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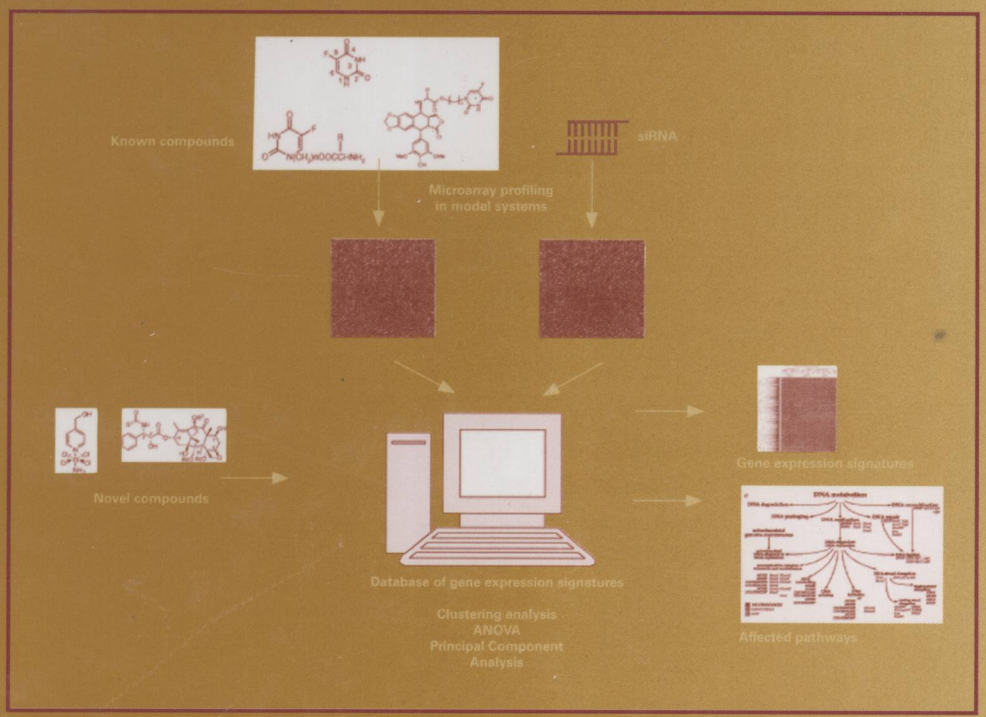
Preclinical Development HANDBOOK

Toxicology



Edited by

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PRECLINICAL DEVELOPMENT HANDBOOK

Toxicology

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WILEY-INTERSCIENCE
A JOHN WILEY & SONS, INC., PUBLICATION

Published by John Wiley & Sons, Inc., Hoboken, New Jersey
Published simultaneously in Canada

Library of Congress Cataloging-in-Publication Data is available.

ISBN: 978-0-470-24846-1

Printed in the United States of America

10 9 8 7 6 5 4 3 2 1

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PREFACE

This *Preclinical Development Handbook: Toxicology* focuses on the methods of identifying and understanding the risks that are associated with new potential drugs for both large and small therapeutic molecules. This book continues the objective behind this entire *Handbook* series: an attempt to achieve a through overview of the current and leading-edge nonclinical approaches to evaluating the nonclinical safety of potential new therapeutic entities. Thanks to the persistent efforts of Mindy Myers and Gladys Mok, the 31 chapters cover the full range of approaches to identifying the potential toxicity issues associated with the seemingly unlimited range of new molecules. These evaluations are presented with a thorough discussion of how the approaches fit into the mandated regulatory requirements for safety evaluation as mandated by the U.S. Food and Drug Administration and other regulatory authorities. They range from studies on potential genotoxicity and cardiotoxicity in cultured cells to a two-year study in rats and mice to identify potentially tumorigenic properties.

The volume differs from the others in this series in that although the methods used by the researchers are fixed by regulation at any one time, these methods are increasingly undergoing change as it is sought to become ever more effective at identifying potential safety issues before they appear in patient populations. Although we will never achieve perfection in this area, we continue to investigate new ways of trying to do so.

CONTENTS

1

PRECLINICAL DRUG DEVELOPMENT PLANNING

NIRMALA BHOGAL, ROBERT COMBES, AND MICHAEL BALLS
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Contents

Preclinical Development Handbook: Toxicology, edited by Shayne Cox Gad

1.1 INTRODUCTION

1.1.1 Overview of Objectives

It is well recognized that productivity in drug development has been disappointing over the last decade, despite the steady increase in R&D investment [1] and advances in techniques for producing potentially new candidate molecules. The principal problems appear to be a lack of efficacy and/or unexpected adverse reactions, which account for the majority of drug withdrawals and drugs undergoing clinical testing being abandoned. This high attrition rate could be dramatically reduced by improving the preclinical testing process, particularly by taking account of multidisciplinary approaches involving recent technologies, and by improving the design of preclinical projects to facilitate the collection and interpretation of relevant information from such studies, and its extrapolation to the clinical setting.

The objective of this chapter is to provide an overview of the early drug discovery and development processes. The main focus is the use of *in vitro* and *in silico* methods. This is because these techniques are generally applied during the earliest stages to identify new targets (target discovery) and lead compounds (drug discovery), as well as for subsequent drug development. They are also used to resolve equivocal findings from *in vivo* studies in laboratory animals, to guide selection of the most appropriate preclinical *in vivo* models, and to help define the mechanistic details of drug activity and toxicity. However, the use of animals in preclinical testing is also considered, since animal data form part of new medicine dossiers submitted to regulatory bodies that authorize clinical trials and the marketing of new products. The drug development process that will be considered is shown in Fig. 1.1. Definitions of the terminology and abbreviations/acronyms used in this chapter are listed in Table 1.1.

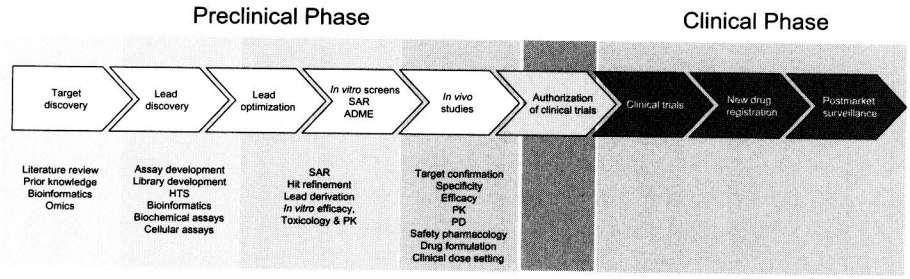

FIGURE 1.1 The key stages of drug discovery and development. A typical series of methods and strategies uses preclinical phases. Note that some of the studies may not be required and the process can be iterative. Refer also to Fig. 1.2 for a more detailed description of toxicity testing planning.

1.1.2 Drug Development Models

An essential part of drug development is the selection of the most appropriate animal, *ex vivo*, *in vitro*, or *in silico* systems, to allow the collection of information that can be interpreted in terms of the effects of a new therapeutic agent in humans or in one or more subpopulations of humans. There are several deciding factors that guide model selection. During early drug discovery screening, the main consideration is whether the chosen model can cope with large libraries of potentially bioactive molecules. It is generally accepted that, while nonanimal models generally lack the sophistication of studies on vertebrate animals and are based on nonclinical endpoints, they are a useful means of filtering out poor candidates during early drug discovery. The possibility of false hits during this stage is accepted as a trade-off, but it is also recognized that data from the use of several techniques and prior information can assist with the weeding out of false hits. The drug development process involves a more extensive evaluation using *in vitro* and *in silico* approaches and preclinical studies in vertebrate animals on a limited number of potential therapeutic agents.

The drive toward the use of systems biology approaches that take into account the roles of multiple biological and physiological body systems earlier in the drug development process has prompted a dramatic change in the way that data from cell-based studies are used. In many instances, data from several tests can be assembled and analyzed by using *in silico* models to gain a systems biology overview of drug ADMET and activity. Advances in comparative genomics have also opened up the scope for using zebra fish (*Brachydanio rerio*) and invertebrate organisms, such as nematode worms (*C. elegans*) and the fruit fly, *Drosophila melanogaster*, during the early stages of drug development. Likewise, advances in information mining, bioinformatics, data interpretation, the omics technologies, cell culture techniques, and molecular biology have the potential to greatly enhance the drug development process. Ironically, up to now, few of these methodologies has been standardized, formally validated, and accepted for regulatory use. Indeed, *in vitro* data are generally considered supplementary to animal data, rather than as an alternative source of information that is useful and applicable in its own right. Nevertheless, *in vitro* approaches provide information about the mechanisms of action

TABLE 1.1 Terminology and Abbreviations

Term	Definition
2D heteronuclear NMR	Two radionuclides are used to construct a two-dimensional map of a binding site by NMR.
Agglomeration	The process of particle attraction and adhesion.
Algorithm	A set of rules to assist with problem solving.
Allometric scaling	The process by which size, blood volume, and anatomical features of an organism are taken into account during extrapolation of information from animals to humans.
Analogue-based minimization	The process of using information about variants of the natural ligand for a target to derive a minimum number of features required of a smaller substance, so that binding affinity, efficacy, and/or specificity for the target in question are retained.
Antisense	A piece of genetic material that is the exact opposite of the natural messenger RNA that encodes a potential protein.
Bioaccumulation	The buildup of a drug or its metabolite(s) in a particular tissue or cell type.
Bioavailability	A measure of the amount of an administered drug that reaches its intended site of action.
Bioinformatics	The management and analysis of information, in order to use computer-based processes to understand biological events.
Biokinetic	Describes the key physiological processes that follow the exposure of an organism to a chemical or drug.
Biomarker	A molecular indicator of a biological event.
Biotechnology product	Replacement therapeutics or recombinant protein or DNA products isolated from or produced by using GM animals, cell cultures, plants, or microorganisms.
Biotransformation	The process by which a substance is chemically or functionally modified within the body, which usually involves the action of specific enzymes.
Combinatorial library	Large libraries of chemicals generated by a combination of acquisition and understanding of the requirements for recognition of a particular target.
Comparative genomics	The study of human genetics by reference to the genetics of other organisms as a means of deciphering human gene organization and function.
Cytotoxicity	A measure of the ability of a substance to damage or kill a cell.
Decision tree	A support tool for selection among competing choices and their possible consequences.
DNAzymes	A DNA-modifying enzyme.
Drug mimetic	A drug or drug-like molecule with a structure or modulatory activity that resembles that of a substance found within the body.
Druggable genome	The sum of the genes, their encoded disease-related proteins, or gene expression regulatory elements, which can functionally be modulated by drugs and drug-like molecules.

Druggable proteins	Proteins that bind drugs with a binding affinity below 10μM.
Drug discovery	The identification of a potential therapeutic agent.
Drug development	The progress of a lead from drug discovery toward a marketable drug.
Drug-like compound	A compound that has a molecular weight typical of a drug (around 500 daltons) and a structure that indicates it may have pharmaceutical properties.
Efficacy	The capacity of an agent to cause the desired biological effect.
Endpoint	The measurable effect of a substance on a biological system.
Epitope	The recognition site on a molecule for a particular molecule or class of molecules.
Eukaryotic	Describes organisms whose cells possess a nucleus and other membrane-bound vesicles, including fungi, plants, and animals.
Ex vivo	Literally, "out of the living"—used to refer to experiments that are conducted on tissues or cells isolated directly from a living organism.
Gene silencing	The process of preventing a gene from being expressed.
Genome	The entire genetic makeup of an organism.
Genomics	The study of the genetic makeup of an organism.
Genotoxicity	The adverse effects of a substance on the genetic makeup of a cell or organism.
Glucuridonation	The process of conjugating the uronic acid of glucose to substances, to detoxify or inactivate them.
Hapten	A substance that must combine with a carrier, in order to induce specific antibody production.
Hematotoxicity	The adverse effects of a substance on blood cells or on the cells or processes that produce specific types of blood cells.
Hit	The product of the high-throughput screening of large libraries of drug-like compounds, fragments, peptides, or proteins, identified by predominantly one-shot affinity, activity, or *in silico* methods.
Homeobox	DNA sequences found throughout the genome of most organisms that regulate gene expression, particularly during early development.
Homolog	A molecule with corresponding structures or functions in two or more species.
Humanized	The product of a process that is aimed to confer more human-like properties on a molecule, cell, or living organism.
Hydrophobicity	The tendency of a molecule to repel or exclude water molecules. (Means the same as lipophilicity.)
Immunogenicity	The ability of a substance to stimulate an immune response.
Immunohistochemistry	The testing of the ability of a tissue to be stained with an antibody.
Immunoprecipitation	The ability of an antibody–molecule complex to pull a second molecule out of solution as a result of interactions between the antibody recognizing molecule and secondary molecule.
Indels	Insertional or deletion mutations in DNA.
In silico	Using computer-based methods and virtual systems.
In vitro	Literally, "in glass"—used to refer to maintenance of tissues, cells, or cell fractions outside the body from which they were derived.