DRUGS

FROM DISCOVERY TO APPROVAL

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FROM DISCOVERY TO APPROVAL

Rick Ng, Ph.D.
Biopharmaceutical Manufacturing Technology Centre
Singapore

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To

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CONTENTS

PREFACE

This book is written as a basic framework to introduce the concepts and processes from drug discovery to marketing approval by regulatory authorities. It is particularly suitable for undergraduates pursuing courses in medicine, pharmacy, science and life sciences. Professionals in the pharmaceutical industry will find this book useful as a quick reference guide.

There are eleven chapters:

- Chapter 1 provides a snapshot about the drug discovery and development processes, as well as the current status in the pharmaceutical industry.
- Chapter 2 describes the all-important steps in identifying disease targets and receptors for drug interaction and intervention.
- Chapter 3 explains the current technologies and methodologies for discovering new small molecule drugs.
- Chapter 4 introduces the various large molecule drugs and the methods for discovering and developing them.
- Chapter 5 summarizes the steps for drug development and preclinical tests.
- Chapter 6 details the processes and conduct of clinical trials with due respect for safety, risks and benefits.
- Chapter 7 describes the major drug regulatory authorities in selected countries such as the United States, European Union, Japan, China and some international organizations.
- Chapter 8 shows the basic procedures for applications to regulatory authorities for clinical trials and drug marketing approvals.
- Chapter 9 discusses the regulatory requirements for drug manufacture, with selected examples.
- Chapter 10 demonstrates the controls required to manufacture drugs that meet the regulatory requirements.
- Chapter 11 gives perspectives about the future events for drug discovery and development.

Some background concepts are introduced in four appendices together with acronyms and glossary.

In writing this book, I am indebted to many friends and colleagues. I am grateful to Dr. Choon Onn Wong, Dr. Dinesh Khokal, Dr. Wang Sijing and Dr. Paul Baker who painstakingly read the entire draft and provided insightful and invaluable suggestions. For the artwork, I thank Mr Jadish Kuchibhatla and my brother-in-law Mr Yong Kit Song. My thanks to Mr Tim Badgery-Parker who worked on the copyediting and Ms Jessica Teo and Ms Stephanie Chiang who helped with proofreading the drafts. I sincerely thank Ms Luna Han of John Wiley & Sons for seeing potential in this book and agreed to publish it.

I thank my family for the encouragement and support that they have provided me.

Rick Ng

CHAPTER 1

INTRODUCTION

1.1 AIM OF THIS BOOK

The process from discovering a new drug to registering it for marketing and commercialization is very complex and lengthy. The intention of this book is to provide an overview about how a drug is discovered, the amount and types of laboratory tests that are performed, and the conduct of clinical trials before a drug is ready to be registered for human use. The role of regulatory authorities in these processes and their control over safety evaluation, clinical trials, manufacturing and marketing approval of a drug are also explained. This book aims to integrate, in a simplified manner, the interrelationships between all these complex processes and procedures.

To establish a frame of reference, it is appropriate to commence with a definition for the term 'drug'. Generally, a drug can be defined as a substance that induces a response within the human body, whether the response is beneficial or harmful. In this context, toxins and poisons can be classified as drugs. However, the term 'drug' used in this book is strictly reserved for a medicinal substance, which provides favorable therapeutic or prophylactic pharmaceutical benefits to the human body. Readers are referred to Exhibit 1.1 for a definition of drug according to the Food and Drug Administration (FDA) of the United States.

It should be noted that the descriptions in this book on discovery and regulatory processes are mainly for ethical drugs, as opposed to over-the-counter (OTC) drugs. Ethical drugs are prescription drugs that require prescriptions by physicians, whereas OTC drugs can be purchased from

Exhibit 1.1 FDA Definition of a Drug

'An active ingredient that is intended to furnish pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of a disease, or to affect the structure of any function of the human body, but does not include intermediates used in the synthesis of such ingredient'.

pharmacies without prescription. The OTC drugs are mainly established drugs that are deemed safe enough to be taken without supervision by a physician.

There is a further differentiation of ethical drugs into new drugs (those covered by patents) and generics (copies of drugs that have expired patents). Most of the descriptions in this book apply to new drugs.

1.2 AN OVERVIEW OF THE DRUG DISCOVERY AND DEVELOPMENT PROCESS

Although human civilization has been experimenting and consuming drugs for many centuries, it is only in the past 100 years that the foundation was laid for the systematic research and development of drugs. Readers are referred to Appendix 1 for a brief description of the history of drug development since the ancient times.

Teams of scientists, clinicians, and statisticians, as well as regulatory, marketing, medical practitioners, and even economists and legal attorneys, are involved in the process of drug discovery and development. Previously, the main scientific personnel in the discovery process have been the synthetic chemists. Now molecular biologists, biochemists, microbiologists and even computer scientists play equally important roles in the drug discovery and development processes. The reason for this is that drug discovery and development has made a quantum leap forward in the past decade with progress in genomics and biotechnology. In addition, advances in laboratory equipment automation and high-speed computing have assisted in analyzing and processing of large data sets. Personnel with different disciplines and expertise are needed to contribute to discover and develop drugs targeting diseases at the cellular and molecular levels.

It is estimated that, on average, a drug takes 10–12 years from initial research to reach the commercialization stage. The cost of this process is estimated to be more than US$500 million. From discovery to marketing approval of a drug, the following stages are involved (Figure 1.1):

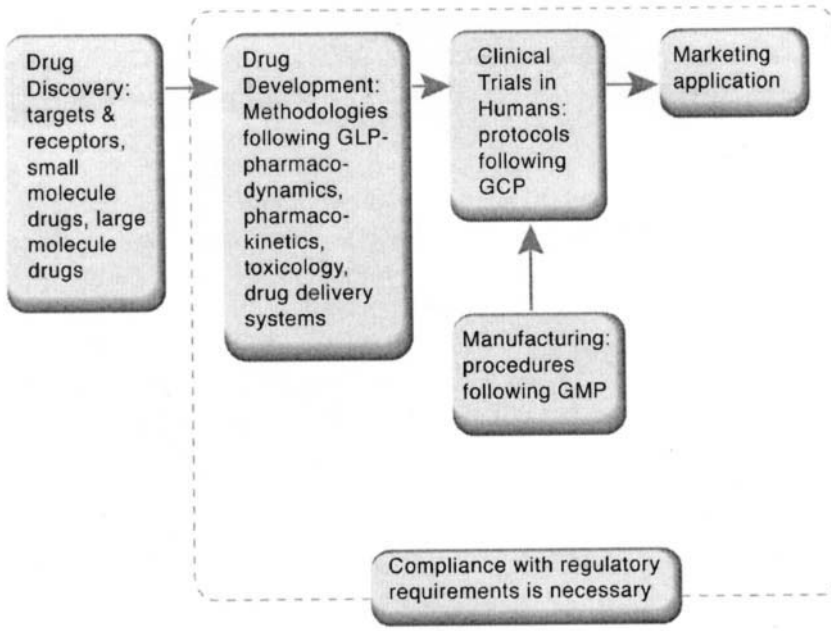

Figure 1.1 The stages from drug discovery to marketing approval

Drug Discovery: The process involves finding out the target that causes the disease. Next, chemical or biological compounds are screened and tested against these targets or assays, which are representative of these targets, to find leading drug candidates for further development. Many new scientific approaches are now used to determine targets (most targets are receptors or enzymes) and obtain the lead compounds; including the use of genomic technology, synthetic chemistry, recombinant DNA (rDNA) technology, laboratory automation and bioinformatics.

Drug Development: Tests are performed on the lead compounds in test tubes (laboratory, *in vitro*) and on animals (*in vivo*) to check how they affect the biological systems. The tests, often called preclinical research activities, include toxicology, pharmacodynamics and pharmacokinetics, as well as optimization of drug delivery systems. Many iterations are carried out, and the leading compounds are modified and synthesized to improve their interactions with the targets, or to reduce the toxicity or improve pharmacokinetics performance. At the end of this process, an optimized compound is found and this becomes a potential drug ready for clinical trial

in humans. The development work has to follow Good Laboratory Practice (GLP) to ensure that proper quality system and ethical considerations are established. Only compounds that satisfy certain performance and safety criteria will proceed to the next stage of clinical trial.

Clinical Trials: These are trials conducted on human subjects. The pertinent parameters for clinical trials are protocols (methods about how trials are to be conducted), safety and respect for human subjects, responsibilities of investigator, institutional review board, informed consent, trial monitoring and adverse event reporting. Clinical trials have to follow regulations and guidelines from the FDA, the European Agency for the Evaluation of Medicinal Products (EMEA) of the European Union (EU) or European Member States, Japan's Ministry of Health, Labor and Welfare (MHLW), or regulatory authorities in other prospective countries where the drug is intended to be registered and commercialized. Clinical trials are conducted in accordance with Good Clinical Practice (GCP).

Manufacturing: The drug designated for clinical trials and large-scale production has to be manufactured in compliance with current Good Manufacturing Practice (cGMP; the word 'current' denotes that regulations do change from time to time and the current regulations have to be applied) following US FDA requirements, EU Directives or International Conference on Harmonization (ICH) guidelines. Regulatory authorities have the rights to conduct inspections on pharmaceutical manufacturing plants to ensure they follow cGMP guidelines so that the drug manufactured is safe and effective. A quality system has to be set up such that the drug is manufactured in accordance with approved procedures. There must also be traceability of materials as well as appropriate tests being conducted on the raw materials, intermediates and finished products. The emphasis is that drugs should be safe, pure, effective, and of consistent quality to ensure that they are fit to be used for their intended functions.

Marketing Application: A drug is not permitted for sale until the marketing application for the new drug has been reviewed and approved by regulatory authorities such as the US FDA, the EU EMEA or Japan's MHLW. Extensive dossiers are provided to the authorities to demonstrate the safety, potency, efficacy and purity of the drug. These are provided in the form of laboratory, clinical and manufacturing data, which comply with GLP, GCP and cGMP

requirements. After the drug has been approved and marketed, there is continuous monitoring of the safety and performance of the drug to ensure that it is prescribed correctly and adverse events (side effects) are investigated. The advertising of drugs is also scrutinized by regulatory authorities to ensure that there are no false representations or claims for the drugs.

The subsequent chapters will elaborate on each of these processes. An example of the complexity, time and cost of developing a new drug is shown in Exhibit 1.2.

Exhibit 1.2 Did You Know?

Total drug development time grew from an average of 8.1 years in the 1960s to 11.6 years in the 1970s, to 14.2 in the 1980s, to 15.3 years for drugs approved from 1990 through 1995. Pharmaceutical companies and regulatory authorities are working together to reduce this time span.

The cost of developing a new drug is more than three times the price of a Boeing 747-400 airplane.

Typically, tens of thousands of compounds are screened and tested, and only a handful make it into the market as drug products. The statistics are such that, of 5000 compounds that show initial promise, five will go into human clinical trials, and only one will become an approved drug.

SOURCE PhRMA (Pharmaceutical Research and Manufacturers of America), http://www.phrma.org/ [accessed Mar 28, 2002].

A recent report puts the cost for developing a drug at US$802 million, although this figure has been challenged by various groups.

SOURCE Ezzel, C., The price of pills, *Scientific American*, July, p. 25 (2003).

1.3 THE PHARMACEUTICAL INDUSTRY

The pharmaceutical industry as we know it today started in the late 1800s. It started with the synthetic versions of natural compounds in Europe (refer to Appendix 1).

Drug discovery and development are mainly carried out by pharmaceutical companies, universities and government research agencies, although there are increasing activities in the start-up and smaller companies

that specialize in particular fields of research. A substantial number of the research findings and potential drugs from the start-ups, smaller companies, universities and research organizations are, however, licensed to the multinational pharmaceutical companies for manufacturing, marketing and distribution. Alternatively, alliances are formed with the multinational pharmaceutical companies to develop or market the drugs, because of the huge cost involved for drug development and commercialization.

In 2002, the combined worldwide pharmaceutical market was around US$400 billion. The distribution of the market (in US$ billion) is shown in Table 1.1. From these data, it is evident that the US, Europe and Japan account for more than 85% of the worldwide pharmaceutical market. The regulatory authorities in these countries are hence very important to the pharmaceutical companies to ensure their products are approved for commercialization.

Table 1.2 shows the top 10 drugs in 2002, according to IMS *World Review 2003*. Exhibit 1.3 provides an explanation of cholesterol and the mechanism of action for Lipitor and Zocor. Exhibit 1.4 gives more information about Prilosec.

Table 1.1 Global pharmaceutical sales by region, 2002

World	2002 sales (US$bn)	% Global sales	% Growth market
North America	203.6	51	+12
European Union	90.6	22	+8
Rest of Europe	11.3	3	+9
Japan	46.9	12	+1
Asia, Africa and Australia	31.6	8	+11
Latin America	16.5	4	–10
Total	**400.5**	**100**	**+8**

SOURCE IMS *World Review 2003* and IMS Consulting, http://www.ims-global.com/insight/ news_story/0302/news_story_030228.htm [accessed Mar 25, 2003].

Table 1.2 The top 10 best-selling products, 2002

Product	Therapy	Company	US$ (bn) (% growth from 2001)
Lipitor (atorvastatin)	Cholesterol reducer	Pfizer	8.6 (20%)
Zocor (simvastatin)	Cholesterol reducer	Merck	6.2 (13%)
Prilosec (omeprazole)	Antiulcerant	AstraZeneca	5.2 (–19%)

Table 1.2 *Continued*

Product	Therapy	Company	US$ (bn) (% growth from 2001)
Zyprexa (olanzapine)	Antipsychotic	Eli Lilly	4.0 (21%)
Norvasc (amlopdipine)	Antihypertensive	Pfizer	4.0 (6%)
Erypo (erythropoietin)	Anemia	Johnson & Johnson	3.8 (18%)
Prevacid (lansoprazole)	Acid reflux disease treatment	TAP	3.6 (3%)
Seroxat/Paxil (paroxetine HCl)	Antidepressant	GlaxoSmithKline	3.3 (13%)
Celebrex (celecoxib)	COX-2 inhibitor and anti-inflammatory	Pharmacia	3.1 (–1%)
Zoloft (sertraline)	Antidepressant	Pfizer	2.9 (12%)

SOURCE IMS *World Review 2003*, http://www.ims-lobal.com/insight/news_story/0302/news_story_030228.htm [accessed Mar 25, 2003].

Exhibit 1.3 Cholesterol and Cholesterol-lowering Drugs

Cholesterol is a fatlike substance (a sterol) that is present in our blood and all the cells. It is synthesized within the body or derived from our diet. Cholesterol is an important constituent of the cell membrane and hormones.

Cholesterol is carried in the bloodstream by lipoproteins such as low density lipoprotein (LDL, or 'bad cholesterol') and high density lipoprotein (HDL, 'good cholesterol'). LDL carries cholesterol from the liver to other parts of the body. LDL attaches to receptors (see Chapter 2) on the cell surface and is taken into the cell interior. It is then degraded and the cholesterol is used as a component for cell membrane. When there is excessive cholesterol inside the cell, it leads to a reduction in the synthesis of LDL receptors.

The number of active LDL receptors is also affected by a condition called familial hypercholesterolemia, in which there is a defective gene coding for the receptor. In either case, the reduction of active receptors means that the LDL carrying cholesterol is unable to enter the cell interior, instead it is deposited in the arteries leading to the heart or brain. These deposits build up over time, and may block blood supply to the heart muscle or brain, resulting in a heart attack or stroke. In contrast, HDL transports cholesterol from other parts of the body to the liver, where it is degraded to bile acids.

An enzyme (see Section 2.6) called HMG-CoA reductase is involved in the biosynthesis of cholesterol. Drugs such as atorvastatin (Lipitor) and simvastatin (Zocor) are competitive inhibitors of HMG-CoA reductase. They inhibit cholesterol synthesis by increasing the number of LDL receptors to take up the LDL.

Exhibit 1.4 Prilosec

Omeprazole (Prilosec, AstraZeneca) is a drug termed as proton pump inhibitor. It turns off the secretions of acid into the stomach. When less acid is produced, there is a reduced amount of acid that can flow back up from the stomach into the esophagus to cause reflux symptoms.

It should be noted that there are normally three names associated with a drug: the trade or proprietary name (for example, Prilosec), generic or non-proprietary name (omeprazole) and a specific chemical name for the active ingredient. In the case of omeprazole, the active ingredient is a benzimidazole: 5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl) methyl] sulfinyl]-1H-benzimidazole. It has an empirical formula of $C_{17}H_{19}N_3O_3S$.

SOURCE AstraZeneca, http://www.priloseconline.com/ [accessed Apr 15, 2002].

The top 10 pharmaceutical companies in 2002 are shown in Table 1.3. These 10 companies account for almost half of the global sales of drugs. In the same period, they spent in excess of US$20 billion in research and development, which is around 10% of their sales revenue.

Table 1.4 shows the R&D investments into drug research by research-based US pharmaceutical companies and the National Institutes of Health (NIH) for the period from 1991 to 2000. The enormous spending on R&D has escalated in recent years. According to a report, PhRMA Annual Report 2000–2001, US pharmaceutical companies have almost double their R&D spending every five years since 1980. Out of every $5 in sales, $1 is put back into R&D.

Pharmaceutical firms have to ensure that there is a pipeline of new and better drugs to return the substantial investments made. It is estimated that large pharmaceutical firms need 4–5 new drugs approved every year to maintain their premium positions. However, most firms are far short of this target, with only about 1–2 new drugs approved per year.

According to the May 2003 report by IMS Health, the sales of pharmaceuticals through retail pharmacies in 13 major world markets for the 12 months to March 2003 grew at 6% to US$284.4 billion. Biopharmaceutical products make up to about 7% of the total pharmaceutical markets of US$400 billion. However, the growth rate for biopharmaceuticals is high, and it is expected that half the total pharmaceutical market will be biopharmaceuticals within the next 10–20 years.

The top five biopharmaceutical companies are listed in Table 1.5.

Table 1.3 The top 10 pharmaceutical companies, 12 months to September 2002

Company	Rank	Market share
Pfizer	1	7.3%
GlaxoSmithKline	2	7.1%
Merck	3	5.1%
Johnson & Johnson	4	4.6%
AstraZeneca	5	4.6%
Novartis	6	4.0%
Bristol-Myers Squibb	7	3.7%
Aventis	8	3.6%
Roche	9	3.1%
Pharmacia	10	3.0%

SOURCE IMS *World Review 2003*, http://www.ims-global.com/insight/ news_story/0302/news_story_030227htm [accessed Mar 25, 2003].

Table 1.4 R&D investments by research-based US pharmaceutical companies and the National Institutes of Health (NIH)

Year	Companies (US$ billion)	NIH (US$ billion)
1991	10.0	8.9
1992	11.5	9.6
1993	12.4	10.8
1994	13.2	11.3
1995	15.2	11.8
1996	17.0	12.4
1997	19.0	13.5
1998	21.0	14.3
1999	24.0	16.0
2000	26.4	17.8

SOURCE Zoon, K.C., FDA CBER: Update — 100 years of biologics regulation, *FDA Consumer Magazine* (2002).

Table 1.5 The top five biopharmaceutical companies, 2002

Companies	Sales (US$ billion)
Amgen	4.0
Genentech	2.2
Serono International	1.4
Biogen	1.1
Immunex	1.0

SOURCE Contract Pharma, http://www.contractpharma.com/JulyAug022.htm [accessed Sep 18, 2002].

1.4 ECONOMICS OF DRUG DISCOVERY AND DEVELOPMENT

The pharmaceutical market is very competitive. It is imperative that pharmaceutical companies (including biotechnology companies), large or small, discover and develop drugs efficiently and within the shortest time span to remain competitive.

Figure 1.2 shows the expenses versus revenues to a company's investment in developing a new drug. Up until the clinical stage, the investment is substantial in the discovery and development processes. The largest cash demand is in the clinical trial stages, where hundreds and thousands of human subjects have to be recruited to test the drug.

A positive return of revenue only occurs after the drug has been approved by regulatory authorities for marketing. The overall profitability of a drug is the difference between the positive returns and the negative expenses within the patent period of 20 years. After that period, if the patent is not extended, there is no further protection on the intellectual rights for the drug.

After patent expiry, generic drugs from other companies are unencumbered by patent rights infringement and can encroach into the profitability of the company that developed the original drug. It is thus crucial that drugs are marketed as quickly as possible to ensure there is maximum patent coverage period and to be 'first to market', to establish a premium position. When cimetidine (Zantac, GlaxoSmithKline) came off patent in the US, it lost almost 90% of sales within four years (from $2085 million in 1995, to $277 million in 1999).

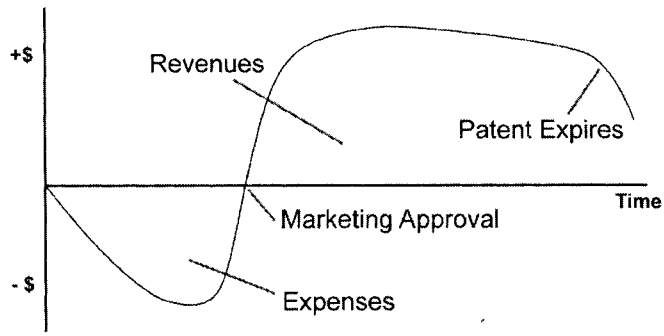

Figure 1.2 Expenses and revenues curve for a new drug

Exhibit 1.5 provides a brief explanation of patents. Patents are the pillars that support the drug industry. In contrast, traditional medicines, which are mainly derived from natural products of plant or animal origins, are not patentable. This is because traditional medicines consist of a multitude of compounds and it is difficult to establish patent claims based on varying quantities of materials.

Exhibit 1.5 Patents

A patent is a right granted by a government for any device, substance, method or process that is new, inventive and useful. The patent discloses the know-how for the invention. In return for this disclosure, the owner of a patent is given a 20-year period of monopoly rights to commercial returns from exploiting the invention.

There are two ways to register patents: either through applying in individual countries, which means multiple applications for different countries, or through designating the desired countries in a single application using the Patent Cooperation Treaty (PCT) mechanism. There are more than 90 member countries belonging to the PCT, including major developed countries.

PCT does not grant patents. Application with PCT goes through two phases: an international phase and a national phase. The international phase is where the application is searched, published and subjected to preliminary examination. Then the application enters into the national phase in each country. The application is subjected to examination and granting procedures in each country.

Another important item for a patent is the priority date. The priority date is established when a patent application is filed for the first time. If the invention is known before this date, then the patent is not granted. Most countries are first-to-file countries, which means that the patent is awarded to the person with the earliest filing date. In the US, patents are awarded to the first person to invent. The inventor can attempt to show the invention was made before another person's filing date to claim priority.

SOURCE The Patent Cooperation Treaty, http://www.wipo.org/ pct/en/index.html [accessed Oct 8, 2002].

1.5 TRENDS IN DRUG DISCOVERY AND DEVELOPMENT

The approach to drug discovery and development can generally be classified into the following areas:

- Irrational Approach
- Rational Approach

- Antisense Drugs
- Biologics
- Gene Therapy
- Stem Cell Therapy– both somatic cell and germ cell.

Irrational Approach: This approach is the historical method of discovering and developing drugs. It involves empirical observations of the pharmacological effects from screening of many chemical compounds, mainly those from natural products. The active component that gives rise to the observed effects is isolated. The chemical formula is determined, and modifications are made to improve its properties. This approach has yielded most drugs available today.

Rational Approach: This approach requires three-dimensional knowledge of the target structure involved in the disease. Drugs are designed to interact with this target structure to create a beneficial response. This is an emerging field in drug discovery.

Antisense Therapy: This is a relatively new approach and it requires the modifications to oligonucleotides that can bind to RNA and DNA (refer to Appendix 2 for a description of cell structure, genes, DNA, RNA and proteins). The antisense drugs are used to stop transcriptional (from DNA) or translational (from RNA) pathways from proceeding, and so interfere with the process of disease.

Biologics: These are mainly protein-based drugs in the form of antibodies, vaccines and cytokines. Their discoveries generally start from an understanding of the biological mechanistic pathways that cause specific diseases. Manufacturing of these drugs is based on recombinant DNA technologies using living organisms such as bacteria, yeast and mammalian and insect cells.

Gene Therapy: The basis of this therapy is to remedy a diseased gene or insert a missing gene. This is a hot new topic that raises many ethical considerations to resolve. The diseased gene is taken out from a patient, fixed outside the body (*ex vivo*) and then reinserted back into the body. In the case of missing gene, a copy of the new gene is inserted into the patient. The aim is for the inserted gene to influence the disease pathway or to initiate manufacture of the missing proteins or enzymes.

Stem Cell Therapy: With stem cell therapy, the aim is to grow body parts to

replace defective human organs and nerves. The stem cells are harvested from very early embryos or umbilical cord blood. Because of the very young age of these cells, they can be directed to grow into organ tissue to replace diseased tissue. The stem cell technology can provide an alternative to organ transplants with perhaps less rejection problems than the current practice of obtaining parts from another donor person. Stem cell therapy using germ cells involves cloning, and there are strict regulatory guidelines on how research is to be conducted.

Human genomic research has discovered many novel disease targets, which can be utilized to develop better and more effective drugs. Regardless of the approach used for discovering new drugs, pharmaceutical and biotechnology companies are now using a full suite of technologies to discover new drugs. These enabling technologies include:

- Microarray for Disease Target Identification
- High Throughput Screening
- Combinatorial Chemistry
- Structure–Activity Relationships: X-ray Crystallography, Nuclear Magnetic Resonance, Computational Chemistry
- Bioinformatics: Data Mining
- Recombinant DNA Technologies.

Detailed discussions of these technologies are presented in Chapters 2–4.

1.6 FURTHER READING

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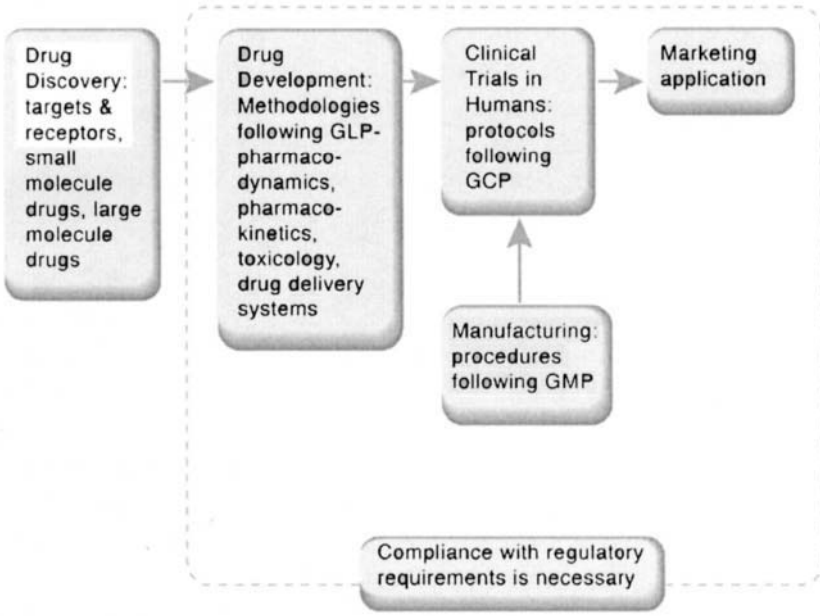
CHAPTER 2

DRUG DISCOVERY: TARGETS AND RECEPTORS



2.1 DRUG DISCOVERY PROCESSES

For a drug to work, it has to interact with a disease target in our body and intervene its wayward functions. An analogy is the lock and key comparison, with the lock being the disease target and the key representing the drug. The correct key has to be found to turn the lock and open the door to treat the disease.

The conventional method for drug discovery under the irrational approach is to scan thousands of potential compounds from natural sources for a hit against specific assays that represent the target (more about this in Chapter 3). This procedure has been compared to finding a needle in a haystack. In our analogy, it is like trying out many keys to find a fit to a lock. As we can imagine such a process is somewhat random and cumbersome. The chances for failure are high, although it should be borne in mind that most drugs on the market today were discovered in this manner.

Further advances in drug discovery led to the rational approach. This approach starts with finding out about the structure of the target and then designing a drug to fit the target and modify its functions. A comparison to the lock and key concept is to determine the construction of pin tumblers in the lock first and then design the key with the appropriate slots and grooves to pick it and open the door. The latest progress in drug discovery is contribution from genomics and proteomics research. Here the emphasis is to identify and validate targets *a priori* to drug discovery. This approach is to find out the target that causes the disease as the first step in drug discovery. After that, the rational approach would proceed. The analogy is to find out the exact diseased lock and then discover a drug to unlock the correct door.

With the foregoing in mind, the typical current drug discovery processes would proceed according to the flow chart in Figure 2.1. This chapter focuses on the medical needs, identification and validation of disease targets; followed by discussions on receptors, signal transduction and assay development. Chapters 3 and 4 focus on lead compound generation and optimization, for small, synthetic drug molecules and large, protein-based macromolecules, respectively. In Chapter 5, we cover drug development and preclinical studies.

2.2 MEDICAL NEEDS

A pharmaceutical organization has to determine which medical area has an unmet clinical need for an effective prophylactic or therapeutic intervention.

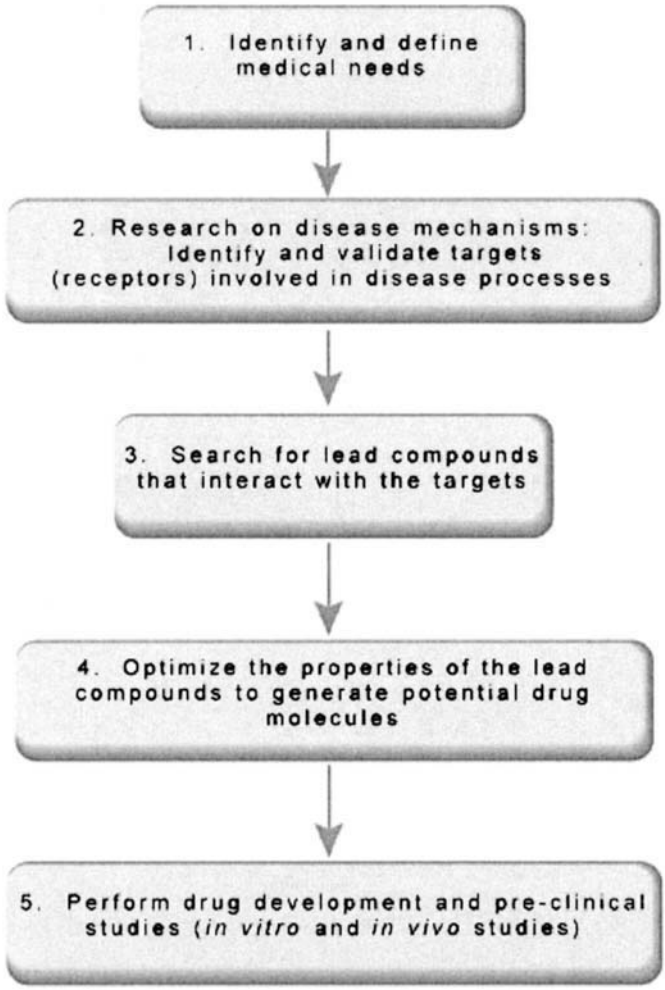


Figure 2.1 Flow chart of drug discovery processes

Next the organization has to evaluate its core competency, technological advantages, competitive barriers and financial resources before committing to develop a drug to fulfill the unmet need. As discussed in Chapter 1, for a monetary outlay that averages US$500 million for each drug development, the organization has to weigh its options carefully. The important factors to consider are:

- Market potential
- Patent, intellectual property portfolio
- Competitive forces and regulatory status
- Core competencies.

Overall, the organization needs to project the expected returns from such an investment and assess the competitive factors and barriers, including government regulations, before deciding which drug to develop.

Table 2.1 shows the therapy classes in terms of global sales in 2001. The top three therapy classes are antiulcerants, cholesterol and triglyceride reducers, and antidepressants. They account for 49% of sales in these top 10 therapy classes. There are significant changes to the growth of some therapy classes within a single year. This is especially true when new and more effective drugs are introduced; their sales can increase dramatically within a short time span and surpass the sales of more 'established' drugs.

Pharmaceutical companies have to be continuously vigilant and forecast the future directions of drug developments and regulatory requirements. They have to use their core competencies to deliver a pipeline of products to remain competitive and profitable in the long term.

2.3 TARGET IDENTIFICATION

2.3.1 Genes

Most diseases, except in the case of trauma and infectious diseases, have a genetic connection. Genetic makeup and variations (see single nucleotide

Table 2.1 Leading therapy classes, 2001

Therapy class	2001 sales (US$ billion)	Global sales	Growth
Antiulcerants	19.5	6%	+14%
Cholesterol and triglyceride reducers	18.9	5%	+22%
Antidepressants	15.9	5%	+20%
Antirheumatics (non-steroidal)	10.9	3%	+16%
Calcium antagonists (plain)	9.9	3%	+4%
Antipsychotics	7.7	2%	+30%
Oral antidiabetics	7.6	2%	+30%
Angiotensin-converting enzyme (ACE) Inhibitors (plain)	7.5	2%	+5%
Cephalosporins and combinations	6.7	2%	0%
Antihistimines (systemic)	6.7	2%	+22%

SOURCE IMS *World Review 2002*, http://www.ims-global.com/insight/news_story/0204/news_story_020430.htm [accessed Jun 8, 2002].

polymorphism in Section 11.5) determine a person's individuality and susceptibility to diseases, pathogens and drug responses.

The current method of drug discovery commences with the study of how the body functions, in both normal and abnormal cases afflicted with diseases. The aim is to break down the disease process into the cellular and molecular levels. An understanding of the status of genes and their associated proteins would help to pinpoint the cause of the disease. Drugs can be tailor-made to attack the epicenter of the diseases. In this way, more specific (fewer side effects) and effective (high therapeutic index, see Section 5.2) drugs can be discovered and manufactured to intervene or restore the cellular or molecular dysfunction.

From the Human Genome Project, we know that there are approximately three billion base pairs that make up the DNA molecule (refer to Appendix 2). Only certain segments of the enormous DNA molecule encode for proteins. These segments are called genes. The estimate is that there are about 30 000–40 000 genes that encode proteins. Exhibit 2.1 provides some information about the number of genes and the complexity of life forms.

From these 30 000 to 40 000 genes, many thousands of proteins are produced. Drug targets are normally protein or glycoprotein molecules

Exhibit 2.1 Genes and Molecular Complexity

The number of protein-coding genes in an organism provides a useful indication of its molecular complexity, although there is as yet no firm correlation between the number of genes and biological complexity.

Single-celled organisms typically have a few thousand genes. For example, *Escherichia coli* (a bacteria commonly found in the intestines of animals and humans) has 4300 genes, and *Saccharomyces cerevisiae* (a fungus commonly known as baker's or brewer's yeast) has 6000 genes. *Caenorhabditis elegan* (a small soil nematode about 1 mm long) has 19 000 genes. *Drosophila melanogaster* (a 3 mm fruit fly) has 13 600 genes. For human beings, the number of genes is estimated at around 35 000.

It was initially thought that the number of human genes was of the order of 100 000. The smaller number of 35 000 was surprising considering the complexity of human beings compared with smaller organisms. The latest view is that, although the number of genes indicates complexity, there is more involved in determining complexity. Each gene may code for more than one protein, to account for human complexity.

SOURCE Ewing, B. and Green, P., Analysis of expressed sequence tags indicates 35,000 human genes, *Nature Genetics*, 25, pp. 232–234 (2000).

because proteins are the ingredients for enzymes and receptors, with which drugs interact. To date, only about 500 proteins have been targeted by the multitudes of drugs in the market. The opportunities that are opened up by the genomics and proteomics research have paved the way for many more targets and new drugs to be discovered.

Exhibits 2.2, 2.3 and 2.4 provide examples of genetic causes of diseases, for example cancer, sickle cell anemia and cystic fibrosis.

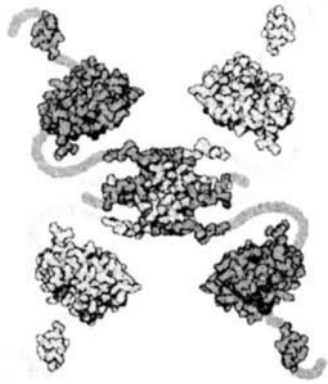
Exhibit 2.2 The p53 Protein in Cancer

The p53 gene is a tumor suppressor gene, which means that its activity stops the formation of tumors via the production of p53 protein. As shown in the picture below, the p53 protein has four identical chains, which are joined together by a central tetramerization domain. The p53 protein molecule wraps around and binds DNA. This wrapping action then turns on another gene, which codes for a 21-kDa protein that regulates DNA synthesis.

Normally a cell grows by cell division and then dies through a process called apoptosis—programmed cell death. The p53 protein triggers apoptosis, which is a 'stop signal' for cell division, to arrest cancer growth.

In the case of cancer growth, the gene that codes for p53 is mutated. The mechanism for programmed cell death becomes inactivated and no longer functions. Cancer cells then just keep on growing and dividing at the expense of surrounding cells, thus leading to tumor formation.

SOURCE Campbell, M.K., *Biochemistry*, 3rd edn., Harcourt Brace College Publishers, Orlando, FL, 1999.



p53 molecule picture: Goodsell, D.S., The Scripps Institute, Featured Molecule: p53 Tumor Suppressor, *Bio.Com*, http://www.bio.com/ [accessed Sep 7, 2002].

Exhibit 2.3 Sickle Cell Anemia

Hemoglobin is a tetramer with four polypeptide chains: two identical α chains (141 residues) and two identical β chains (146 residues).

In people with sickle cell anemia, there is just one mutation in each of the β chains. The glutamic acid in position 6 is substituted by valine. This substitution, two residues out of a total of 474, is sufficient to cause the red blood cell to deform and constrict blood flow by blocking the capillaries.

SOURCE Campbell, M.K., *Biochemistry*, 3rd edn., Harcourt Brace College Publishers, Orlando, FL, 1999.

Exhibit 2.4 Cystic Fibrosis

Cystic fibrosis (CF) is a hereditary disease of abnormal fluid secretion. It affects cells of the exocrine glands, such as intestine, sweat glands, pancreas, reproductive tract, and especially the respiratory tract. The disease affects about one in 2500 infants of the Caucasian population to varying degrees of seriousness. Patients produce thickened mucous that is difficult to get out of the airway. This leads to chronic lung infection, which progressively destroys pulmonary function.

CF is caused by the absence of a protein called cystic fibrosis transmembrane conductance regulator (CFTR). This protein is required for the transport of chloride ions across cell membranes. On the molecular level, there is a mutation in the gene that encodes for CFTR. As a result, CFTR cannot be processed properly by the cell and is unable to reach the exocrine glands to assume its transport function.

SOURCE Karp, G., *Cell and Molecular Biology, Concepts and Experiments*, John Wiley & Sons, New York, 1996.

2.3.2 Targets

There are a number of techniques used for target identification. Radioligand binding was a common technique until recently. Now DNA microarrays, expressed sequence tags, and *in silico* methods are used.

Radioligand binding The classical way to discover drug targets or receptors is to bind the potential receptors with radioligands (see Exhibit 2.5) so that targets can be picked out from a pool of other receptors. Bound receptors are then separated from the radioligands, cloned and their nucleotide sequence decoded. Potential drug molecules are then studied with these receptors or their nucleotide sequences to determine their interactions in terms of biochemical and functional properties.

Exhibit 2.5 Radioligands

Ligands are molecules that bind to a target. They may be endogenous (that is, produced by the body), such as hormones and neurotransmitters, or exogenous, such as drug molecules. Ligands (exogenous or endogenous) with high specificity for particular targets are labeled with radioisotopes. The tissue known to contain the target is mixed with a known quantity of the radioligands. Those targets bound with radioligands are separated by rapid filtration or centrifugation, followed by washing with cold buffers to remove unbound ligands. Scintillation counting techniques are used to reveal the amount of bound radioligands.

The target bound with radioligands can be isolated and its amino acid sequence determined. The sequence information enables classifications of the target based on previously known targets. Targets that do not appear to show homology to known ligands and that have no known endogenous ligand are called 'orphan' targets. Active research is ongoing to find molecules of compounds to interact with these orphan targets as possible sites for therapy.

Sequence information can be used to clone the target by using recombinant technology. In this way, biochemical pathways of the target can be studied in detail, rendering the development of a drug molecule with higher chances of success.

DNA microarray DNA microarray, also known as DNA or gene chips, is a new technology to investigate how genes interact with one another and how they control biological mechanisms in the body. The gene expression profile is dynamic and responds to external stimuli rapidly. By measuring the expression profile, scientists can assess the clues for the regulatory mechanisms, biochemical pathways and cellular functions. In this way, microarrays enable scientists to find out the target genes that cause disease.

The heart of the technology is a glass slide or membrane that consists of a regular array of genes (Figure 2.2). Thousands of genes can be spotted on the array, using a photolithography method. Samples from healthy and diseased cells are mixed with the genes on the array. In this way, many genes can be studied and their expression levels in healthy and diseased states can be determined within a short time. The gene that is responsible for a particular disease can be identified. Exhibit 2.6 presents a more detailed explanation of microarrays.

Expressed sequence tags and in silico methods Expressed Sequence Tags (ESTs) are short nucleotide sequences of complementary DNA with about 200–500 base pairs. They are parts of the DNA that code for the expression of particular proteins. EST sequencing provides a rapid method to scan for all the protein coding genes and to provide a tag for each gene on the genome.