of the regulatory group. The Hygienic Laboratory was renamed the National Institute of Health in 1930, and the National Institutes of Health (NIH) in 1948. In 1955, the Laboratory of Biologics Control was made an independent regulatory organization within the NIH after the release of a faulty polio vaccine from Cutter Laboratories. Oversight for biologics, including serum, vaccines, and blood products, was transferred from the NIH to the FDA in 1972 as the Center for Biologics Evaluation and Research (CBER). The CBER was assimilated by the Center for Drug Evaluation and Research (CDER) in 2008; however, the independent biologics review continues within the respective review divisions.

The major components of the FDA include the CDER, the Center for Veterinary Medicine, the Center for Devices and Radiological Health, the Center for Food Safety and Applied Nutrition, and the National Center for Toxicological Research (NCTR). The centers that are based in the Washington, DC, metropolitan area have regulatory responsibility and interact directly with pharmaceutical companies on specific drug development issues and drug approvals. In contrast, the NCTR, located in central Arkansas, has a primary research function to address toxicology issues that are important to the decision-making activities of the centers.

The legislation governing drug development is located in Part 21 of the Code of Federal Regulations. Specifics for the investigation of new drugs are covered in Section 312 (Investigational New Drug [IND]) and outline the necessary data to initiate human studies, including expectations for pharmacology and toxicology information. Guidance documents have been periodically issued by the FDA to help clarify the regulations and lay out the expectations of drug developers. There is a key distinction here: the regulation specifies what the law mandates, whereas a guidance or guideline describes performance that will satisfy legal requirements.

### 1.2.3 European Drug Law

The current regulatory framework in the EU arose from a harmonization of national drug laws to form the European Medicines Agency (EMA) in 1995 (see the EMEA website: http://www.ema.europa.eu/ema/). Much of the basis for the national laws stemmed from the adulteration of foodstuffs and then spread into the area of medicines. Similar to the FDA, the EMA is responsible for protecting and promoting public and animal health through the evaluation and supervision of medicines for human and veterinary use. The agency is responsible for the scientific evaluation of marketing applications for both human (Committee for Human Medicinal Products [CHMP]) and veterinary (Committee for Veterinary Medicinal Products [CVMP]) therapeutic and prophylactic agents. There are six scientific committees that carry out the functions of the EMA: the CHMP, the CVMP, the Committee for Orphan Medicinal Products, the Committee on Herbal Medicinal Products, the Pediatric Committee, and the Committee for Advanced Therapies. All the committees are composed of representatives from all EU member states and European Economic Area–European Free Trade Association states. The agency also works with a network of more than 4500 European experts that serve on the scientific committees, working parties, or scientific assessment teams.

The EMA is responsible for coordinating the existing scientific resources provided by the member states for the evaluation, supervision, and pharmacovigilance of medicinal products. The agency also provides advice relating to the evaluation of the quality, safety, and efficacy of medicinal products for human or veterinary use, in accordance with the provisions stated in the EU governing legislation. The primary pharmaceutical legislation is Regulation 2309/93 and Directives 2001/82/EC and 2001/83/EC, which lay out the requirements for the content of a marketing application and approval criteria and establish the Clinical Trials Directive, which governs the Investigative Medicinal Product Dossier (IMPD). The IMPD is used for initial data review prior to beginning human studies in the EU in the same manner that the IND is used in the United States. As with the FDA, the EMA issues guidelines, position papers, and points to consider to clarify the legislation or to provide advice to applicants.

Another key role of the EMA is to provide scientific advice and protocol assistance to drug developers [EMA/4260/2001 rev 9; EMA/821278/2015]. This centralized procedure ensures consistency of advice across applicants and provides broad involvement of internal and external European experts. It is important that the applicant knows that only the question asked will be answered. The advice is also not legally binding, but an applicant must justify any deviations in the marketing application.

### 1.2.4 Japanese Drug Law

The Japanese Pharmaceutical Affairs Law was first enacted in 1948 and revised several times between 1961 and 2005 (also see the Pharmaceuticals and Medical Devices Agency [PMDA] website: http://www.pmda.go.jp/english/index.html). The legislation provides the basic organization for regulation and guidance and the requirements for clinical trials and marketing approval. The Ministry of Health, Labor, and Welfare (MHLW) is the cabinet-level office of the Japanese parliament and is known as the "Korosho" or "Koseirodosho" in Japanese. The PMDA is the MHLW section, analogous to the FDA and EMA. The Japanese name for the PMDA is "Iyakuhin Iryokiki Sogo Kiko" or "Kiko" for short. The group relies heavily on the guidelines put forward by the International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH), which will be discussed later.

Unlike its previous structure, one team now handles a clinical candidate from the initial clinical trial stage through marketing approval. The first interaction of the Kiko team with a drug developer is at the submission of a clinical trial notification (CTN) [PMDA Notification (0307001–0307007) 2007; MHLW (No. 0331003) 2005]. This document is very similar in structure to the IMPD of the EU and IND in the United States and is an explanatory document that presents the rationale of the clinical study. The marketing application process follows a very stepwise fashion from receipt of the CTN to when a recommendation is made to the MHLW.

### 1.2.5 International Harmonization

Until the early 1990s, drug development was governed by several sets of regulations that often varied widely in requirements across the different regions of the globe. Differences in regional regulations and expectations resulted in the conduct of repetitive studies or the inclusion of more animals in dose groups than was necessary. As a result, the ICH was convened to bring the key stakeholders from the three major regions together. The six parties to the ICH represented the pharmaceutical manufacturers and regulatory authorities from the United States, the EU, and Japan. The result of these discussions was agreement on the acceptable standards and requirements for the development and registration of pharmaceutical agents in these regions.

The agreed-upon guidelines can be found on the websites of each of the central regulatory authorities or from the ICH directorate (www.ich.org). The guidance documents cover key topics in nonclinical, manufacturing, and clinical development and are the basis for the studies that are evaluated by the toxicologic pathologist. The "Safety Guidelines" section covers the broad areas of carcinogenicity testing, genotoxicity, drug exposure, single and repeated dose toxicity studies, developmental and reproductive toxicity studies, preclinical development of biotechnology-derived pharmaceuticals, safety pharmacology studies, immunotoxicity studies, preclinical evaluation of anticancer drugs, and general guidance on safety studies necessary to conduct human clinical trials and marketing authorizations. As new topics arise, the ICH Steering Committee decides whether or not a new guidance is needed. If so, it follows the same procedure that was used to develop the existing guidance documents and any subsequent revisions (see the ICH website for the most current versions of each guidance).

One additional area of harmonization was in the format and content of the marketing application called the Common Technical Document (CTD). The CTD is the dossier containing all critical and supportive information on the nonclinical, manufacturing, and clinical development of the candidate drug presented in such a way as to facilitate the assessment of the data by the reviewing health authorities. Full details of the CTD can also be found on the ICH website (www.ich.org /products/ctd.html). With respect to the nonclinical portion of the CTD, there are three main sections where the data are housed and summarized. Briefly, Module 4 (Safety) contains the individual study reports, including all individual animal data. Module 2.6 contains the textual and tabular

summaries for the individual reports, and Module 2.4 contains the integrated nonclinical overview. Each of the modules builds an integrated and interpretive data pyramid that, hopefully, facilitates the review, understanding, and assessment of the data contained in the *in vitro* and *in vivo* studies.

It is important to be aware of increasing interaction of the regulatory agencies from the various parts of the world that may not necessarily be associated with the development of the ICH Guidelines. These interactions occur through various meetings and telephone discussions among the various regulatory agencies and may be related to specific issues in drug development. Therefore, the regulatory personnel in one region of the world are more likely to be informed of the views of their peers in other agencies than may have occurred in the past. This increased interaction tends to foster a more consistent although, by no means uniform, approach to review and approval of therapeutic agents.

### 1.2.6 Current Regional Regulatory Differences

Despite the ICH process and guidelines, regional differences in requirements for drug development and market authorization still exist (Wang et al. 2010). Most countries around the globe use ICH requirements as a primary basis, but differences in timing of nonclinical studies or need for additional studies can and do occur. The toxicologic pathologist needs to be aware of these differences and determine how the pathology evaluation can aid in obviating the need for additional animal studies. For instance, local irritation of parental drug products can be assessed by the pathologist as part of the general toxicity studies by describing not only what was present but also what was not present at the injection site. This small addition can preemptively prevent a question from a regulator or a request for a specific local tolerance study. This is where awareness by the pathologist of the regulatory environment and registration expectations can reduce the number of animal studies conducted and aid the drug development timeline.

### 1.2.7 Regulatory Review Process

Even with the harmonized format, each region still has specific ways to review data. For example, the marketing application in Japan looks for more of a scientific story than other regions and typifies the regional differences in data review (Figure 1.1). In the review of the Japan New Drug Application (NDA), the linear development of the candidate drug is of the most interest. In this way, the rationale for each study must be justified by the results of previous studies; dose selection is based on previous results and not solely on dose multiples or maximum tolerated dose (MTD).

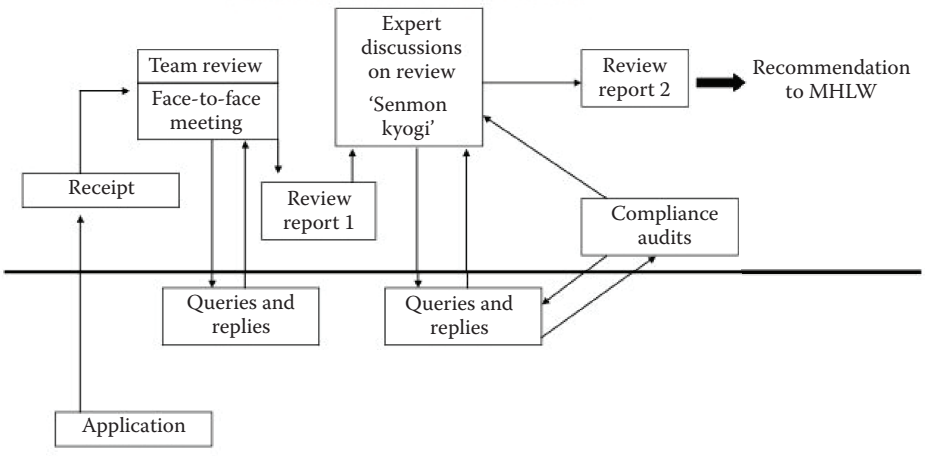


**FIGURE 1.1** Stylized review process used by the PMDA for evaluation of new drug applications in Japan.

As a result, the entire thought process in the preclinical development of the drug is laid out for the reviewer in such a way that this development continuum, from early pharmacology to carcinogenicity testing, is evident in the data presentation.

The European system, on the other hand, begins with the critical review presented by the company in the integrated nonclinical overview (Figure 1.2). This summary is also known as "Module 2.4." The "expert opinion" presented in the summary is the initial basis of review by the rapporteur and co-rapporteur for assessment of the preclinical dossier. If further detail is required to make an assessment, the more detailed study summaries are then referred to, which also contain tabular summaries of each study. Finally, the individual study reports may be used to answer more specific questions about results presented in the integrated overview.

The system followed in the United States tends to begin with the individual data from each study where the assessment is built from the bottom up (Figure 1.3). It is in this style of review that

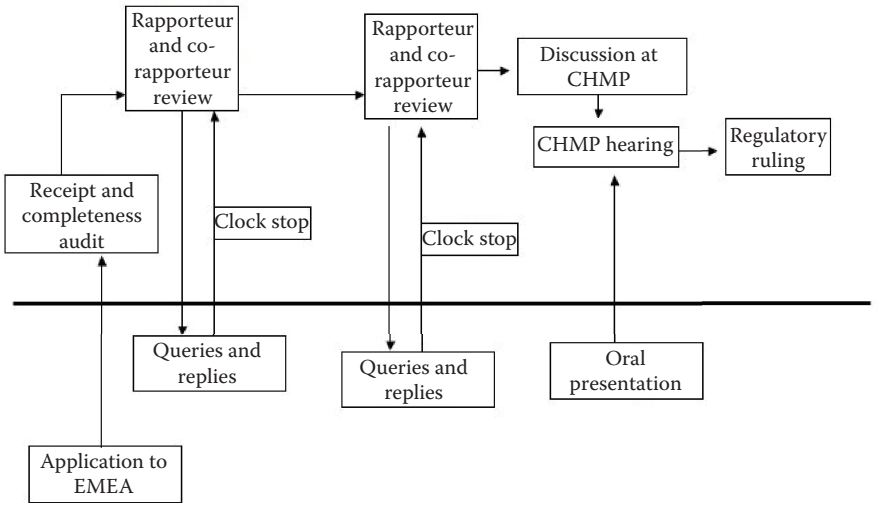


**FIGURE 1.2** Stylized review process used by the CHMP for evaluation of new marketing authorization applications in Europe.

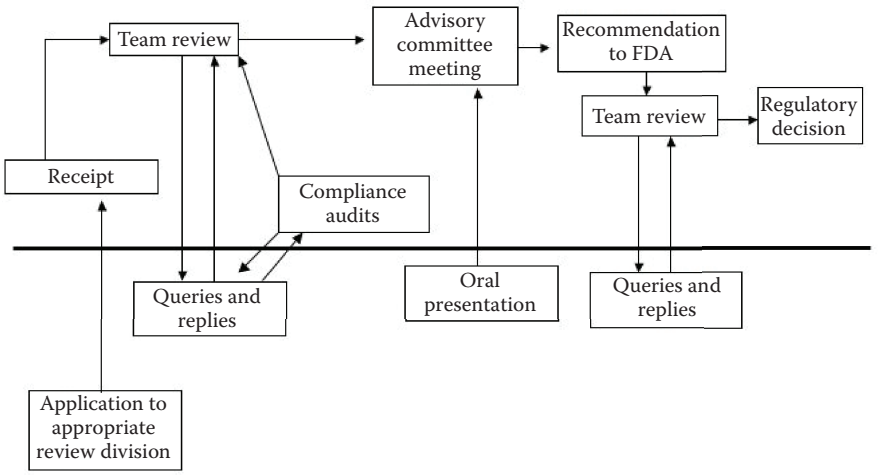


**FIGURE 1.3** Stylized review process used by the FDA review divisions for evaluation of new drug applications in the United States.

variations in nomenclature used in the pathology evaluation or clinical observations in individual studies can create confusion for the reviewer. Utilization of standardized diagnostic criteria and nomenclature, such as the Standardized System of Nomenclature and Diagnostic Criteria guidelines or the International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice (Society of Toxicologic Pathology website: https://www.toxpath.org/ssndc.asp), hopefully minimizes this confusion.

## 1.3 SEQUENCE OF SMALL-MOLECULE DRUG DEVELOPMENT

It is valuable for the toxicologic pathologist to have a basic understanding of the drug development process to understand how the pathology data are used. The drug development process is very complex with essential contributions from multiple groups with specialty scientific expertise and administrative capabilities. The following provides generalized descriptions of activities, responsibilities, and interactions that lead to successful drug development. Obviously, there is no single approach to drug development, and the experience of a toxicologic pathologist will vary depending on the company and the therapeutic area(s) of interest to the company. As an example, the drug development process tends to be shorter and more limited for anticancer drugs than for drugs intended for extended (perhaps lifetime administration) use to treat a chronic disease such as hypertension or diabetes. The development of an antibiotic requires special consideration in the drug development process for various reasons, including the short periods of intended use in most cases and the potential to alter normal bacterial flora in animals used in toxicity evaluation. While the size of the drug development program influences the processes and structure in various companies, basic differences in organizational and management philosophy are also important factors in the drug development process, irrespective of the size of the company. The interactions between areas of varying scientific expertise tend to be more fluid and informal in smaller organizations than in the very large organizations. Likewise, the breadth of responsibilities is frequently greater in very small companies in contrast to large companies.

The drug discovery and development sequence is generally divided into several major steps, each consisting of important intermediate steps that ultimately contribute to successful development. The major steps include Drug Discovery, Nonclinical Development, and Clinical Development, which is in turn subdivided into Phase I, Phase II, and Phase III (Tonkens 2005). The toxicologic pathologist may be involved during most, if not all, of the steps in drug discovery and development, although the type of involvement may be very different, depending on the stage (see Chapter 5).

### 1.3.1 Selection of Areas for Drug Development

Medical needs are too diverse for any one company to have active efforts to develop drugs for all disease processes; thus, decisions about which areas to develop an expertise and specialization must be made. This decision is based on broad corporate input and may follow many different scenarios. However, there are a few basic points that are always considered in the selection of a therapeutic area or disease process for potential drug intervention. Return on investment (ROI) potential is always considered in a decision to enter into a specific drug development effort. This requires specialized expertise to determine multiple factors. Foremost is the current or near-term availability of competitive drugs for the same indication. If a very effective medication is on the market with limited safety issues, the potential for a new drug to successfully enter this commercial area is reduced. However, the presence of a commercially successful drug certainly does not preclude future sales opportunities if a new drug can be developed that is either more efficacious or has a better safety profile, or both. Indeed, much of drug development is focused on development of better therapeutic agents. The number of drugs in development to address diseases for which there is no currently marketed therapy is rather small, although this area has been rapidly expanding in the early twenty-first century, particularly for drugs with relatively small patient populations. A drug being developed for a

therapeutic application for which a current drug exists can include a potential drug that acts through the same mechanism as the marketed drug or may act through a new or novel biologic mechanism to ameliorate the disease or disease symptoms. Both approaches can be very important to the progressive development of more effective drugs. Indeed, medical research, including the development of safe and effective drugs, generally progresses through steady improvement of existing knowledge and experience rather than on totally new or novel approaches. However, when identified, new and novel approaches and therapeutic agents can make very substantial improvements in medical therapy and, therefore, are of great interest and importance in pharmaceutical development. In fact, innovation and bringing truly transformative drugs and integrated healthcare solutions to patients is the life blood and future of the pharmaceutical industry in a world where healthcare reform and healthcare cost containment are urgent priorities.

The determination of market potential may result in the development of a required product profile for success. In other words, the commercial section of a company may determine that a drug will be useful and can be successfully marketed if a certain profile can be achieved. This profile could specify a certain reduction in symptoms, frequency of required administration, or an acceptable vs unacceptable side-effect profile. In some cases, a significantly improved side-effect profile compared with a marketed drug may be an important factor in deciding to pursue a drug development effort. The process for making the determination of need from a commercial perspective is based on input from prescribing physicians, patients (including patient advocacy groups), and the experience and expertise of the individual or group making the assessment. While the sales potential must always be considered in identifying research objectives, there are a number of equally important considerations, as noted below.

"Unmet medical need" is a very important determinant in selecting an area for current and future drug development. It should be recognized that there are various degrees of unmet medical need. Obviously, if there is no therapy marketed for a specific indication and the indication is a significant medical entity in terms of the disease impact on patients, there is a high unmet medical need. However, an unmet medical need may also occur when there is a currently marketed therapy, but there is general agreement that this therapy is far from adequate to cure or control the disease or ameliorate symptoms. As noted above, progress in human therapy is frequently incremental rather than through de novo identification of the perfect therapeutic agent entering the market first.

Scientific opportunity and tractability are important requirements for modern drug development and are determined by the scientific basis for the rational interference or alteration of a disease process or symptoms. Scientific opportunity is generally identified through progress in basic biomedical research, which continually evolves through the intricate interaction of basic science investigation and, subsequently, is tied to a proposed hypothetical clinical application. Scientific opportunities generally come from research in academia and government laboratories, most notably, the NIH in the United States, from counterpart laboratories elsewhere in the developed world, and, more recently, from expanding laboratories in emerging markets. Therefore, it is imperative that individuals in drug discovery be up to date on advances in biomedical research so that possible scientific opportunities and advances in therapeutic modalities that open up previously intractable targets are not missed. Likewise, pathologists supporting or working in drug discovery must be familiar with the basic medical advancements in the areas of interest to the company so that they may fully interface in the early assessment of toxicity. For the toxicologic pathologist, it is also important to be up to date on the scientific opportunities to assess toxicity through new or novel technologies and approaches (see Chapters 5, 6, and 9). Certainly, the advancements in the last decade in toxicogenomics and metabolomics and the role that they have in identifying safety biomarkers are a recent example of the need for the toxicologic pathologist to be knowledgeable of new approaches for assessing toxicity.

Expertise of staff is another important criterion in the selection of areas for drug development by a company. Expertise must be assured in at least two different arenas. First, the company must have staff that are scientifically and technically competent and have a superior knowledge of the

area being pursued. There is no substitute for understanding the basic science supporting a drug development initiative. The company may not have staff with basic scientific knowledge in a given area and the staff, in turn, must have the ability to retool to address a new scientific opportunity. However, in some cases, the company may need to seek additional scientific expertise if it is choosing to enter into a specialized area of drug discovery/development wherein the staff lacks the needed experience and background. The second arena regarding scientific expertise is the ability to take basic knowledge and expertise and apply them to successfully determine how they can be utilized to address a basic medical issue or disease process. While this point may seem self-evident, it is not always so easy to achieve in practice. There are many well-trained, highly skilled scientists in the world that have excellent skills to address basic biological and biomedical processes, and yet lack the ability or, in some cases, the interest to apply that expertise to create solutions to medical issues. The expertise and efforts of these basic biomedical researchers are very important for medical advancement since an understanding of fundamental biologic processes underlies the entire drug discovery and drug development efforts in the world; however, such expertise alone is inadequate to result in lifesaving therapies.

### 1.3.2 Scientific Expertise Required for Drug Development

Expertise in multiple scientific disciplines is required for effective drug development. The expertise of the toxicologist and toxicologic pathologist is an essential component of the drug development process to meet the responsibilities as outlined in Chapter 2 (“Nonclinical Safety Evaluation of Drugs”). Drug metabolism and pharmacokinetics (DMPK) expertise is essential to meet the responsibilities as outlined in Chapter 8 (“Toxicokinetics and Drug Disposition”) and is particularly important for the toxicologist and toxicologic pathologist since the interpretation and understanding of toxic effects are frequently dependent on information generated by this scientific discipline. Since these areas are covered in separate chapters, they are not discussed further here. However, the role of several other areas of scientific expertise will be mentioned briefly since they are not addressed in other chapters.

Synthetic chemistry expertise is obviously essential for the development of small-molecule drugs, but the diversity of required expertise may not be readily apparent. Chemistry expertise is essential in the early phases of the discovery process to produce the myriad of small molecules that enter into evaluation by the discovery biologists. Indeed, a very close collaboration between the discovery biologist and the chemist is essential for success. The chemist has the expertise to produce molecules that are subtle variations of a basic chemical structure but maintain the ability to interact with the pharmacologic target, whether it is an enzyme, a receptor, or a gene product. Simply stated, the drug discovery process for small molecules cannot be initiated in the laboratory until a chemist provides material for assessment, first by the discovery biologist and, subsequently, by the pharmacokineticist and toxicologist for evaluation of early toxicity assessment. The amount of material required is substantially increased as a molecule progresses in the drug discovery and development process. Larger-scale batches of drug are required for safety assessment than was required for discovery support, but this amount is very small compared with the requirement for human clinical trials. In clinical trials, the amount of drug required progressively increases as the number of humans receiving the potential drug increases from perhaps a handful of individuals receiving the drug for short periods in Phase I studies to thousands of individuals receiving the a drug for prolonged periods in Phase III studies. Obviously, the chemistry expertise and the facilities used by that expertise are very different for the support of the various phases of drug discovery and development.

Analytical chemistry expertise is also essential in drug development. Once the molecule has been produced, the material must be characterized. While purity is determined using batches of a drug in the early stages of development, such as the material used in early and all subsequent toxicity studies, progressive development of a molecule requires progressively more detailed evaluation

of the batch of material used. Identification of impurities must be made for material used in later development phases and must conform to the ICH guidelines.

Pharmaceutical science expertise is also essential. This group is frequently involved in the selection, development, and evaluation of the pharmaceutical characteristics of salt forms of a new molecular entity, which has implications for material used in nonclinical safety evaluation. Pharmaceutical science expertise is utilized to determine the stability of drug substance and drug product, particularly for the support of clinical trials and, subsequently, for the commercial form of the drug to be marketed. This group develops the final marketed product formulation, which requires careful compounding of the drug with the appropriate and acceptable excipients.

Physicians are obviously required to evaluate and oversee the administration of the potential pharmaceutical to humans at all phases of clinical development. They are ultimately responsible for the safety of the clinical trial subjects, from the volunteers involved in Phase I trials to patients receiving the drug to assess efficacy in Phase III trials. Physicians overseeing the clinical trials generally have specialty expertise in the diseases for which the drug is being developed and would frequently have specialty medical certification in the relevant area. However, the physician must also be knowledgeable about the rules, regulations, and acceptable approaches regarding the design, performance, and monitoring of clinical trials.

Project management can play various roles across different organizations, but this group generally ensures that the drug development program progresses according to earlier defined objectives and timelines. Project management leads and supports the interaction of the various project team members, which generally represent the multiple scientific disciplines that are involved in the development process at any given stage of development, including nonclinical safety.

### 1.3.3 Stages of Drug Development

Drug development is typically divided into three steps or areas: discovery, nonclinical development, and clinical development. While this categorization is still useful to gain a basic understanding of the various areas of drug development, it is also misleading for modern drug development where the three areas are, or should be, very interactive and integrated rather than being viewed as separate steps, as was common in the past. For example, the results of early toxicity studies may result in modification of the molecular structure in the discovery area. Likewise, specific adverse event findings, such as elevated serum ALT, in a clinical study may result in assessment of liver toxicity in specially designed toxicity studies. For the purpose of the present discussion, the three areas of drug development are maintained, but their interrelationship will be stressed. In addition, it is important to recognize that the division of these activities across administrative departments may be very different from one company to another.

### 1.3.4 Drug Discovery

Once a decision has been made to pursue a therapeutic indication and a target has been identified in the disease process, the drug discovery activities start in earnest in the laboratory. While there are no standardized steps taken depending on the target selected and the approaches preferred by the specific company, the following would not be unusual. The discovery support chemists and the discovery biologists work together to identify a molecular structure that has the potential to interact with a target. Typically, a large number of molecules are synthesized using one or several chemical structures sometimes referred to as "scaffolds." Binding assays may be used to determine the interaction with the target, as well as *in vitro* functional assays to assess the effect(s) of the molecule on the target. Once a molecular structure or a small number of molecular structures show the potential for interaction with the target, the functional activity of the molecule on the disease process is assessed typically using *in vitro* cellular-based assays followed by *in vivo* animal efficacy model(s). This is frequently a major challenge since animal models of the disease process may be limited or

incompletely correlate to the human disease process. In addition, the marker for clinically relevant action with a disease process may not be apparent. Despite these deficiencies and challenges, the *in vivo* assessment of the molecule is a very important step before proceeding further in the selection of molecules for drug development where resource requirements are much greater.

Either prior to, in conjunction with, or immediately after the evaluation of the molecule in *in vivo* efficacy models, basic pharmacokinetic characteristics of the molecule are established. *In vitro* cell systems may first be used to determine if the molecule can cross into and through intact cells. It is very important to know that the drug has been absorbed *in vivo* to interpret the presence, or lack of an effect, in the efficacy model. During this discovery step, chemical synthesis pathways provide a greater quantity of material for the next stage in development or may need to be refined to provide material of greater purity.

Early toxicity evaluation generally occurs during the discovery step in most organizations today. This is in contrast to approaches of several decades ago where toxicity evaluation rarely occurred in the discovery phase. This previous approach has been largely abandoned since a molecule, fully evaluated for pharmacologic characteristics but not for toxicity potential, frequently resulted in rapid termination due to severe toxicity in the initial toxicity studies, resulting in a substantial waste of resources. There is no standard approach for limited assessment of toxicity in discovery, with wide variation, depending on the philosophy and approaches of the individual company and previous knowledge of toxicity, and with pharmacologically related molecules or chemical scaffolding. However, *in vitro* cytotoxicity assays may be used in conjunction with assessment of target binding selectivity and specificity followed by short-term animal studies, again dependent on the preferred approach of the organization. Such studies would be more common if a previous and related molecule may have failed in toxicity evaluation after progressing into development.

Again, it is stressed that there is no uniform approach across companies as to which activities are included in discovery vs the early, nonclinical development phase. In addition, the activities included in discovery may be altered based on experience with the molecules of the chemical class or molecules that are chemically unrelated but that are designed for interaction at the same target. This lack of uniformity, based on experience, should be viewed positively since decisions are being made based on scientific knowledge and judgment, and not based on a standard "cookie cutter" approach.

### 1.3.5 Nonclinical Development

Nonclinical development begins once a molecule has been accepted or approved for entry into the drug development program. This is generally a very important decision requiring formal review, since the decision to progress a molecule from discovery to development results in a substantially increased resource requirement. Therefore, a company must frequently make priority decisions on the use of development resources since there may be multiple competing molecules available to enter into development.

Toxicology and, therefore, the toxicologic pathologist have a very central role in this step of drug development. The toxicology profile, as well as the related pharmacokinetic and an initial drug metabolism profile developed at this step, is the primary basis for progressing a molecule into humans in the first phase of clinical development. While nonclinical development must, by necessity, precede clinical assessment where humans are given the molecule, nonclinical development activities continue to support the next stage of clinical development.

For additional details on nonclinical development activities related to safety assessment as well as DMPK of small molecules, the reader is referred to Chapters 2 and 8. Chapter 3 provides details on the nonclinical safety evaluation of advanced therapies (eg., cell therapy, gene therapy, and expression knockdown therapy) while Chapter 4 provides details on the nonclinical safety evaluation of medical devices.

### 1.3.6 Clinical Development

Clinical development starts with the first administration of drug to humans and continues until the drug is submitted to regulatory agencies for approval or is removed from clinical development for any one of various reasons, but most often, owing to a lack of efficacy or an unacceptable side-effect profile. The clinical development program is divided into three phases that are relatively distinct. While these phases will be different based on disease indication, the following assumes a therapeutic drug for long-term use. The approach for an anticancer drug would be substantially different. It is also important to realize that the following phases generally consist of multiple studies and not a single one.

Phase I clinical trials will be initiated in the United States after an IND submission to the FDA, with comparable submissions preceding human studies in other parts of the world. The initial study in Phase I is frequently referred to as First-in-Human studies. Typically, this phase involves administration of the drug to small numbers of volunteers, frequently several dozen, in a controlled setting where the effects can be readily monitored under appropriate medical supervision. The objective of Phase I is to determine safety, including the identification of adverse events, obtain the first human pharmacokinetic data, and determine pharmacodynamic effects, when possible. Volunteers initially receive single or multiple administrations of the drug over a few days, although follow-up is often for longer periods to ensure safety. The dose of the drug will generally be progressively increased to obtain dose-related data on safety, pharmacokinetics, and pharmacodynamics, which provide the crucial information for selecting doses for subsequent phases of evaluation. The highest dose administered in the first segment of a Phase I study may be determined in advance of the study based on toxicity data, including the data generated by the toxicologic pathologist. This highest dose prior to study start may be exceeded, based on careful assessment of initial human data at lower doses. The availability of accepted, sensitive biomarkers for both pharmacologic activity and toxicity provides an important resource for determining the acceptable, and potentially efficacious, dose for subsequent human studies. Therefore, the role of the toxicologic pathologist in the development and validation of biomarkers in laboratory animals can have important, direct implications on the clinical development program at all three phases of clinical development. Multiple Phase I studies may be required to generate the data necessary to proceed to the next phase of clinical investigation. As noted earlier, this phase may be different, depending on the therapeutic indication, such as for anticancer drugs where Phase I consists of administration of the drug to cancer patients in place of volunteers.

Phase II clinical studies are frequently divided into two steps, designated as Phase IIa and Phase IIb. Phase IIa studies are primarily designed to determine preliminary dose range that would be most appropriate for later clinical studies and generally include a relatively small number of patients with uncomplicated forms of the disease for which the drug is being developed. In this case, more extensive pharmacokinetic, pharmacodynamic, and adverse event data are generated, usually with multiple administrations of the molecule compared with a more limited number of administrations investigated in Phase I. These data may be similar to or significantly different from the data generated in Phase I since the pharmacokinetics, pharmacodynamics, and adverse events may be modified by the disease process. Phase IIb studies, also performed in patients, are definitive dose-range response studies and generally provide the first substantial proof of efficacy, though preliminary evidence for efficacy may be noted in the Phase IIa studies. These studies are well controlled to provide critical information to support the progression to Phase III studies.

Phase III studies are definitive and provide proof of efficacy and safety in a population with the targeted disease. This patient population has fewer restrictions for entry in the study than may occur with Phase IIb studies. These studies most often are substantially larger than previous ones and would typically include hundreds to thousands of patients, with some studies having more than 10,000 subjects, though fewer than 100 patients may be necessary for certain orphan indications. The size and complexity of these studies make them very expensive, frequently in the range of several hundred millions of dollars per study. Two Phase III studies are generally required, in part to

demonstrate reproducibility of results though, under unusual circumstances, a single positive Phase III study may be accepted for marketing approval.

In addition to the basic studies in the three phases described, additional special human studies may be required for regulatory approval and are generally performed concurrent with the Phase IIb and Phase III studies. The specific studies required can be based on a variety of factors and are not uniform from one development program to the next. Drug interaction studies are, however, very important and generally, if not always, included. First, an interaction with food is very important and must be known prior to the pivotal studies. The presence or absence of food in the gastrointestinal tract can substantially alter drug absorption and thereby alter plasma drug levels and efficacy. The potential for interference of drug metabolism (drug–drug interaction) is another important aspect to be addressed, particularly for interactions with commonly used drugs, notably those that may be taken as concomitant medications with the drug under development. Special studies to address ethnic and gender sensitivities and cardiac liabilities, particularly effects on the electrocardiogram QT interval, have been increasingly required in recent years. Other specialty human studies that may be required could include evaluations in special populations, including those with renal or hepatic insufficiency, and particularly where these routes are the major excretory routes of the drug. Other special studies may focus on evaluation of potential toxicity endpoints noted or suspected in either preclinical or clinical studies.

### 1.3.7 Postmarketing

Ideally, all relevant studies would be successfully completed prior to marketing approval. However, it is not uncommon for a drug to be approved contingent on postmarketing (Phase IV) studies that generally focus on special human populations or to further address potential human adverse effects. In rare cases, toxicology studies may be required after marketing approval. However, for nonclinical studies, a postmarketing requirement most frequently pertains to the completion of carcinogenicity studies that are in progress but not completed prior to NDA submission.

### 1.3.8 Decision Process for Advancement or Termination during Drug Development

Most molecules that enter any stage of drug discovery or development do not become marketed drugs for many different reasons, though toxicity (in animals or humans) and lack of efficacy, either in preclinical discovery models or subsequently in human trials, are the major reasons for their attrition. The process for making the decision on advancement or termination of a drug from development is very different from one company to another; however, there are several basic points of which toxicologic pathologists should be aware.

As with other aspects of drug development, the process is affected by the size of the company. In small companies, the final decision is generally made by the head of research and development or the chief scientific officer. However, in large organizations, the sheer number of molecules being addressed requires evaluation of the possibility of success at other levels, as delegated by management, though those decisions may be reviewed at a higher level.

Project Teams generally have the responsibility to successfully develop a drug candidate and are generally not given the sole responsibility for determining the future fate of a given development compound. Indeed, in practice, the Project Team frequently becomes the advocate for advancing the molecule. Given this responsibility, it is not surprising to see the Project Team strongly advocating for moving a molecule forward in development because of faith in future success and a sense of ownership, both of which may lead to bias in assessment.

Project Teams are not the only potential source of advocacy. Clearly, the drug discovery team that was the origin of the molecule in question expends much effort, in some cases leading to emotional attachment to the potential drug candidate. This group has seen the very positive aspects of the molecule that allowed it to progress into clinical development but is generally less aware of

shortcomings identified further into development. In some cases, other groups will have the same attachment to a molecule if the group was instrumental in recommending the in-licensing or purchase of a molecule after due diligence efforts. In addition, it is not rare for a molecule to have an internal advocate when the basis of such advocacy is not obvious. Whether Project Teams, licensing due diligence teams, or other advocacy groups, it is always hard to accept failure when members of these teams have spent much time and given their best efforts to advancing a potential drug candidate. However, the failure of a potential drug is inherent in the world of drug development and must ultimately be accepted as part of the drug development process. All advocacies for progression of a molecule must be taken seriously and treated with respect, but ultimately should not alter the final decision on the basis of a full and comprehensive assessment of the attributes and deficiencies of a molecule, based on the information available at the time the decision is being made.

Irrespective of the person or group responsible for determining the future fate of a molecule in development, there are basic and multiple criteria that must be considered. Obviously, the newest data from nonclinical and clinical investigations must be of primary consideration. While severe toxicity with a minimal safety margin or lack of efficacy would be obvious criteria for ending development, most decisions are not that simple. Nearly every molecule that becomes a successful drug has had toxicity identified in nonclinical studies (perhaps at high multiples of exposure compared with human exposure at therapeutic doses) or in clinical studies as adverse events. Therefore, considerable judgment is required to make a decision about the importance of each type of toxicity/adverse event. On the other side, lack of efficacy may appear to be a sure predictor of imminent termination of development and usually is. However, it is not uncommon to see a molecule progress in clinical development, perhaps using a higher dose or altered clinical protocol. Again, judgment is of the essence. It is also interesting that a molecule that lacks efficacy in clinical trials for the primary indication may continue in development for another potential indication.

In addition to evaluation of the most recent data developed from company research, other factors are important in the consideration of whether to promote a given molecule. Scientific data may dampen the perceived potential that a molecule will actually be effective therapy. It is not uncommon for basic medical research to identify a mechanism of the disease process that could be altered by drug intervention but then later be disproven. Therefore, it is important to reassess whether such changes have occurred before committing to continuation of development. The commercial and competitive status of a potential drug changes continually. It is indeed very rare that a single company will be working on a new approach for drug intervention of a disease. In most cases, multiple companies will have become aware of the scientific opportunity at about the same time and will have initiated drug discovery efforts at roughly the same time. This creates a very competitive atmosphere where excellent progress may be occurring in one company while another company may be having technical problems in their program. If a molecule is slipping behind the development of competitors, particularly if there is more than one competitor, the financial value of the future drug may be seriously impaired. While this may seem reasonable and obvious, it is again not as easy as portrayed since the competitors are not sharing status information.

In summary, evaluation of a molecule at a given stage of development may result in one of several different decisions. Development could be continued along the original development plan, it could change, or it could be terminated altogether. However, other decisions, such as altering the indication, may also occur. It is important to note that drugs terminated from development are not necessarily terminated permanently. "Terminated" molecules are sometimes reconsidered at a later stage when more information is available, such as information on the basic biology of a disease process, including diseases other than the original indication. This point is more important than it first appears, particularly to those outside of the pharmaceutical industry, as there is a belief that any molecule in which development has been stopped should be made available for investigation by anyone who might request the molecule. However, this attitude is based on a lack of understanding of how frequently a molecule that is terminated from clinical development may come back with substantial commercial success at a later point in time.

### 1.3.9 Role and Responsibility of Toxicologic Pathologist in Drug Development

While very diverse scientific and administrative expertise is required to achieve successful drug development as previously noted, it is very obvious that the toxicologic pathologist has a significant and central role to play in the drug development process. The toxicologic pathologist must be astute in the identification of tissue components altered by administration of the drug to animals. However, this expertise and contribution do not fulfill the responsibility and, especially, the opportunities offered to the toxicologic pathologist. First, interpretation of the lesion is essential. This includes being able to outline potential mechanisms that have led to the development of the lesion and suggestions for approaches to support the hypothetical mechanism. This responsibility may be initially accomplished by interactions with colleagues in the safety assessment group, particularly the Study Director and, ultimately, the leadership of the safety assessment group. Interaction with the discovery group is very important, particularly when the toxicity may be related to the pharmacologic effect(s) of the drug or where the discovery group may be aware of a potential drug effect on a nontarget site causing what is referred to as an off-target effect.

Another major responsibility of the toxicologic pathologist is effective communication, an essential attribute that is often not adequately considered or practiced. Communication should never rely solely on the preparation and distribution of the pathology narrative report and summary tables but also upon interpretation of lesions relative to the biologic mechanisms and pharmacology of the development compound. The pathologist may also need to demonstrate the lesions through the use of selected photomicrographs, in some cases in conjunction with simplified diagrams. The pathologist is successful only when the other members of the drug development team, irrespective of the scientific background, can understand the effects caused by the drug and the potential implications. The lesions should be put into the context of the clinical pathology observations and basic concepts of lesion pathogenesis. Once communication has been effectively achieved with regard to the pathology findings, the toxicologic pathologist should be willing and able to participate in the discussion of future actions to address the issues identified.

## 1.4 APPROACHES TO DRUG DEVELOPMENT OF BIOTHERAPEUTICS

The development of biotherapeutics has become increasingly important over the last several decades. While there are a number of similarities between the development of small molecules and biotherapeutics, there are also a number of distinct differences. Some general differences in approach to the development of a biotherapeutic compared with small molecules should be understood and are discussed here and presented in Table 1.1. While the toxicity of a small molecule is most often,

**TABLE 1.1**
**Typical Drug Safety Packages**

| Biomolecule Development | Small Molecule Development |
|---|---|
| • Range finding studies | • Safety pharmacology |
| • 1-, 3-, 6-month studies | • Acute studies |
| • Safety pharacology | • Range finding studies |
| • Development toxicity studies | • 1-, 3-, 6-month studies |
| • Irritation/tolerance | • 1 year non-rodent |
| • Others as needed | • Genotoxicity studies |
| • Linear time: 2–2.5 years | • Carcinogenicity studies |
| | • Development toxicity studies |
| | • Route specific studies |
| | • Industrial toxicity |
| | • Linear time: 4.5–5 years |

though not always, related to the chemical structure of the parent molecule or metabolite, or a result of creating reactive metabolites, the toxicity of a biotherapeutic is more often related to the intended pharmacodynamics of the molecule than with small-molecule pharmaceuticals. The innate toxicity of small molecules allows dose selection to follow a predictable pattern to achieve an MTD. As the biotherapeutic may not establish an MTD, dose selection may be less obvious. Often, the maximum feasible dose that can be administered is used, but this results in exposures many hundreds or even a thousandfold greater than that necessary for saturation of the target receptor or to achieve a maximum pharmacologic response. As such, the ICH process specifically addresses the topic of dose selection, indicating that a multiple above the clinical exposure may be used as the highest dose in nonclinical animal studies (ref ICH S6).

Owing to the protein nature of most biotherapeutics, immunogenicity in animals is also an issue that must be addressed. It is well recognized that immunogenicity in animals is not predictive of effects in humans, but it may limit the exposure of the animals to the drug candidate and result in toxicity mediated by the anti-drug antibody (ADA) reponse. In this instance, several methods are available to increase exposure for the necessary duration of the study.

The protein base of the biotherapeutic also creates another divergence from small-molecule development. Small molecules most often are removed from the body via metabolic conversion in the liver and excretion through the bile or urine. Biologics, on the other hand, are metabolized by the patient in the same manner as an endogenous protein where it undergoes catabolism by peptidases and reincorporation of the amino acids into new proteins. Therefore, the traditional radioactive molecule studies for small molecules that examine routes of metabolism and metabolic products and distribution to host tissues are not required or expected for biotherapeutics.

For monoclonal antibodies, a tissue-binding study evaluating the potential for off-target binding and toxicity in animal and human tissues is presently required. As the monoclonal antibodies intended to be therapeutics have been optimized to bind to specific human receptors, and often have modified Fc regions as a part of their structure, these molecules often make poor reagents for what is, in essence, an immunohistochemical screen for target distribution. As such, the value of this assay is questionable when a full toxicity profile in animals can be determined to establish potential risks for patients.

Beyond these high-level differences, the number and types of toxicity studies conducted with a biotherapeutic to support clinical studies in humans and marketing authorization are also divergent from small molecules. Specifics of these differences are discussed in Chapter 2.

### 1.4.1 Approaches to Drug Development of Oligodeoxynucleotide Therapeutics

The development of DNA/RNA-based therapeutics has been an active area of investigation for over 20 years. These oligodeoxynucleotide (ODN) drugs fall under the auspices of small molecules for development rather than as biologics in spite of their chemical structure. The two most advanced classes are the single-stranded antisense oligonucleotides (ASOs) and the double-stranded silencing RNAs (siRNAs). The overall toxicity profile of these agents does not vary as much as with small molecules, though. More often, there are common toxicities to the class related primarily to protein binding, backbone chemistry, and sequence. The phosphorothioate ASOs are regarded as the first generation of antisense drugs. Modifications to the 2′-position on ribose sugar of nucleobases has improved potency by increasing hybridization affinity with the target mRNA and improved the pharmacokinetic profile through decreasing metabolism, giving rise to the so-called second generation antisense therapeutics (Henry et al. 2008). Similar chemical modification may also be made to siRNAs to improve their stability or otherwise optimize their drug-like properties.

The toxicology evaluation for ASOs and siRNAs follows the traditional path of evaluating a range of doses in both a rodent and nonrodent species treated by the same route and duration as intended to use in clinical trials. For ASOs, consideration must be given to the potential for hybridization-dependent

toxicities (i.e., those arising from hybridization to mRNA) and hybridization-independent toxicities (i.e., those arising from the interaction of the oligonucleotide with a specific protein/receptor or resulting from accumulation). The hybridization-independent effects can be either sequence-independent, which is the case with most toxicities, or occasionally, sequence-dependent. The majority of observations in toxicology studies are related to hybridization-independent effects. Since the ASOs tend to have common charge-to-mass ratios and pharmacokinetic behavior, the toxicologic properties also tend to be similar. Thus, while there is more quantitative variation in the toxicology properties between sequences than observed in the pharmacokinetic properties, there are a number of changes that are considered common to each chemical class (Henry et al. 2008). The same characterizations also hold generally for siRNAs.

Owing to the nucleic acid nature of these drugs, protein binding plays an essential role in pharmacokinetics and also in toxicity profile. There are typically large differences in protein binding between ASOs and siRNAs. As such, some differences in the toxicity profiles would not be unexpected. Overall, the number and types of toxicity studies conducted with an antisense oligonucleotide and siRNAs to support clinical studies in humans and marketing authorization are very similar to small molecules. Specifics on these novel biotherapeutics are discussed in Chapter 3.

### 1.4.2 Approaches to Drug Development of Gene Therapy Products

Gene therapy refers to medicinal products that introduce genetic material into a patient's DNA to replace faulty or missing DNA sequences, thereby treating the underlying cause of a disease or medical condition. Guidance documents for gene therapy products have been developed by the Center for Biologics Evaluation and Research (CBER-FDA, 2013) and the EMEA (2008). Both documents discuss preclinical study considerations and specific recommendations for investigating the safety of gene therapy products as the nature of risks posed by gene therapy products can differ from those commonly seen with other types of pharmaceuticals.

Specific considerations describe not only the expectations for the biologic product but also the delivery system to be used as a majority of the biologic effects seen tend to result from the delivery system/vector particle/virus, the transgene(s)/expression vector, and the gene product(s). Most importantly, the animal model(s) chosen to evaluate preclinical safety and efficacy should provide an assessment of the expected human pharmacodynamics and duration of effects as much as possible.

As with other biologic products, the animal models should establish the scientific basis for the desired pharmacodynamic action; the biodistribution of the product, including persistence, shedding, and uptake by other cells of the vector and/or complete product; potential target organs of toxicity and sites of biologic activity; and recommendations for initial starting dose and dose escalation scheme for the clinical studies. The toxicity studies for a gene therapy product can become complicated due to the nature of the endpoints that need to be evaluated. For example, when an adenoviral vector is utilized and administered systemically, liver and/or kidney toxicity and the occurrence of inflammatory cytokine storms need to be examined. Furthermore, toxicity of the complete gene therapy product (virus; other microorganisms; vector particle; and/or delivery system plus the expression vector, including the cassette and transgene) should be examined, taking into consideration the intracellular positioning of the product (nuclear or mitochondrial) and the number of expression vector/transgene copies. The consequences of overexpression of the transgene product or immunogenicity of the product also need to be taken into consideration. Some gene-therapy products are intended to integrate into the host genome. As such, the genetic alteration could lead to activation or inactivation of neighboring host genes, resulting in a neoplastic event. Due to the unique nature of each gene therapy product and lack of broad clinical experience with this therapeutic class, early consultation with the health authorities regarding the development plan should be considered (CHMP/GTWP/125459/06).

## 1.5 TIME AND RESOURCE UTILIZATION IN DRUG DEVELOPMENT

Drug development is a high-risk and high-cost endeavor. This simple statement has broad implications for toxicologic pathologists who choose to work in the pharmaceutical industry. The high risk provides not only a level of excitement but also a level of instability for the employees in the business compared with other employment opportunities. The nature of the business provides an opportunity for those with a more entrepreneurial outlook. However, it must be recognized that the degree of stability and opportunity for entrepreneurial applications is very different from one company to another. While the large companies have been viewed as having greater employment stability compared with small companies, largely due to a greater stability of revenue, this fact has changed in the past decade as downsizing and altered career paths have affected many individuals. To provide a better understanding of the stability (or lack thereof), and to understand the opportunity for contributions by the toxicologic pathologist, a few basic facts of the economics of pharmaceutical companies must be understood. Additional details are available through multiple sources, but notably through the efforts of the Tufts Center for the Study for Drug Development (TCSDD). Their web site (http://csdd.tufts.edu) gives a full listing of publications and other publicly available information. The *Outlook 2010* report from the TCSDD provides specific, up-to-date information on various topics, including R&D efficiency, the regulatory environment, biotechnology trends, and prescription drug policy (Outlook 2010).

To those outside of the pharmaceutical industry, the public announcement of a new drug may appear to be the culmination of a rather straightforward, albeit protracted, effort by the company; however, this is far from the truth. The retention rate of molecules in various stages of development is very low. Estimates generally indicate that only 1 in 10,000 molecules that are considered in early drug discovery actually makes it to the market. While molecule attrition occurs in the drug discovery phase, significant losses occur even after a very thorough analysis of the potential of the molecule to become a drug and the probability of success that occurs before the molecule moves from discovery to drug development. In nonclinical development prior to first administration to humans, approximately one in three molecules progress from first safety and pharmacokinetic studies to human administration. The loss may occur because of unexpected safety concerns arising in the toxicology studies, most often when a toxicologic pathologist has identified the specific target-organ toxicity of concern. However, significant loss may also occur because of the unacceptable absorption, distribution, or metabolism characteristics of the molecule. It should, however, be noted that there is a serious attempt to reduce loss of molecules at the nonclinical phase of development through closer scrutiny for potential toxicity, as well as better characterization of pharmacokinetic parameters, prior to the decision to commit the major resources that are necessary in drug development.

Despite the attrition of many molecules prior to human administration, the loss of molecules in subsequent drug development is still substantial. Unacceptable characteristics of a molecule may still be identified in nonclinical studies after human administration has begun. For example, toxicities may be identified after more chronic animal treatment (e.g., 3 or 6 months) that was not noted after administration for shorter periods. Such late-appearing toxicity may occur because of the nature of the toxicity, or toxicity at a later time point may first become apparent because greater numbers of animals are generally used in chronic vs. short-term studies. Identification of reproductive and carcinogenic effects is rarely, if ever, noted early since these studies are not completed until the molecule has progressed into the intermediate or later stages of clinical development.

Since toxicities and pharmacokinetic characteristics of a molecule are not always similar among species, it is no surprise that molecules may have unexpected characteristics in humans compared with the effects in nonclinical *in vitro* and whole animal models. Only approximately 1 in 10 molecules that enter clinical development become marketed drugs (Outlook 2010). Importantly, these success rates have not budged over recent decades despite all of the advances in medicine and technology. This lack of success is due to a variety of reasons, including unanticipated safety issues, unacceptable pharmacokinetic characteristics, and lack of efficacy. Safety issues may be unique to humans. Alternatively, a molecule may be progressed into humans with knowledge of a nonclinical

toxicity at substantially higher drug exposures than anticipated in the human but greater human exposure is required for efficacy obviating the safety margin. Pharmacokinetic characteristics are a very important point of evaluation in early clinical trials where the drug plasma profile may not be acceptable owing to various reasons, including an unacceptable, short half-life in plasma. However, the greatest reason for attrition in the clinical program is a lack of efficacy despite the best of efforts during the drug discovery stage. Unfortunately, lack of efficacy cannot be determined until late Phase II or, more usually, in late Phase III after substantial expenditures during clinical evaluation.

Drug development is very expensive and is becoming more expensive with time. The TCSDD (Outlook 2015) estimated a cost of $54 million to develop a new drug in 1979. By 1991, the cost was estimated to be $231 million, and by the mid-2000s, $1 billion. In 2015, the estimate had increased to $2.6 billion per successful drug. The advent of biotherapeutics in the last several decades has also incurred comparable increases in cost estimates for each successful drug, resulting in an estimate of $1.2 billion per marketed drug by 2006. This cost is, in part, related to the resources used for unsuccessful molecules prior to the termination of development. The increasing cost with time is multifactorial and the basis of considerable disagreement. Factors that purportedly have driven the increase in cost with time include more difficult disease processes being addressed, enhanced expectations of physicians, more stringent requirements of regulatory agencies, and perhaps increased demands for greater efficacy.

Drug development time increased over several decades until the average time was over 9 years by the 1990s (Outlook 2010). This included not only the actual time for development by the company prior to submission to the regulatory agencies for consideration for marketing approval but also the time required for review by the regulatory agencies. Subsequent to the early 1990s, serious attempts have been made to reduce development time. Foremost has been the reduction in time during the approval phase when the health authorities review data for consideration of approval. This reduction followed the passage of The Prescription Drug User Fee Act of 1992 that established user fee charges to the submitting pharmaceutical company. These fees were designated to increase the staffing of the FDA to allow more rapid review, but the act also established time targets for completion of the review process. During the 1990s, many pharmaceutical companies went through "re-engineering" reviews in an attempt to identify and eliminate unnecessary steps or management practices that were hindering rapid drug development. The activities taken by the reviewing agencies and the pharmaceutical companies have generally resulted in progress in reducing the drug development time to approximately 7 years, even though clinical trials continue to increase in complexity.

Irrespective of the specific causes for the enormous expenditures required for successful drug development, the current projection of cost vs successful marketed drug is not sustainable. While costs for clinical development have more than doubled from 2005 to 2015 (Outlook 2015), the number of approved drugs each year has remained the same or decreased over this same period, although there is now an indication that the number of US drug approvals is rebounding due in part to the FDA Safety and Innovation Act. These basic economic realities are driving the current changes in the drug development arena, resulting in mergers, acquisitions, partnerships, and downsizing activities in the last decade. The toxicologic pathologist should be cognizant of these changes and monitor them in the future since they are likely to have an important impact on the toxicologic pathologist's role in drug development in the years to come.

## 1.6 FUTURE CHANGES IN DRUG DEVELOPMENT

Drug development is a continually changing and evolving process. Numerous changes have occurred in the past and are currently ongoing. Additional changes will occur in the future. While it is impossible to comprehensively predict the future, there are certain changes in drug development occurring today that are affecting the process, although the magnitude of these changes may not be universally agreed upon by the various players in the field.

"Personalized medicine" and "precision medicine" have become buzzwords in the pharmaceutical field and have extended into the lay press. In its most simplistic form, personalized medicine simply indicates that medical developments, but more specifically, drugs in the future, will be tailored to the individual with a given disease rather than be focused on use by all or at least most individuals with that disease. The need for drugs that better control a disease process in a given individual has been evident for some time as documented by the variable efficacy of a given drug within a diseased population. While such variability has long been appreciated, the basis for variability in efficacy from one individual to another was generally not clear in the past. With the extensive advancements in our understanding of genetics in the last one to two decades, the basis for the difference in response is now better appreciated for some diseases, most notably in cancer where treatment of an individual is now being determined based on the specific testing and identification of the genetic lesions that are drivers for a tumor's growth. This approach has a very positive outcome since the efficacy rate has been increased in many defined populations. However, several obstacles must be overcome before such personalized approaches will become common in drug therapy. First, the genetic basis of disease processes that might guide personalized approaches to therapy is unclear in many diseases, particularly the common chronic diseases where the genetic basis is likely to be multifactorial. Second, developing drugs for personalized medicine has a significant economic barrier. If a developed drug is appropriate for only a subset of individuals with a disease, the market is reduced, resulting in the necessity of recouping the development cost from a smaller number of treated patients, which translates into higher costs per treated patient. However, this higher cost must be considered in relation to effective treatment rather than simply the number of individuals treated. The toxicologic pathologist must be aware of and monitor this trend since there will likely be an impact on drug toxicity evaluation. Simply stated, the advent of developing drugs for defined patient populations through personalized medicine should remind the toxicologist and toxicologic pathologist of the potential for "personalized toxicity." While this term has not been used generally, the concept of personalized toxicity is well known in medicine and in drug development through the occurrence of rare, adverse events in treated patients who have generally been referred to as "idiosyncratic" events. In summary, personalized medicine approaches may drive greater concern for understanding and avoiding the idiosyncratic events in humans, thereby putting greater pressure on toxicologists and toxicologic pathologists to refine toxicity assessment approaches to identify and prevent such personalized toxicity in humans in the future.

Expanding globalization of business, including the business of research and development, has already had dramatic effects on how they operate and how responsibilities are distributed globally. The globalization trend will surely continue although the impact over the next decade is difficult to predict. The impact on the toxicologic pathologist is that responsibilities are likely to change concurrent with changes in employer expectations. While globalization in the past has been generally business—and not science—based, the rising scientific expertise around the world will likely change this situation. Whether globalization continues on the basis of business acumen or scientific skill, globalization will surely continue and will alter how scientists, including toxicologic pathologists, participate in the scientific process.

Technical advancements and advancements in the basic understanding of disease processes have provided the opportunity to develop therapeutics that could never have been predicted in the not-too-distant past. Likewise, continuing technical and scientific advancements will set the basis for new approaches and opportunities in drug development in the future, even though the specifics cannot be predicted today.

In summary, drug development is not and never has been static. The changes in the future will likely be much greater than those in the past because of the persistent increase in the rate of change. Toxicologic pathologists have a great opportunity to participate in this exciting future, but only if they remain adaptable and scientifically current.

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# 2 Nonclinical Safety Evaluation of Drugs

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## CONTENTS



## 2.1 INTRODUCTION

Drug development is often divided into three distinct areas composed of (1) drug discovery with subsequent lead optimization, (2) nonclinical drug development, and, finally, (3) testing of the potential drug in clinical trials (Figure 2.1). The transition between these areas is a continuum and forms the basis of translational research and medicine. Importantly, the development of new drugs involves the evaluation of both animal model (nonclinical) and human (clinical) safety information. Drug development is a highly regulated process in which specific regulatory agency criteria, including Good Laboratory Practice (GLP) regulations, must be followed (OECD 1998). GLPs apply to nonclinical studies conducted for the assessment of the safety of chemicals (including pharmaceuticals) to man, animals, and the environment. GLPs help assure regulatory authorities and sponsors that the data submitted are a true reflection of the results obtained during the study and can, therefore, be relied upon when making risk/safety assessments.

The regulatory authority for pharmaceutical development and marketing approval in the United States is the US Food and Drug Administration (FDA: http://www.fda.gov); in the European Union, the European Medicines Agency (EMA: http://www.ema.europa.eu); and in Japan, the Ministry of Health, Labor, and Welfare (MHLW: http://www.mhlw.go.jp/english). These three regions work together to harmonize the nonclinical safety requirements as part of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). The ICH is unique in bringing together the regulatory authorities and pharmaceutical industry to discuss scientific and technical aspects of drug registration. Since its inception in 1990, the ICH has gradually evolved to respond to the increasingly global face of drug development. The ICH's mission is to achieve greater harmonization worldwide to ensure that safe, effective, and high-quality medicines

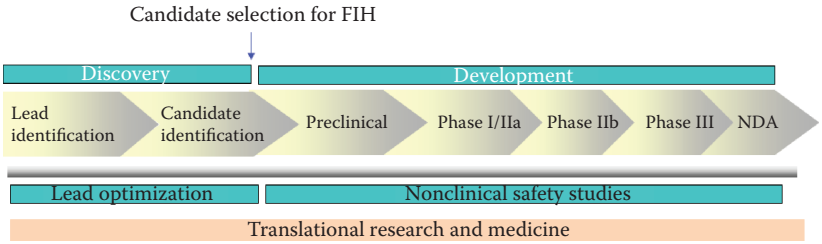


**FIGURE 2.1** Stages of drug development and the role of translational research and medicine.

are developed and registered in the most resource-efficient manner. The reader is encouraged to visit these and other regulatory websites for more detailed information (http://www.ich.org).

A major milestone in pharmaceutical development is the transition from nonclinical safety studies to the first-in-human (FIH) clinical trial. Major goals of nonclinical toxicology testing are to ensure human safety, aid in establishing a clinical start dose, and identify potential target organs of toxicity along with safety biomarkers that can be utilized in the clinical setting.

An important aspect of interpreting the results of nonclinical safety studies is assessing the risk/benefit relationship, which is based on the effects observed in the nonclinical animal studies that may be predictive of adverse events in the clinic. Traditionally, in toxicology, compound-related effects would be expected to follow a dose–response pattern in regard to incidence and severity, so a dose level and exposure could be identified where the effects do not occur or are interpreted as not being adverse. To be effective, this requires an evaluation of whether or not the effects observed in the animal model are important or even relevant to human risk assessment (Dorato 2007).

The toxicologic pathologist needs to identify, assess, and interpret the impact of the clinical pathology, biomarkers, and histopathologic observations from the nonclinical animal safety study and determine if there is a real difference between control and treated groups and if the effects observed are adverse or translatable to humans. It would not be expected that all nonclinical "adverse" effects have equal impact in assessing potential human risk. Factors to consider regarding the translation of a potential adverse event in the clinic include the possibility of patient monitoring via an assessable biomarker and if the effect is expected to be reversible. The role of the toxicologic pathologist in nonclinical safety studies includes identifying potential target organs of toxicity, determining the potential for reversibility of the toxicity, and providing data to help determine a no-observed-effect level (NOEL), the dose that did not result in any changes to the animal, or a no-observed-adverse-effect level (NOAEL).

The NOAEL is identified as the dose that produced no significant adverse effect in the animal in that specific nonclinical toxicology study. All of the parameters evaluated in a toxicology study, such as clinical signs, body weight, clinical pathology endpoints, macroscopic observations obtained at the necropsy, and histopathology data contribute to the identification of a NOAEL. The abundant and complex nature of the toxicology study data combined with the lack of precision in the scientific process often creates difficulty in identifying a NOAEL (Black 2007; Kerlin et al. 2016; Ramaiah et al. 2017). A NOAEL has been defined as the highest dose (or exposure) that does not cause biologically important or toxicologically relevant increases in the frequency or severity of adverse effects. Minimal toxic effects could still be observed at the NOAEL, but if they are deemed nonadverse to the animals under study and would not be expected to endanger human health or be precursors of serious events, they can be interpreted as nonadverse. For pharmaceutical development, the lower of the two NOAELs identified in the rodent and nonrodent repeat-dose nonclinical toxicology studies is used to help calculate the starting dose in the clinic. The identification of a NOAEL provides a basis for moving forward into clinical trials; however, it is understood that this approach is not risk free (Butler et al. 2017). The Society of Toxicologic Pathology (STP) has published recommendations to help achieve a consistent approach for interpretations of test article-related effects that may

be "adverse" and determine the NOAEL. Importantly, adverse findings in the study report should be defined in relation to effects on the test species used and also within the context of the given study (Kerlin et al. 2016).

Histopathology data are pivotal in establishing the NOAEL, and the toxicologic pathologist plays a vital role in nonclinical drug safety data generation, interpretation, and risk assessment. Toxicologic pathologists with a clinical pathology specialty are also involved in the interpretation of biomarkers of organ injury (e.g., liver enzymes) that may be identified from the animal toxicology studies (Schultze et al. 2008). Biomarkers of organ injury help enable clinical monitoring for potential adverse effects. Although nonclinical data are limited in the early stages of clinical drug development (e.g., Phase I), the animal toxicology studies must be adequately designed to characterize potential toxic effects under the conditions of the supported clinical trial per guidelines provided by the ICH (M3 [R2] 2009).

In general, the nonclinical safety assessment for marketing approval of a pharmaceutical includes general toxicology studies, development and reproductive toxicology (DART) studies, safety pharmacology studies, and genotoxicity studies. For drugs that are intended for a long duration of use or have special cause for a cancer concern, an assessment of carcinogenic potential is also conducted. Specialized nonclinical studies, such as a phototoxicity study, an immunotoxicity study, a juvenile animal toxicology study in support of a pediatric indication, an abuse liability animal study for central nervous system (CNS) drugs, or a toxicology study investigating the effects of an intended marketing of combination of drugs may also be necessary based on specific need or regulatory agency recommendation.

As this chapter addresses nonclinical safety assessment for more conventional small molecule drugs and large molecule drugs (e.g., monoclonal antibodies, bi-specific antibodies, peptides), the reader can refer to Chapters 3 and 4 of this textbook, respectively, for the nonclinical safety evaluation of advanced therapeutic modalities (e.g., cell-based nucleic acids) and medical devices. The principles outlined here for the toxicologic pathologist also pertain to these other modalities.

## 2.2 LEAD OPTIMIZATION SAFETY ASSESSMENT

Over the past decade, the importance of discovery toxicology, responsible for facilitating the selection of the best quality candidate with the fewest safety liabilities, has become a mainstream practice in the pharmaceutical industry. Years before a possible drug candidate is nominated to move forward into development and clinical trials, researchers identify and investigate a putative biologic target believed to be critical for modifying the disease of interest. Scientists then begin the process of screening a series of compounds, by use of many different technologies (e.g., computational analysis, high-throughput screens, and *in vitro* models) with the goal of identifying a short list of molecules that possess the desired biologic properties, target engagement, and specificity (Naven and Louise-May 2015). Lead optimization can be defined as the drug discovery period in which a "short list" of lead molecules are "optimized" to improve a variety of attributes, such as target specificity, potency, pharmaceutical properties, pharmacokinetic (PK) properties, and reduced safety liabilities. The goal of lead optimization is to rank-order this shorter list of candidate molecules and select the top candidate with the best profile that would then move into more formal, nonclinical drug development.

Attrition due to safety concerns in drug discovery and early drug development is not an uncommon event (Butler et al. 2017). Much of the attrition due to toxicity may be identified preclinically, as compounds entering clinical development have typically cleared many safety hurdles via extensive *in silico*, *in vitro*, and *in vivo* lead optimization screening activities (Roberts et al. 2014; Cook et al. 2014).

Prior to initiation of exploratory animal toxicology studies during lead optimization, other safety liabilities are oftentimes first assessed and screened *in silico* and *in vitro*, owing to ease, throughput, and minimal compound requirements. *In vitro* screening assays include those to detect genotoxicity, cardiac arrhythmia, and secondary (off-target) pharmacology.

Variations of the "abbreviated" or "miniaturized" or "mini-Ames" assay are commonly utilized, owing to very small compound requirements, as an early screening to detect compounds that are mutagens (i.e., cause DNA damage), since a positive result indicates that the chemical may also act as a carcinogen (Ames et al. 1973). These mutagenicity tests use multiple strains of *Salmonella typhimurium* engineered to be histidine deficient (*his*$^-$), necessitating histidine in the culture media for growth. The bacteria are plated and, over time, only those that have mutated back to *his*$^+$ survive; revertants indicate that the molecule is Ames positive. An additional genotoxicity test, often conducted during lead optimization, is an *in vitro* clastogenicity assay (Fenech 2000). A positive clastogen is an agent that can cause structural damage to the chromosome or induces aneugenic aberrations, resulting in the loss or gain of chromosomes. In general, a genotoxic molecule would not be a good candidate to move forward into drug development because of the increased risk of the molecule being a carcinogen in humans.

Cardiac arrhythmias, such as Torsades de pointes (TdP), is a specific and rare variety of ventricular tachycardia that can progress to ventricular fibrillation. Prolongation of the QT interval, observed on an electrocardiogram (ECG), precedes the onset of this serious and often life-threatening arrhythmia. A common cause of long QT syndrome is a block of the hERG (human ether-a-go-go-related gene) ion channel. The hERG ion channel is a major contributor to cardiac repolarization and several marketed drugs have been reported to block the hERG channel, resulting in acquired long QT syndrome and TdP (Redfern et al. 2002; Roden 1998). The potential for drug-induced hERG binding, therefore, is now routinely evaluated and screened in a high-throughput assay during lead optimization (Bowlby et al. 2008).

*In vitro* screening to identify other possible safety liabilities, such as "off-target" (secondary) pharmacology, is a growing and evolving field that is becoming more standard during lead optimization (Bowes et al. 2012; Papoian et al. 2015; Whitebread et al. 2016). *In vitro* profiling involves the screening of compounds against a broad range of targets that can include receptors, ion channels, enzymes, and transporters. These targets are often distinct from the intended pharmacologic target and may be the cause of unexpected toxicities observed either in the animal study or in the clinic. Various protocols are commonly available and, in general, consist of binding assays, functional assays, and enzyme assays that can provide important information on the pharmacological activity of a drug candidate in addition to possible unanticipated side effects. Current regulatory guidance does not indicate which targets of interest need to be screened via *in vitro* pharmacology profiling. However, when the data suggest possible off-target activity, the extent of that risk, under clinical conditions and drug exposures, need to be carefully assessed.

Testing strategies increasingly rely on *in vitro* data as a basis to characterize early steps or key events in toxicity at relevant dose levels in human tissues (Meek and Lipscomb 2015; Wetmore 2015). For example, assays to screen for possible interference with bile salt export pump function, which may affect human liver injury (Morgan et al. 2010; Soroka and Boyer 2014), are becoming more common, in addition to those assays used to identify mitochondrial function and impairment (Dykens and Will 2007). Results of the *in vitro* screening may prompt specific animal studies to help better understand or reduce the putative risk. Quantitative *in vitro–in vivo* extrapolations (QIVIVE) can contribute to the incorporation of these integrated testing strategies (Blaauboer 2015).

In general, nonclinical safety evaluation is starting earlier in drug discovery. Exploratory pathology (non-GLP-based studies) approaches are now utilized to determine potential toxicities that could be limiting for progression into drug development and clinical trials. Identifying both exaggerated pharmacology (i.e., on-target) and chemically-based (i.e., off-target) toxicities can contribute to the intelligent design and modification of the molecule of interest.

Genetically engineered mouse models that either overexpress the target of interest or specifically have the target of interest gene "knocked-out" (KO) are utilized in drug discovery to obtain information on the intended target that is to be investigated (Boverhof et al. 2011). Utilization of such models has become routine in the pharmaceutical industry (Bolon and Galbreath 2002; Rudman

and Durham 1999). The evaluation of KO mice in the literature, or histopathologic evaluation to phenotype the model, can identify possible safety liability concerns that may need further investigation. Phenotyping of transgenic mice serves as one approach to elucidating putative safety liabilities associated with a specific target of interest and is proving to be helpful in furthering our understanding of disease processes (Cohen 2004a).

Evaluation of phenotypic differences between genetically modified mice and their wild-type controls includes a wide range of endpoints such as clinical signs of behavior, macroscopic observations at necropsy, and clinical and anatomic pathology parameters (Kramer et al. 2007). Combining phenotypic data from the genetically modified animal model with target organ toxicities identified in more routine toxicology studies can aid in the understanding of the pathogenesis of potential safety findings. Moreover, the effect of novel pharmaceutical candidates on certain safety endpoints can be estimated in KO mice. For example, the generation of viable and fertile animals with null mutations for a potential target protein implies that pharmacologic inhibition of the molecule *in vivo* would elicit no major developmental adverse effects. KO and other genetically engineered mice, however, are often structurally normal, even if functional abnormalities are apparent; in other cases, these mice have both structural and functional defects. Subtle phenotypes may sometimes be unmasked using pharmacologic challenges or other physiologic stressors (Bolon and Galbreath 2002; Doetschman 1999). Genetically engineered mouse models have been used to assess drug specificity, investigate mechanisms of toxicity, and screen for mutagenic and carcinogenic activities of therapeutic candidates (Boverhof et al. 2011).

Toxicologic pathologists, collaborating with their drug discovery colleagues, are able to provide early toxicology data that have the potential to facilitate the selection of a lead compound. During the testing of compounds for efficacy in the specific animal model of human disease, additional valuable information may be gained from these studies to help inform on possible toxicities (Bass et al. 2009; Fielden and Kolaja 2008; Sasseville et al. 2004). A complement of toxicologic endpoints, including clinical pathology, macroscopic assessment, and light microscopic evaluation, can be incorporated into these efficacy studies. If there is an adequate supply of the test compound, a cohort of animals that are administered a higher dose (i.e., 10-fold above the proposed efficacious dose) could be added to the study to facilitate the identification of possible target organs of toxicity. The goal of this early screening is to provide toxicology data to support the selection of the molecule with the highest "probability of success" regarding safety concerns in later stages of evelopment.

Other approaches to improve candidate selection are to conduct dedicated exploratory toxicology studies prior to candidate nomination. The value of conducting these studies is to identify unwanted toxicities evident after repeat administration for a short duration (e.g., 3–14 days), as well as to identify putative toxicities based on a known cause of concern (e.g., prior knowledge based on class of compound or the literature). Clinical pathology data and histopathologic evaluation of tissues provide important information during lead optimization in drug development. While the approach of conducting short-term exploratory toxicology studies is to mitigate the risk of identifying a safety liability later in development, it is important to recognize that toxicities may still arise after long-term exposure (e.g., 4 weeks or greater) that were not identified in the shorter-term studies. Different strategies in study conduct and design (e.g., rising-dose approach in the beagle, single gender, limited group size) are utilized by pharmaceutical companies with the availability of drug substance often being the critical component of the exploratory study design.

The benefits of conducting exploratory safety studies are many. Results of these studies can provide the data to move the best candidate forward, allow to test for a specific cause of concern, and provide data to help understand both on-target and off-target toxicity (Bass et al. 2009). Toxicologic pathologists play an important role in these exploratory studies in the generation and interpretation of pathology data that can influence decisions on progressing candidates forward or terminating them, owing to an unwanted safety concern.

## 2.3 NONCLINICAL ANIMAL TOXICOLOGY STUDIES FOR SMALL MOLECULES

In the 1920s, J.W. Trevan proposed an experiment in mice to determine the dose of a chemical that would cause a 50% death rate, termed the median lethal dose ($LD_{50}$). Pharmacologists branching into toxicology subsequently proposed acute testing in several species, on the basis of observations regarding species differences in responsiveness to both the pharmacologic and acute toxic effects of chemicals. When the guidelines for repeat-dose toxicity experiments were developed in the early 1940s, the concept of using more than one species was automatically included. In response to demands from the US FDA and other national and international regulatory bodies in the 1960s, the protocols for toxicology testing became highly formalized with requirements to conduct all studies in a rodent and non-rodent species (Zbinden 1993). Because of advances in animal toxicology study designs and endpoints, the $LD_{50}$ approach is no longer utilized because we no longer need to depend on crude estimates of achieving lethality.

Currently, the conduct of toxicology studies is based on historic precedence and ICH recommendations, centered on the assumption that the choice of animal models and the design of the toxicology studies are truly predictive of possible human hazard (Olson et al. 2000; Monticello 2015). The default rodent species for toxicology studies is the rat, due to the larger size compared with the mouse, which permits easier manipulation (e.g., oral gavage dosing, blood collection) and greater blood volumes. The purpose-bred beagle is the default nonrodent owing to the domesticated nature of the dog, the consistent quality of health, decades of multiple generations of controlled breeding, and the overall lack of background pathologies that could confound results of a toxicology study. Today, there is a wealth of historic toxicology and pathology data on the rodent (mouse and rat) and purpose-bred beagle. The historic database on the cynomolgus monkey and minipig continues to expand (Heining and Ruysschaert 2016; Mecklenburg and Romeike 2016; Monticello and Haschek 2016; Schaefer et al. 2016; Snyder et al. 2016).

An internationally agreed-upon guidance, ICH M3 (R2) (2009), is the standard reference for nonclinical safety programs that are needed to support both human clinical trials and the eventual marketing authorization of small molecular new chemical entities (NCEs). A global guidance decreases the likelihood of inter-regional differences in nonclinical safety requirements, promotes the timely conduct of clinical trials, decreases overall development costs, and reduces animal use according to the 3Rs: initiative of reduce, refine, and replace (Goldberg and Locke 2004). For small molecules, the nonclinical safety studies need to be conducted in both a rodent model and a nonrodent with assurance that major human metabolites and the parent molecule are present and, therefore, qualified by at least one or both of the nonclinical safety species.

Standard animal toxicology studies should include assessment of drug exposure, primarily parent drug plasma concentration. In general, the drug plasma concentrations obtained in the nonclinical studies help guide both exposure limits and safety monitoring in the clinic. This approach is sufficient when the metabolic profile in humans is similar to at least one of the animal species used in the nonclinical safety studies. Metabolic profiles across species can differ both quantitatively and qualitatively; however, there are cases when clinically relevant metabolites have not been adequately evaluated in the nonclinical safety studies (CDER 2008). If the metabolite is active, for example, and binds to the therapeutic target or other unintended targets, it could result in an unanticipated safety liability; however, this phenomenon is uncommon.

The identification of potential differences in drug metabolites between the animal species used in the nonclinical safety assessment program and humans should be conducted early in the drug development process (Baillie et al. 2002). For example, if the nonhuman primate (NHP) has a more similar metabolic profile to humans compared with the beagle, the NHP may be selected as the nonrodent test species for that drug candidate. Metabolites identified only in human plasma or metabolites present at disproportionately higher levels in humans than in any of the animal test species may need to be qualified and evaluated in a dedicated animal toxicology study with the specific metabolite as the test article. Human metabolites that can raise a safety concern are those formed at

greater than 10% of total drug-related exposure and at greater levels in humans than the maximum exposure achieved in the toxicology studies (ICH M3 [R2] 2009).

An important part of the nonclinical safety evaluation program is determining the relevance of drug-related toxicities in animals to humans. Certain toxicologic findings often occur more frequently in some animal species but not others. An animal species that has a drug profile similar to the human in terms of pharmacology and PK would be considered more relevant such that the drug-related findings may be given more consideration. Recently, a nonclinical to clinical translational database was created and analyzed, which demonstrated the value of animal testing in providing safe entry into phase I clinical trials (Monticello et al. 2017). Results of this database indicated that while animal toxicology studies can demonstrate great value in the positive predictive value for certain test species and organ categories, the negative predictive value was the stronger predictive performance measure across all test species (rodent and nonrodent) and target organs, indicating that an absence of toxicity in animal studies strongly predicts a similar outcome in the clinic (Monticello et al. 2017). Data from the nonclinical safety studies are also used to determine the margin of safety of a drug, defined as the multiple (in doses or exposure) between the NOAEL defined from the most sensitive nonclinical animal toxicology study (rodent vs nonrodent) and the targeted maximum clinical efficacious dose in humans.

For both small and large molecules, the repeat-dose toxicology studies follow the principles outlined in ICH M3 (R2) (2009) with regard to the timing of nonclinical studies relative to clinical development. In principle, the duration of the nonclinical animal toxicology studies should be equal to or exceed the duration of the proposed human clinical trial (Table 2.1). Approval and market authorization of small-molecule drugs (nononcology indication) require longer-term testing of a 6-month study in the rodent and a 9-month study in the nonrodent. Examples of study design and group size numbers for a standard toxicology study are presented in Table 2.2.

**TABLE 2.1**
**Recommended Durations of Repeat-Dose Toxicology Studies to Support the Conduct of Clinical Trials (Non-Oncology Products)**

| Duration | Rodent | Non-Rodent |
|---|---|---|
| Up to 2 weeks | 2 weeks | 2 weeks |
| 2 weeks to 6 months | Same as clinical trial | Same as clinical trial |
| Greater than 6 months | 6 months | 9 months |

*Source:* ICH M3 (R2), Guidance on Non-Clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Biopharmaceuticals. June 2009. Retrieved June 2011 from http://www.ich.org. With permission.

**TABLE 2.2**
**Study Design Examples for Standard Toxicology Studies**

| | | Rodent | | Non-Rodent | |
|---|---|---|---|---|---|
| | | Main Study Animals | Recovery (Control and High-Dose Groups Only) | Main Study Animals | Recovery (Control and High-Dose Groups Only) |
| **Study Duration** | **Dose Groups** | ***n*/sex/Group** | | ***n*/sex/Group** | |
| 4 or 13 weeks | Control, low, intermediate, high | 10 | 5 | 3 | 2 |
| 26 weeks or greater | Control, low, intermediate, high | 20 | 5 | 4 | 2 |

It is important for the pathologist to know the age of the animals on a toxicology study if reproductive organs will be evaluated, in order to differentiate between immature sexual organs and compound-related toxicity. To properly evaluate effects on spermatogenesis, for example, animals should be sexually mature by at least the termination of the study. Rats are sexually mature at 9 weeks, whereas mice, at 7 weeks. Beagles should be 9–12 months of age to minimize confounding aspects of immaturity (Lanning et al. 2002). Male cynomolgus monkeys are more likely to be sexually mature at greater than 5 years of age and greater than 5 kg of body weight (Smedley et al. 2002).

## 2.4 NONCLINICAL ANIMAL TOXICOLOGY STUDIES FOR BIOPHARMACEUTICALS

The regulatory review processes applied to biopharmaceuticals are the same as those applied to an NCE (small molecules). Regulatory guidelines specific to issues and challenges associated with the unique properties of biopharmaceuticals have been generated to harmonize the nonclinical testing required for the development and worldwide approval of these large molecules. The primary nonclinical guidance document for biopharmaceuticals is the ICH S6, "Preclinical Safety Evaluation of Biotechnology-Derived Biopharmaceuticals" (1997). General principles addressed in this guidance include selection of a relevant animal model, dosing route and frequency, and the specification of the test material. A recent addendum further clarifies the topics of species selection, study design, immunogenicity, reproductive and developmental toxicity, and assessment of carcinogenic potential (ICH S6 [R1] 2011).

Biopharmaceuticals (or large molecules) are defined as products in which the active substance is produced by, or extracted from, a biologic source. Since the first FDA biologic approval of insulin in 1982, there have been more than 250 additional biopharmaceutical approvals, including recombinant and monoclonal antibody (mAb)-based products and recombinant vaccines (Shankar et al. 2006). Recently, from the period of 2010 to 2016, biopharmaceuticals have represented over 20% of all new and approved drugs (Mullard 2017). Some biopharmaceuticals have had safety-related regulatory actions postapproval, such as a notification letter sent to healthcare professionals, a modification to the drug insert label, or an added "black box" warning to the label (Giezen et al. 2008). Such safety warnings have included general disorders, administration side effects, infections, immune system disorders, and tumor risk.

Regulatory guidance from the ICH S6 outlines special considerations in the design and conduct of toxicology studies for biopharmaceuticals. Unique properties of biopharmaceuticals can create various challenges in conducting nonclinical safety assessment studies owing to their complex structural and biologic nature. The goals for conducting a nonclinical safety program for a biopharmaceutical are similar to those for a small molecule, including identification of potential adverse effects and target organs of toxicity and determination of potential safety biomarkers that can be monitored in clinical trials.

Toxicology studies of up to 6 months in duration are needed for regulatory approval and marketing of a biopharmaceutical intended for chronic use (ICH S6 [R1] 2011). On the basis of a retrospective analysis of nonclinical and clinical safety data for approved biopharmaceuticals, the 6-month toxicology testing paradigm was determined to be adequate for predicting human safety with biopharmaceuticals (Clarke et al. 2008). When there are two pharmacologically relevant species for the biopharmaceutical candidate (one rodent and one nonrodent), both species are used for the short-term (<6 months) general toxicology studies in support of FIH clinical trials. If the toxicity profile of the biopharmaceutical is similar between the rodent and nonrodent from the short-term studies, or if the toxicity profile is understood from the mechanism of action, only one species is needed for the 6-month study. Applying the 3Rs of animal research, the rodent species should be considered for the longer-duration toxicology study.