PRECLINICAL DEVELOPMENT HANDBOOK

Toxicology

SHAYNE COX GAD, PH.D., D.A.B.T.
Gad Consulting Services
Cary, North Carolina

WILEY-INTERSCIENCE
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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.

1

PRECLINICAL DRUG DEVELOPMENT PLANNING

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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.

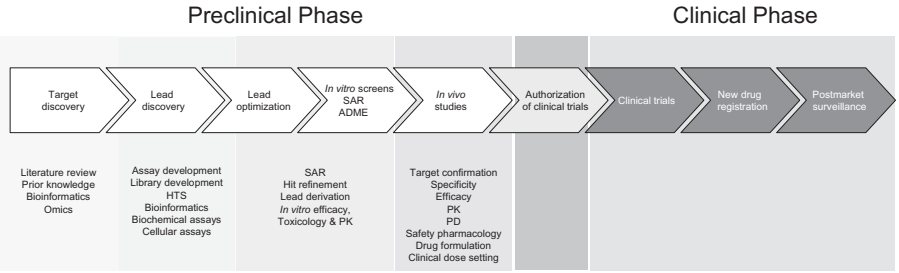

FIGURE 1.1 The key stages of drug discovery and development. A typical series of methods and strategies uses preclinical phases. Note that some of the studies may not be required and the process can be iterative. Refer also to Fig. 1.2 for a more detailed description of toxicity testing planning.

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 10 μM.
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.

TABLE 1.1 ***Continued***

Term	Definition
In vivo	Literally, "within the living"—used to refer to experiments conducted on intact living organisms.
Isozyme	Variants of enzymes that catalyze the same reaction(s) but differ from each other in primary structure and/or electrophoretic mobility.
Karyotype	The chromosomal complement of an organism.
Lead compound	A compound identified by hit generation that has suitable physicochemical and functional properties to serve as a starting point for the development of a potentially marketable drug.
Lipophilicity	The affinity of a molecule for a lipophilic environment.
Log *P*	The octan-1-ol/water partition coefficient—used to express lipophilicity.
Macroparticle	Particulate matter of a crystalline nature, generally exceeding a 10 nm diameter.
Margin of safety (MOS)	A ratio of the maximum amount of a substance that causes no effect in animals and the actual exposure (intended or otherwise) of the human population.
Meta-analysis	A statistical process for combining information from different sources.
Metabolic competence	The ability of a system to metabolize.
Metabonomics	The study of metabolic responses to drugs and chemicals.
Microfluidics	Small-scale systems comprised of chambers connected by a fluid matrix.
Molecular dynamics	Computer simulations of the movement of atoms, based on changes in the energy required to maintain certain conformations.
Monte Carlo simulation	A statistical method for studying systems, especially those with large numbers of coupled degrees of freedom.
Nanomedicines	Therapeutic agents based on the use of nanoparticles.
Nanoparticle	A microscopic particle with a unit size not exceeding 100 nm.
Oligonucleotide	A short stretch of synthetic DNA.
Omics	Technologies relating to the study of the genome, proteome, or metabolic responses of cells, tissues, and organisms.
Organotypic	An *in vitro* system designed to preserve or reconstitute the 3D structure of a tissue or organ, to mimic the *in vivo* situation.
Patch clamping	A process for measuring electrical activity across a living membrane by using electrodes.
Permeability	The ability to cross a living membrane.
Phage display	A system whereby a protein is displayed on the surface of a bacterial virus (a bacteriophage).
Phagocytic	Describes the engulfing of a molecule, a microorganism or part of an organism, by leukocytes (a type of white blood cell).
Pharmacokinetic	Describes the uptake, biotransformation, and distribution of a pharmaceutical agent and its metabolites in the tissues, and their subsequent elimination.
Pharmacophore	A collection of electrical and molecular features that define interactions between a molecule and its binding site on its target.

Plasma clearance rate	The speed at which a substance is removed from the blood.
Polymorphisms	Genetic differences within the population that occur for a given gene at a frequency of 1% or more.
Posttranslational modification	The process by which a protein is altered after it is synthesized, by the additional or removal of specific moieties.
Potency	The comparative ability of a drug to induce the desired effect.
Prokaryote	Cellular organisms that lack a distinct nuclear membrane or membrane-bound organelles (e.g., bacteria).
Proteome	The total protein complement encoded by the genome of an organism.
Proteomics	The study of protein expression patterns in specific cells, tissues, or organisms.
Quantum dot	Nanocrystals comprised of a semiconductor metal core.
Reactive oxygen species	Oxygen radicals or super-radicals that are capable of causing cellular damage.
Recombinant DNA technology	The process of DNA manipulation in an artificial environment.
Redox	The process of loss of oxygen or gain of hydrogen by one molecule accompanied by the gain of oxygen or loss of hydrogen by another molecule.
Reporter gene	A gene that is expressed in response to an upstream biochemical event, which can be used to monitor that event.
Reverse pharmacology	The screening of a library of compounds against one particular target to identify a lead for drug development.
RNA aptamers	RNA-based molecules that bind to enzymes.
RNAi	RNA interference—process of silencing or dampening protein expression.
Signal-to-noise ratio	Measure of the signal strength (change being observed) against the background within an experiment.
Therapeutic agent	A chemical, protein/peptide, DNA, stem cell, natural product, or biotechnology product that forms the active component of a finished pharmaceutical product.
Therapeutic index	The ratio between the toxic dose and the therapeutic dose of a drug, which is related to the MOS.
Three Rs	The principles of *replacement* of animal experiments, *reduction* of the number of animals used in a given study, or *refinement* of the procedures used, in order to minimize suffering and distress.
Toxicogenomics	The use of genomics and bioinformatics to identify and characterize mechanisms of action, based on changes in gene expression as monitored by the production of mRNA transcripts.
Toxicokinetic	Describes the uptake, biotransformation, distribution, and effects of a directly or indirectly toxic substance and its metabolites in the tissues, and their subsequent elimination.
Transcription	The process of messenger RNA production from a gene.
Transgene	A gene or variant of a gene that is inserted into the genetic makeup of an organism.
Vector DNA/RNA	Carrier DNA/RNA that may also facilitate the expression and/or cellular uptake of foreign genetic material by cells and tissues.
Xenobiotic	A chemical or other substance that is not a natural component of the makeup of the organism exposed to it.

TABLE 1.1 *Continued*

Abbreviation/Acronym	Full Name
ADME(T)	absorption, Distribution, Metabolism, Elimination (Toxicity)
ADR	Adverse Drug Reaction
BBB	Blood–Brain Barrier
BCS	Biopharmaceutics Classification System
BRET	Bioluminescent Resonance Energy Transfer
cAMP	Cyclic Adenosine Monophosphate
CASE	Computer Automated Structure Evaluation
CBER	Center for Biologics, Evaluation and Research
CDER	Center for Drugs, Evaluation and Research
CoMFA	Comparative Molecular Field Analysis
COMPACT	Computerized Optimized Parametric Analysis of Chemical Toxicology
CRE	Cyclic-amp Responsive Element
CYP	Cytochrome P450
CYP450-DMO	Cytochrome P450-Dependent Monooxygenase
DEREK	Deduction of Risk from Existing Knowledge
ECVAM	European Centre for the Validation of Alternative Methods
ELISA	Enzyme-linked Absorbance Assay
EMEA	European Medicines Agency
EPA	Environmental Protection Agency
ERE	Estrogen Responsive Element
FACS	Fluorescence-Activated Cell Sorting
FDA	Food and Drug Administration
FRET	Fluorescent Resonance Energy Transfer
GFP	Green Fluorescent Protein
GPCR	G-Protein-Coupled Receptor
HESC	Human Embryonic Stem Cell
HTS	High-Throughput Screening
IAM	Immobilized Artificial Membrane
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
ICH	International Conference for Harmonization
IND	Investigational New Drug
LC-MS/MS	Liquid Chromatography and Tandem Mass Spectrometry

LD_{50}	Lethal Dose that kills 50% of a test group (of animals)
Log P	Octan-1-ol/water partition coefficient
MAP kinase	Mitogen-Activated Protein Kinase
MCASE	Multi-CASE
MDCK	Madin–Darby Canine Kidney
MOS	Margin of Safety
MS	Mass Spectrometry
MTD	Maximum Tolerated Dose
NCE	New Chemical Entity
NCTR	FDA National Center for Toxicological Research
NMR	Nuclear Magnetic Resonance
OECD	Organization for Economic Co-operation and Development
PAMPA	Parallel Artificial Membrane Permeation Assay
PBPK	Physiologically Based Pharmacokinetic
PCR	Polymerase Chain Reaction
PK	Pharmacokinetic
pK_a	The acid-ionization constant
PTFE	Polytetrafluoroethylene
QSAR	Quantitative
QSAR–ES	Quantitative Structure–Activity Relationship—Expert System
QT Interval	The time between the start of the Q wave and the end of the T wave in the heart's electrical cycle
SAR	Structure–activity Relationship
SPA	Scintillation Proximity Assay
SPR	Surface Plasmon Resonance
TOPKAT	The Open Practical Knowledge Acquisition Toolkit
UFAW	Universities Federation for Animal Welfare

of a drug that is vital for the design of *in vivo* animal studies and can add substantial weight to the product dossier submitted to regulatory bodies.

Increasingly, predictions about the ways in which a particular chemical is likely to interact with its desired cellular target are made by undertaking *in silico* modeling. These results are used to filter out poor candidate molecules according to chemical class and structural or functional features during drug discovery. However, filtering of this kind is sometimes impossible, so lead identification still relies to some extent on serendipitous finds from random libraries, rather than on rational lead discovery. For instance, for new chemical entities (NCEs) for which there are no data, i.e., are first-in-class, *in silico* screenings are difficult to handle, particularly where there is also limited knowledge of the structure of the active site of the target. Also, there might be a lack of important information for other compounds. For example, predicting drug effects can be seriously compromised when ADME data on the behavior of a molecule in different tissues and species are lacking. This is confounded by the reality that this kind of information for different individuals will always be limited. Both of the above situations are most evident in the case of large molecules, such as (1) peptides and proteins with complex structures and multiple conformations, (2) humanized products that could be differentially immunogenic in different species, and (3) nanoparticle formulations.

1.1.3 Information Required Prior to Drug Authorization/Approval

Once a new therapeutic candidate has been successfully identified from preclinical studies, the next stage involves the authorization of clinical studies. The information required prior to the authorization of any clinical trial is crucial for the design and execution of preclinical studies, irrespective of whether the aim is to define drug action or provide safety information. Such information includes (1) manufacturing quality, (2) physicochemical properties, (3) efficacy, (4) proposed mechanism of action, (5) selectivity, (6) ADME, and (7) possible adverse effects in humans.

In the United States, the Food and Drug Administration (FDA) handles drug approvals. The FDA has fast tracked this process for treatments for serious diseases where no therapies currently exist [2]. Drug developers are required to submit an Investigational New Drug (IND) Application, in which evidence from preclinical studies is provided for review by the FDA. The FDA decides whether it is reasonably safe for the company to test the drug in humans. Under the FDA's jurisdiction, the Center for Drugs, Evaluation and Research (CDER) and the Center for Biologics, Evaluation and Research (CBER) are responsible for reviewing different types of therapeutic agent applications (Table 1.2). Note that these changes in jurisdiction mean that biological products, the testing of which was at one point based on limited animal tests (because of their poor predictivity), are likely to require more stringent testing under the CDER [3].

The FDA has exclusive executive control over decisions regarding drug approvals in the United States. However, in Europe, it is possible to have a drug approved by a number of different routes. This is because companies can apply either via the EMEA (European Medicines Agency) for pan-European approval or via one or more national agencies. However, since November 2005, all new drugs for the major diseases, including AIDS, cancer, diabetes, and neurodegenerative disorders, and

TABLE 1.2 CDER and CBER:[a] Review of New Therapeutic Agent Applications

CDER

- Traditional small molecule therapeutics
- Growth hormone, insulin, and other endocrine peptide therapeutics
- Monoclonal antibodies
- Proteins (e.g., cytokines, enzymes, and other novel proteins), except those specifically assigned to the CBER, namely, vaccines and blood products that are assigned to CBER
- Immunomodulatory agents (but not vaccines)
- Growth factors intended to modulate hematopoiesis *in vivo*
- Combination products where the primary mode of action is that of an agent assigned to the CDER

CBER

- Products composed of human, bacterial, or animal cells or fragments of cells, for use as preventative or therapeutic vaccines
- Gene therapy products
- Vaccines
- Allergenic extracts used for the diagnosis and treatment of allergic diseases
- Antitoxins, antivenins, and venoms
- Blood and blood products from humans or animals
- Combination products where the primary mode of action is that of an agent assigned to the CBER

[a]The CDER and CBER are afforded jurisdiction by the U.S. FDA.

medicinal products developed by means of biotechnological processes must be approved via the EMEA.

With the globalization of the pharmaceutical industry, the International Conference on Harmonization (ICH) guidelines have, since 1990, set out to standardize drug applications in terms of their content and format. Japan, the United States and the European Union (EU) comply with these requirements for the quality, safety, and efficacy assessment of new drugs. These guidelines operate alongside national requirements. Quality assessment guidelines are provided to standardize the assessment of drug stability (shelf-life), and the management of risks due to impurities, such as residual solvents and infectious agents, such as viruses (which can be present when a drug is isolated from plants, animals, humans, or cell lines). The guidelines also require the standardization of cell lines, test procedures, acceptance criteria, and procedures for formulation and development. Efficacy guidelines are also provided, to standardize the conduct, interpretation, and reporting of clinical trials.

There are some important practical considerations that should be borne in mind when conducting preclinical studies. The most comprehensive guidelines are those provided for drug safety testing, which cover a number of toxicological endpoints, including carcinogenicity, genotoxicity, reproductive and developmental toxicity, and immunotoxicity. Some of the guidelines apply generically to all new drugs, while others focus on specific types of therapeutic agents, such as biotechnology products. These guidelines are essential reading for researchers engaged in drug development and are considered in more detail throughout the remainder of this chapter.

Another important source of reference is the Organization for Economic Co-operation and Development (OECD). By ratifying the convention of the OECD, many European countries, Australia, Japan, New Zealand and the United States have agreed to abide by a set of test guidelines for assessing the human health effects of chemicals [4], which apply equally to the testing of therapeutic agents. Later, we refer to a number of nonanimal methods and refinements of animal procedures accepted by the member countries of the OECD.

1.2 FINDING NEW DRUG TARGETS

1.2.1 Background

Until relatively recently, drug development focused on a limited number of targets, against which NCEs with a desired effect could be selected. These "druggable" targets were once most extensively investigated by using animal models. However, greater access to recombinant DNA technology means that most early screens are now conducted primarily by using different genetically engineered cell lines expressing putative targets that can be arrayed into high density plastic plate formats suitable for interactions between the targets and potential lead chemicals (for methods, see later discussion).

Overington et al. [5] derived a consensus figure for the number of therapeutic drug targets for the FDA-approved drugs that were available in 2005. They identified 324 drug targets for all classes of approved therapeutic agents, which were targeted by in excess of 1357 drugs, of which 1204 were small molecules and 166 were biologicals. Cell surface receptors and channels represented the targets for >50% of all the FDA-approved drugs. A further 10% of the drugs, including monoclonal antibodies, also target other cell surface proteins. Most of the remaining targets were enzymes, nuclear receptors, DNA, or ribosomes. These targets represent a minute fraction of the genome, and a mere 3% (266 proteins) of the predicted proteome.

According to this survey, on average 5.3 new druggable targets are discovered each year. This means that many more potential drug targets remain to be discovered. Whether a potential drug target will be a good therapeutic target, however, depends on whether (1) it plays a key role in gene regulation, (2) it is selectively expressed in certain disease states or tissues, and (3) it has a definable and unique binding site.

Often, a further important piece of information is the nature or identity of the endogenous modulator. For example, >1000 G-protein-coupled receptors (GPCRs) have been cloned from various species, including 160 distinct human subtypes with known ligands, although these represent only a limited set of targets for current therapeutic agents. A further 100 or so are orphan receptors, for which there is currently no known natural ligand. In such cases, the starting point is the gene, from which the protein receptor can be expressed and used to screen large combinatorial libraries of chemicals in the search for a modulator. Such a reverse pharmacology strategy uses the orphan receptor as a "hook" for screening libraries and hit generation, where little is known about the natural ligand. In many cases, receptor models use the crystal structure of rhodopsin as a template, as this is the only GPCR whose structure has been resolved. The importance of GPCRs is emphasized by the fact that, although >20% of the top 200 current best-selling drugs interact with these cell

surface receptors, they generate worldwide sales of drugs such as cimetidine, losartan, and ropinerole of over $20 billion (U.S.) [6].

1.2.2 Impact of New Technologies on Target Discovery

Comparative genetics can provide much relevant information, particularly with regard to the role of human-specific genes and the suitability of animal models for drug development. The application of microarray techniques, standards, and resources that permit the comparison of gene expression patterns across species and between cell types and tissues has started to provide some insight into the metabolic and biochemical differences between health and disease states. A good example of this is the Cancer Genome Anatomy Project (www.ncbi.nlm.nih.gov/CGAP) [7], in which mutational sites in cancer cells have been identified.

A cursory examination of the 373 completed genome sequences for archeal, prokaryote, and eukaryote [8] species suggests that, although genome size increases from archea through prokaryotes to eukaryotes, genome size is not directly linked to the number of genes within the functional genomes, nor with evolutionary status. It is, however, clear that, as the complexity of organisms increases, so does the complexity of gene regulation and the level of genetic redundancy—the ability of several genes to rescue loss-of-function of another gene. Nevertheless, for highly conserved genes, such as those that are involved in early development, and homeobox genes, studies on early life stages of species such as zebra fish and invertebrate models can indicate the roles of genes. However, in general, such studies are more relevant to safety pharmacology than to mechanistic and efficacy studies. It is worth bearing in mind that computational predictions and statistical analyses have suggested that the bacterial *Escherichia coli* and human genomes account for 35 common metabolic pathways, namely, those that are important in biosynthesis and in degradation and respiratory processes [9], and that, possibly as a result of bacterial infection, a number of bacterial genes have become permanently integrated in the human genome [9, 10]. This opens up the possibility of using bacterial studies to decipher a limited number of biochemical pathways affected by drugs, as well as for genotoxicity testing.

Unicellular eukaryotes, such as yeast, share remarkable genetic and functional similarities with multicellular eukaryotes. The most useful yeast strain in terms of dissecting protein and gene interactions is *Saccharomyces cerevisiae*. At 12,100 kilobases, the *S. cerevisiae* genome is much smaller than the human genome. However, because its gene density is 50 times greater than that of the human genome, genes found in the *S. cerevisiae* genome resemble around 30% of the genes associated with diseases in humans [11]. Since the entire genome of *S. cerevisiae* encodes no more than 6000 proteins, it is relatively straightforward to investigate gene function in yeast and make genome-wide microarray measurements. Such data, together with information from other sources, have made it possible to identify a number of putative drug targets [12] and protein–protein interactions [13], thereby facilitating the development of extensive maps of protein and gene interactions. Such studies in *S. cerevisiae* have been particularly useful in neurodegenerative and ageing research and in studies on diseases that arise as a consequence of mitochondrial DNA damage. One example is the observation that yeast mutants for α-synuclein result in a large change in yeast sexual reproduction, as well as causing cytotoxicity,

both endpoints of which are suited to high-throughput screening assays for new treatments for Parkinson disease [14].

Subsequent studies on yeast-based models of Parkinson disease have suggested that there is substantial scope for using yeast for the high-throughput screening of chemicals for drug discovery [15]. For example, *S. cerevisiae* possesses three distinct G-protein-coupled receptors (GPCRs), which are involved in pheromone (Ste2 and Ste3 receptors) and glucose sensing (Gpr1) [16]. These receptors are related, albeit to a limited extent, to the vastly expanded human GPCR repertoire. By coupling heterologously expressed human GPCRs to the yeast MAP kinase pathway (associated with yeast mating and growth arrest), in yeasts where the MAP kinase pathway is linked to reporter gene expression [17], it is possible to monitor receptor recognition and activation by simple growth or colorimetric reporter assays.

Caenorhabditis elegans is another organism that can be used in early drug discovery. This nematode worm is transparent, has a short life span, is a mere 1 mm in length and 80 μM in diameter, reproduces every 3 days by self-fertilization to produce over 300 offspring, and is a multicellular organism composed of exactly 959 somatic cells. It displays many of the basic features of higher eukaryotes, including the possession of muscle, excretory cells, and neural cells, and has been extensively used to increase understanding of the mechanisms of gene regulation and gene function. Antisense knock-out or knock-down of gene expression can be achieved simply by feeding the worm with *E. coli* bacteria transformed with plasmid DNA containing antisense DNA. More recently, RNA interference (RNAi) has been used to manipulate the genomes of organisms such as *C. elegans*, although the possibility of transmission of RNA silencing to subsequent generations can occur [18]. Like all multicellular organisms, *C. elegans* exhibits programmed cell death (apoptosis) [19], in a way that is very similar to that seen in higher organisms as part of ageing and disease processes. Similarities between the signaling pathways involved in the regulation of cell proliferation in *C. elegans* and humans suggest that this organism might provide information on the regulation of cell proliferation, which will be of relevance to cancer therapeutics. The entire 302-cell nervous system of this worm has been mapped by electron microscopy, and although the average human possesses somewhere in the order of 100 billion neurons, it seems that neurotransmission is similar in the two species. Thus, *C. elegans* possesses the major classes of ion channels, receptors, transporters, and neurotransmitters that make it a suitable candidate for some forms of drug screening, such as the discovery of new dopaminergic drugs. Similarly, *D. melanogaster* shares much of its basic neurobiology with higher organisms, including humans. It possesses the same neurodegenerative states, neurotransmission mechanisms, and receptor homolog that are found in humans as key targets for neurally active therapeutic agents, making studies with these organisms useful for the development of treatments for conditions such as Parkinson's disease [20].

1.2.3 Data Mining

Novel drug targets can also be found in other ways, including data mining. This involves analyzing the literature, to determine the biochemistry underlying particular human diseases, and human physiology. In addition, human population genetics studies can be undertaken, to determine the roles of human genes, how they interact, the consequences of population differences at the gene level, and, ultimately, the complete physiology of the human body. In the last-named case, since the