THIRD EDITION

# DRUGS

## From Discovery to Approval

RICK NG

WILEY Blackwell

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Third Edition

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To
Cherry, Shaun and Ashleigh

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# PREFACE

In 2001, I decided to write a book about pharmaceuticals to cover the topics in drug discovery and development, manufacturing, and regulatory compliance. My intention is to have a book that is relevant, informative, and easy to read and understand. My target readership is the pharmaceutical professionals, healthcare students, and general public who wish to have practical information about pharmaceuticals. The result was this book – *Drugs: From Discovery to Approval*, first published in 2004 and then the second edition in 2009.

For this third edition, I have completely revised the contents to include the latest advances and developments. I have added new information and examples. There are now two case studies at the end of each chapter to provide more in-depth perspectives on current issues facing the pharmaceutical industry.

I am particularly grateful to Dr. Wayne Gordon, who read through the entire manuscript and provided many helpful suggestions. Dr. Loh Kean Chong and Dr. Dinesh Khokal have read through all the editions, and their contributions are very much appreciated. I also wish to thank Dr. Peter New and Mr. Chris Sweeney for their perceptive comments on different sections of this book. I am indebted to Mr. Ryan O'Connell, who meticulously checked through the manuscript and corrected the errors and inconsistencies.

I acknowledge the assistance of Ms. Mindy Okura-Marszycki (Senior Acquisitions Editor) and Ms. Stephanie Dollan (Editorial Assistant) from John Wiley and Sons, Inc. My thanks go to them for supporting this edition and arranging access to John Wiley's database for my research and reference.

My family has given me unqualified support and encouragement throughout the time I spent writing and revising these three editions. I am thankful to my wife and children for believing in me and I dedicate this book to them.

Rick Ng
March 2015

# CHAPTER 1

# INTRODUCTION

## 1.1 AIM OF THIS BOOK

The pharmaceutical industry is one of the most regulated industries in the world. From discovering a new drug to registering it for marketing and commercialization, pharmaceutical organizations have to negotiate through very complex and lengthy processes. These processes are necessary to ensure drug products are safe and efficacious, and that their benefits far outweigh their risks.

The intention of this book is to provide an overview of how a drug is discovered, the number 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. Regulatory authorities play an important role in these processes, overseeing the safety and efficacy of drugs through legislation. This book aims to integrate, in a simplified manner, the relationships between all these complex processes and procedures.

To establish a frame of reference, it is necessary 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 that 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 medicinal substances or pharmaceutical products, which provide 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.

*Drugs: From Discovery to Approval*, Third Edition. Rick Ng.

**Exhibit 1.1 FDA Definition of a Drug**

A drug is defined as:

- A substance recognized by an official pharmacopeia or formulary.
- A substance intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease.
- A substance (other than food) intended to affect the structure or any function of the body.
- A substance intended for use as a component of a medicine but not a device or a component, part or accessory of a device.
- Biological products are included within this definition and are generally covered by the same laws and regulations, but differences exist regarding their manufacturing processes (chemical process vs biological process).

*Source*: Data from 1. Food and Drug Administration 2012, *Drugs@FDA Glossary of Terms,* viewed January 6, 2014, http://www.fda.gov/drugs/informationondrugs/ucm079436.htm; 2. World Health Organization 2014, *International Nonproprietary Names,* viewed Jun 19, 2014, http://www.who.int/medicines/services/inn/en/

It should be noted that there are normally three names associated with a drug: the trade or proprietary name (e.g., Lipitor), generic name, or nonproprietary name (e.g., atorvastatin), and a specific chemical name for the active ingredient (e.g., [*R*-(*R**,*R**)]-2-(4-fluorophenyl)-β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate).

The International Nonproprietary Names (INN) of the World Health Organization (WHO) are the unique standard names, also known as generic names, for the active pharmaceutical ingredients in drug products.

The descriptions in this book on discovery and regulatory processes are primarily concerned with 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 pharmacies without prescriptions. OTC drugs are mainly established drugs with long histories of use and are deemed to be safe to be taken without supervision by physicians.

There is a further differentiation of ethical drugs into new drugs (those covered by patents) and generics (copies of drugs that have expired patents – refer to Case Study #10.2). Most of the descriptions in this book apply to new drugs.

## 1.2 AN OVERVIEW OF THE DRUG DISCOVERY TO APPROVAL PROCESS

Although human civilization has been experimenting and consuming drugs for many centuries, it is only in the past hundred years that a foundation has been laid for the systematic research and development (R&D) of drugs. Readers are referred to

Appendix 1 for a brief description of the history of drug development since ancient times.

Today, personnel from many fields are involved in the process of drug discovery and development, namely, scientists, clinicians, medical practitioners, and statisticians. Even persons from seemingly disparate occupations, such as economists, lawyers, and regulatory staff, play vital roles. Previously, the main scientific personnel involved in the discovery process have been synthetic chemists. However, drug discovery and development has made a quantum leap forward in recent times with progress in genomics/proteomics and biotechnology. This has led to an increased importance in the role of molecular biologists, biochemists, microbiologists, engineers, and even computer scientists in the discovery and development of drugs. 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 to progress from initial research to the commercialization stage. The cost of this process was estimated to be more than US$1 billion in the early 2000s, compared to US$140 million in the 1970s. It should be noted that this expenditure is not because of the cost of developing successful drugs alone; it is exacerbated by the amortized cost of failed drugs that do not demonstrate sufficient benefits over risks during clinical trials. From discovery to marketing approval of a drug, the following stages are involved (Figure 1.1):

***Drug Discovery:*** The process begins with discovering the target that causes or leads to the disease. Next, chemical or biological compounds are screened using specific assays and tested against these targets to find leading candidates for further development. Many new scientific approaches are now used to determine targets (most targets are

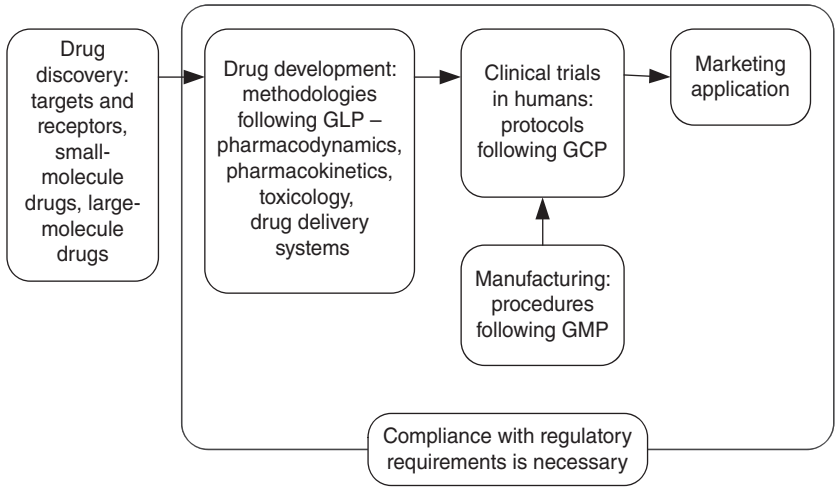



**Figure 1.1** The stages from drug discovery to marketing approval.

receptors or enzymes) and obtain the lead compounds, including the use of genomic and proteomic technologies, synthetic chemistry, recombinant DNA (rDNA) technology, laboratory automation, and bioinformatics.

***Preclinical Drug Development:*** Tests are performed with 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 (sometimes also called nonclinical, these terms are used interchangeably) 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, to reduce toxicity or to improve pharmacokinetic performance. At the end of this process, an optimized compound is found and it 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 Development:*** Clinical trials using the drug dosage form intended for marketing are 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 investigators, institutional review board, informed consent, trial monitoring, and adverse event reporting. These parameters form the basis of Good Clinical Practice (GCP). Clinical trials must follow regulations and guidelines from the US FDA, the European Medicines Agency (EMA) of the European Union (EU) or European Member States, Japan's Pharmaceuticals and Medical Devices Agency (PMDA), or regulatory authorities in other prospective countries where the drug is intended to be registered and commercialized.

***Manufacturing:*** The drug designated for clinical trials has to be manufactured in compliance with current Good Manufacturing Practice (cGMP; the word "current" denotes that regulations change from time to time and the current regulations have to be applied) following US FDA requirements, EU Regulations or Directives, or International Conference on Harmonization (ICH) guidelines (refer to Chapter 7). Regulatory authorities have the right 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 an audit trail, that is, traceability of materials, processes, and personnel involved, 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 they are fit for their intended functions.

***Marketing Application, Approval, and Postapproval:*** 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 EMA, or Japan's

PMDA. Extensive dossiers and samples, if required, are provided to the authorities to demonstrate the safety, potency, efficacy, and purity of the drug. These are provided in the form of laboratory (pharmacology, toxicology), clinical (on humans), and manufacturing data, which comply with GLP, GCP, and GMP requirements, respectively. After the drug has been approved and marketed, the safety and performance of the drug is continually monitored to ensure that it is prescribed correctly, and adverse events (side effects) are reported and investigated. The advertising of drugs is also scrutinized by regulatory authorities to ensure there are no false representations of or claims about the drugs. Furthermore, the commercial-scale manufacture of drugs must comply with GMP, and manufacturing facilities are inspected by regulatory authorities at periodic intervals. Variations to manufacturing processes, materials or specifications, and changes to labeling are to be reported to the regulatory authorities, and in some cases variations require prior regulatory approvals before implementations.

The subsequent chapters 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. A time span of 10–12 years is now generally ascribed to the discovery and development process, despite many new methods and technologies being utilized in an attempt to shorten the period. Pharmaceutical companies and regulatory authorities are working together to reduce this time span to control costs and expedite the time frame for drug marketing approval to cater to unmet medical needs.

The average cost of developing a new drug is estimated to be about US$1–1.2 billion, including expenditures on failed projects. This amount is about three times the price of an Airbus A380-800 at US$400 million, or five times that of a Boeing B-787 Dreamliner at US$250 million.

Typically, tens of thousands of compounds are screened and tested, and only a handful makes it into the market as drug products. The statistics are such that, of the 5,000–10,000 compounds that show initial promise, five will go into human clinical trials, and only one will become an approved drug.

*Source*: Data from 1. Pharmaceutical Research and Manufacturers of America 2013, *Statement on Prescription Medicine Cost Growth* – Press Releases Apr 30, 2013, viewed December 16, 2013, http://www.phrma.org/phrmapedia/search?search_api_views_fulltext=R%26D+cost, 2. Airbus 2013, *New Airbus Aircraft: List Prices for 2013,* viewed December 16, 2013, http://www.airbus.com/presscentre/pressreleases/press-release-detail/detail/new-airbus-aircraft-list-prices-for-2013/, 3. Boeing 2013, *Commercial Airplanes: Jet Prices,* viewed December 16, 2013, http://www.boeing.com/boeing/commercial/prices/, 4. Tufts Center for the Study of Drug Development 2006, *No shortage of controversy in biotech drug creation costs,* viewed December 16, 2013, http://www.bizjournals.com/sanfrancisco/stories/2006/12/04/newscolumn3.html

## 1.3 THE PHARMACEUTICAL INDUSTRY

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

Drug discovery and development are primarily carried out by pharmaceutical companies, universities, and government research agencies, although there are increasing activities in smaller companies and start-ups that specialize in particular fields of research. A substantial number of the research findings and potential drugs from start-ups, smaller companies, universities, and research organizations are, however, licensed to multinational pharmaceutical companies that have the resources for clinical trials, manufacturing, marketing, and distribution. Alternatively, alliances are formed with the multinational pharmaceutical companies to develop or market the drugs. A primary reason for these business relationships is the huge cost involved in drug discovery, development, and commercialization.

In 2012, the combined worldwide pharmaceutical market was around US$962 billion. The distribution of the market (in US$ billion) is shown in Table 1.1. From this data, it is evident that the United States, Europe, and Japan accounted for more than 70% of the worldwide pharmaceutical market. Therefore, the regulatory authorities in these countries are very important to the pharmaceutical companies to ensure their products are approved for commercialization.

Table 1.2 shows the top 10 best-selling drugs in 2012; five of them are small molecule synthetic drugs (refer to Chapter 3) and five are biopharmaceuticals (biologics) or large molecule drugs (refer to Chapter 4). Six years ago only one biopharmaceutical, Enbrel, was in the top 10 list. All of the top 10 drugs are "blockbuster" drugs, meaning each has sales exceeding US$1 billion. While the majority of drugs have been based on small molecules for many years, biopharmaceuticals have become increasingly important in the past three decades since the first one was introduced. The biopharmaceutical market has grown substantially compared to that of the small molecule drugs. For comparison,

**TABLE 1.1 Global Pharmaceutical Sales by Region, 2012**

| World | 2012 sales (US$ billion) | % Global sales | % Growth from previous year |
|---|---|---|---|
| North America | 348.7 | 36.2 | −1.0 |
| Europe (EU + non-EU) | 221.8 | 23.1 | −0.8 |
| Asia (including Indian subcontinent/ Africa/Australia) | 168.3 | 17.5 | +12.8 |
| Japan | 112.1 | 11.7 | 0.0 |
| Latin America | 72.5 | 7.5 | +10.9 |
| Others | 38.7 | 4.0 | +4.5 |
| **Total** | **962.1** | **100.0** | **+2.4** |

*Source*: Adapted from IMS Health Reports: Global Pharmaceutical Market by Region. Available at: http://www.imshealth.com/deployedfiles/imshealth/Global/Content/Corporate/Press%20Room/Total_World_Pharma_Market_Topline_metrics_2012-17_regions.pdf. Accessed December 14, 2013.

**TABLE 1.2 The Top 10 Best-selling Products, 2012**

| Product | Therapy | Company |
|---|---|---|
| Seretide/Advair | Asthma, chronic obstructive pulmonary diseases | GlaxoSmithKline |
| Humira | Rheumatoid arthritis, autoimmune diseases | Abbott |
| Crestor | Cholesterol lowering | AstraZeneca |
| Nexium | Peptic ulcer and gastroesophageal diseases | AstraZeneca |
| Enbrel | Rheumatoid and psoriatic arthritis | Amgen |
| Remicade | Crohn's disease, rheumatoid arthritis, and ulcerative colitis | J&J, Merck |
| Abilify | Schizophrenia, bipolar disorder, depressive disorder | Otsuka/Bristol-Myers-Squibb |
| Lantus | Insulin analog for diabetes | Sanofi-Aventis |
| Rituxan/Mabthera | Lymphomas, leukemias, transplant rejection, autoimmune diseases | Biogen Idec/Roche |
| Cymbalta | Depressive and anxiety disorders | Eli Lilly |

*Source*: Data from IMS. *Top 20 Global Products 2012.* Available at: http://www.imshealth.com/deployedfiles/ims/Global/Content/Corporate/Press%20Room/Top-Line%20Market%20Data%20&%20Trends/Top_20_Global_Products_2012_2.pdf. Accessed December 14, 2013.

**TABLE 1.3 The Top 10 Best-selling Biopharmaceuticals, 2013**

| Product | Therapy | Company |
|---|---|---|
| Humira | Rheumatoid arthritis, autoimmune diseases | Abbott |
| Enbrel | Rheumatoid and psoriatic arthritis | Amgen |
| Remicade | Crohn's disease, rheumatoid arthritis, and ulcerative colitis | J&J, Merck |
| Neulasta | Neutropenia | Amgen |
| Rituxan | Lymphomas, leukemias, transplant rejection, autoimmune diseases | Biogen Idec/Roche |
| Lantus | Insulin analog for diabetes | Sanofi-Aventis |
| Avastin | Cancers: colorectal, lung, breast, glioblastoma, kidney, and ovarian | Roche |
| Epogen | Anemia | Amgen |
| Herceptin | Breast cancer | Roche |
| Lucentis | Macular degeneration | Roche |

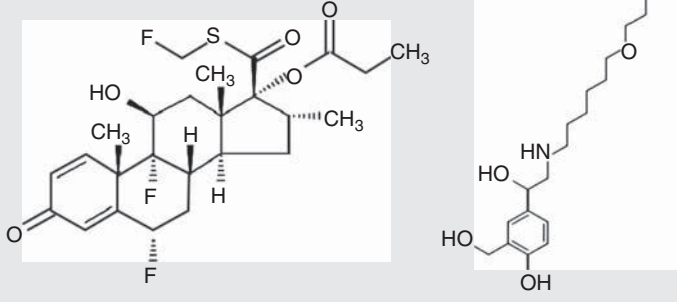
*Source*: Data from Drug.com 2014, *US Pharmaceutical Sales – Q3* 2013, viewed December 16, 2013, http://www.drugs.com/stats/top100/sales

Table 1.3 presents the top 10 best-selling biopharmaceuticals in 2013. Of the top 100 drugs in United States in Q3 2013, 32 are biopharmaceuticals, up from 18 in 2006. Exhibits 1.3 and 1.4 describe the top two drugs, Seretide/Advair and Humira, and their mechanisms of action in the treatment of asthma and inflammation, respectively.

**Exhibit 1.3 Seretide/Advair – Treatment of Asthma and COPD**

Seretide and Advair are different trade names of the same drug marketed by GlaxoSmithKline in different parts of the world. It is a combination of oral and inhalation product consisting of fluticasone and salmeterol. The active ingredients are fluticasone propionate and salmeterol xinafoate. Seretide/Advair is used in the management of asthma and chronic obstructive pulmonary disease (COPD).

*Fluticasone* *Salmeterol*




Fluticasone propionate is a corticosteroid with anti-inflammatory actions. It targets the human glucocorticoid receptor as an agonist. Corticosteroids have been shown to inhibit multiple cell types (e.g., mast cells, eosinophils, basophils, lymphocytes, macrophages, and neutrophils) and mediate the production of histamine, eicosanoids, leukotrienes, and cytokines that are involved in the asthmatic response.

Salmeterol xinafoate is a long-acting β2-adrenergic agonist. Its mechanism of action is to stimulate the enzyme adenyl cyclase that catalyzes the conversion of adenosine triphosphate (ATP) to cyclic-3′, 5′-adenosine monophosphate (cyclic AMP). An increase in cyclic AMP relaxes the bronchial smooth muscle and inhibits production of hypersensitive mediators such as mast cells.

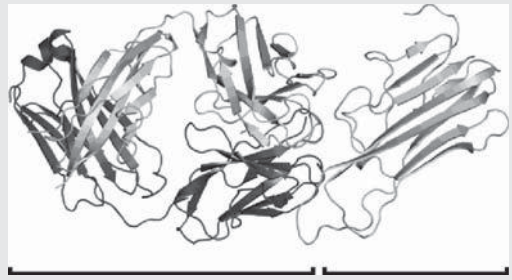
Seretide/Advair is supplied in 100, 250, or 500 mcg fluicasone propionate and 50 mcg salmeterol xinafoate formulated as inhalation powder. The powder is contained within blister packs in a metered dosed disposable inhalation device.

*Source*: Data from Food and Drug Administration 2003, *Advair,* viewed December 17, 2013, http://www.accessdata.fda.gov/drugsatfda_docs/label/2003/21077slr019_advair_lbl.pdf

**Exhibit 1.4 Humira – Treatment of Rheumatoid Arthritis and Autoimmune Diseases**

Humira is a tumor necrosis factor (TNF) blocker indicated for treatment of rheumatoid arthritis, juvenile idiopathic arthritis, psoriatic arthritis, ankylosing spondylitis, Crohn's disease, ulcerative colitis, and plaque psoriasis. The active ingredient, adalimumab, is a recombinant human IgG1 monoclonal antibody that targets the human TNF. Adalimumab is produced by recombinant DNA technology using a mammalian cell system. It has 1,330 amino acids and a molecular weight of approximately 148 kDa.

*Crystal structure of Humira (adalimumab) interacting with TNF*




*Source*: Protein Data Bank in Europe http://www.ebi.ac.uk/pdbe-srv/view/entry/3wd5/summary.html, viewed Nov 8, 2014. Reproduced with permission of European Bioinformatics Institute.

Humira is supplied in prefilled pen or prefilled syringe. Each prefilled syringe delivers 0.8 mL (40 mg) of the drug product. Other ingredients are sodium chloride, monobasic sodium phosphate dihydrate, dibasic sodium phosphate dihydrate, sodium citrate, citric acid monohydrate, mannitol, polysorbate 80, and water for injection, USP. Sodium hydroxide is added as necessary to adjust the pH to 5.2.

*Source*: Data from 1. Hu, S et al. 2013, 'Comparison of the inhibition mechanisms of Adalimumab and Infliximab in treating TNFalpha-associated diseases from a molecular view', *J. Biol. Chem.*, 288, 27059-27067; 2. Food and Drug Administration 2013, *Humira,* viewed Nov 11, 2014, http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/125057s310lbl.pdf

The top 10 pharmaceutical companies (according to market capitalization) in December 2013 are shown in Table 1.4. These 10 companies account for more than half of the global drug sales. In the same period, the top three companies collectively spent in excess of US$25 billion in R&D; this amount comprises more than 15% of their sales revenues, demonstrating the importance of R&D for these companies.

**TABLE 1.4 The Top 10 Pharmaceutical Companies: Dec 18, 2013**

| Rank | Company | Market Capitalization* (US$ billion) |
|---|---|---|
| 1 | Roche | 230.86 |
| 2 | Pfizer | 199.42 |
| 3 | Novartis | 188.80 |
| 4 | Merck & Co | 143.11 |
| 5 | Sanofi-Aventis | 135.50 |
| 6 | GlaxoSmithKline | 126.01 |
| 7 | Bayer | 112.13 |
| 8 | Bristol-Myers-Squibb | 86.59 |
| 9 | AbbVie | 86.25 |
| 10 | Amgen | 85.01 |

*Source*: Data from Google Finance 2013, *Market Summary 2013,* viewed December 18, 2013, http://www.google.com/finance?ei=2YOyUqjXJMidkgWc1QE

*Market capitalization is based on Dec 18, 2013 data, Roche acquired Genentech in 2009.

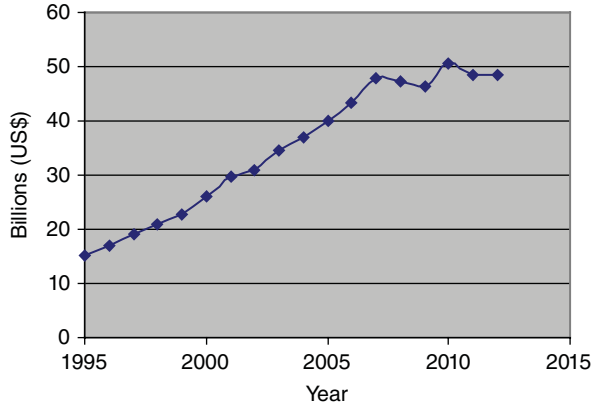



**Figure 1.2** R&D investments by research-based US pharmaceutical companies. (*Source*: Pharmaceutical Research and Manufacturers of America. 2013 *BIOPharmaceutical Research Industry Profile*. Washington, DC: PhARMA. Available at: http://phrma.org/sites/default/files/pdf/PhRMA%20Profile%202013.pdf. Accessed December 18, 2013. Reproduced with permission of the Pharmaceutical Research and Manufacturers of America.)

Further examples of R&D investments into drug research by research-based US pharmaceutical companies from 1995 to 2012 are shown in Figure 1.2. According to reports by the Pharmaceutical Research and Manufacturers of America (PhRMA), US pharmaceutical companies have almost doubled their R&D spending every 5 years since 1980. Out of every five dollars earned in sales, one dollar is put back into R&D. In 2012, the US pharmaceutical industry spent US$48.5 billion in developing new drugs.

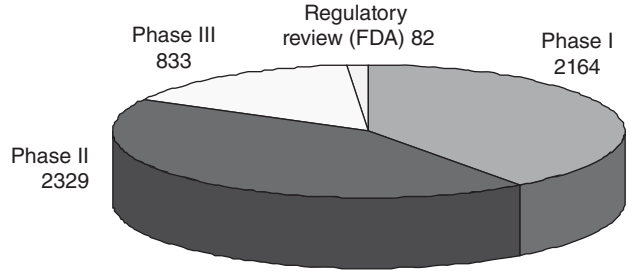



**Figure 1.3** New drugs in development by regulatory phases, 2011. (*Source*: Pharmaceutical Research and Manufacturers of America. 2013 *BIOPharmaceutical Research Industry Profile.* Washington, DC: PhARMA. Available at: http://phrma.org/sites/default/files/pdf/PhRMA%20Profile%202013.pdf. Accessed December 18, 2013. Reproduced with permission of the Pharmaceutical Research and Manufacturers of America.)

Pharmaceutical firms have to ensure there is a pipeline of new and better drugs to return the substantial investments made. It is estimated that large pharmaceutical firms need four–five new drugs approved every year to maintain their premium positions. However, most firms fall far short of this target, with only about one–two new drugs approved per year. From 2000 to 2012, it is estimated that around 400 new drugs were approved by FDA. Figure 1.3 presents a snapshot of the pipeline of new drugs at various phases of development in 2011. The areas of development are neurology, cardiovascular, cancer, psychiatry, diabetes, human immunodeficiency virus (HIV/AIDS), and infectious diseases.

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 10 biopharmaceutical companies in 2012 are listed in Table 1.5. The rise of Roche, the largest pharmaceutical company with an extensive portfolio of biopharmaceutical products, shows the importance of biopharmaceuticals.

## 1.4 ECONOMICS OF DRUG DISCOVERY AND DEVELOPMENT

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

Figure 1.4 shows the expenses versus revenues regarding 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 to thousands of human subjects are recruited to test the drug.

A positive return of revenue occurs only 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.

**TABLE 1.5 The Top 10 Biopharmaceutical Companies: 2012**

| Rank | Company | Market Capitalization* (US$ billion) |
|---|---|---|
| 1 | Roche | 230.86 |
| 2 | NovoNordisk | 76.9 |
| 3 | Amgen | 60.1 |
| 4 | Gilead Sciences | 40.2 |
| 5 | BiogenIdec | 34.3 |
| 6 | Teva | 34.2 |
| 7 | Baxter | 32.3 |
| 8 | Celgene | 28.4 |
| 9 | Merck | 21.2 |
| 10 | CSL | 21.1 |

*Source*: Data from Genetic Engineering and Biotechnology News 2012. Top 25 Biotech Companies. Available at: http://www.genengnews.com/insight-and-intelligenceand153/top-25-biotech-companies/77899671/?page=2. Accessed December 19, 2013.

*Market capitalization is based on Dec 18, 2013 data, Roche acquired Genentech in 2009.

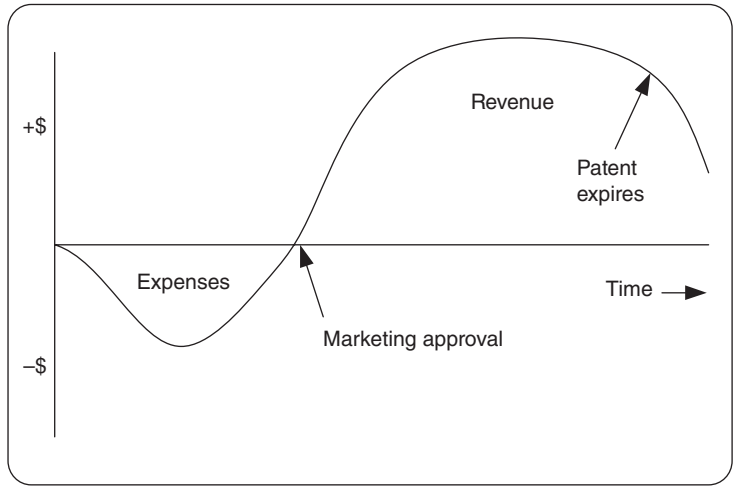



**Figure 1.4** Expenses and revenues curve for a new drug.

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 patented drug. It is thus crucial that drugs are marketed as quickly as possible to maximize the length of patent coverage period and to be "first to market," to establish a premium position. When atorvastatin (Lipitor, Pfizer) lost its patent exclusivity in November 2011, the sales of this best-ever-selling drug plunged from US$13.7 billion in 2006 to $US3.45 billion in 2013. Clopidogrel (Plavix,

**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 all information pertaining to the invention. In return for this disclosure, the owner of a patent is granted a 20-year period of monopoly rights to the commercial returns from the exploitation of 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 140 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 aspect of 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, meaning that the patent is awarded to the person with the earliest filing date. In the United States, patents are awarded to the first person to invent. The inventor can claim priority by proving the invention was made before another person's filing date.

Source: Data from World Intellectual Property Organization, *The Patent Cooperation Treaty,* viewed January 6, 2014, http://www.wipo.int/patentscope/en/patents/

Bristol-Myers Squibb) experienced a similar nosedive in sales, dropping from $US7.09 billion in 2011 to $US0.3 billion in 2013, a 95% loss within two years of the patent expiry in May 2012 (refer to Case Study #1.2).

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 on the basis of varying quantities of materials. Refer to Section 11.15 for further discussions on patents and marketing exclusivities.

## 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 approach
- RNA interference approach
- Biopharmaceuticals/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 many drugs that are 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. Having been established during the past three decades, the rational approach has become an important field in drug discovery.

***Antisense Approach:*** 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). Antisense drugs are used to stop transcriptional (from DNA) or translational (from RNA) pathways from proceeding and so interfere with the process of disease.

***RNAi Approach:*** This uses short interfering RNA (siRNA, sometimes called small interfering or silencing RNA) to interfere with the expression of a particular gene. The siRNAs are double-stranded RNAs of 20–25 nucleotides. It is envisaged that if the biological pathway of a disease is identified, siRNA could interfere to turn off the activity of the gene involved in the pathway and provide therapeutic effect.

***Biopharmaceuticals/Biologics:*** These are mainly protein-based drugs in the form of antibodies, vaccines, and cytokines. Their discovery generally starts from an understanding of the biological mechanistic pathways that cause specific diseases. Manufacture of these drugs is based mainly on rDNA technologies using living organisms such as bacteria, yeast, and mammalian and insect cells.

***Gene Therapy:*** This therapy is based on remedying a diseased gene by inserting a missing gene or modified gene in the cells. This is an emerging field that raises many unresolved ethical considerations. The cells with the diseased gene are taken out of a patient, modified outside the body (*ex vivo*), and then reinserted back into the body. In the case of a missing gene, a copy of the new gene is inserted into the patient's cells. The aim is for the inserted gene to influence the disease pathway or to initiate synthesis of missing proteins or enzymes.

***Stem Cell Therapy:*** The aim of stem cell therapy is to grow body parts to replace defective human organs and nerves. 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. Recent research has enabled adult stem cells to behave similarly to embryonic stem cells and differentiate into different cell types and tissues. The stem cell technology can provide an alternative to organ transplants with perhaps lower rates of rejection than the current practice of obtaining organs from a donor. Stem cell therapy using germ cells involves cloning, and there are strict regulatory guidelines on how research is to be conducted.

Through the Human Genome Project many novel disease targets have been discovered, 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 are as follows:

- Microarray for disease target identification
- High-throughput screening
- Combinatorial chemistry
- Structure–activity relationships: X-ray crystallography, nuclear magnetic resonance, computational chemistry
- Genomics and proteomics
- Metabolomics
- Systems biology
- Nanotechnology
- Bioinformatics: data mining
- rDNA technologies.

Detailed discussions of these technologies are presented in Chapters 2–5.

## 1.6 CASE STUDY #1.1

### 1.6.1 Roche, Pfizer, and Novartis

This case study introduces the top three pharmaceutical companies in the world and their products.

***Roche, 2012:*** Roche is the largest pharmaceutical company in the world. The corporate headquarters is located in Basel, Switzerland.

In 2012, Roche sales amounted to 45.5 billion CHF (Swiss franc, 1CHF = US$1.11, January 2014). Roche's R&D expenditure was 8.48 billion CHF. Table 1.6 clearly demonstrates that pharmaceutical companies are heavily research-based; Roche and Pfizer's R&D expenditure is comparatively more than other technology companies with much higher market capitalization. Roche employed 82,089 people in 2012. The company specializes in oncology and its top five products in 2012 were the following:

**TABLE 1.6 R&D Expenditures in R&D-Based Companies**

| Company | Market Capitalization, US$ Billion (December 18, 2013) | Revenue, US$ Billion (2012) | R&D Expenditure, US$ Billion (2012) |
|---|---|---|---|
| Roche | 230.86 | 45.50 | 8.48 |
| Pfizer | 199.42 | 58.99 | 7.87 |
| Apple | 495.55 | 170.91* | 4.48 |
| Google | 362.40 | 50.18 | 6.59 |
| Microsoft | 305.37 | 77.85* | 10.41 |
| Intel | 125.27 | 53.34 | 10.15 |
| Boeing | 101.81 | 81.70 | 3.30 |

*Source*: Data from Google Finance 2013, *Market Summary* 2013, viewed December 18, 2013, http://www.google.com/finance?ei=IH-yUpCVE8KRkQXR6wE

*Denotes July 2012 to June 2013.

- Rituxan/MabThera – an antibody for the treatment of hematological cancers such as non-Hodgkin's lymphoma, chronic lymphocytic leukemia, transplant rejection, and autoimmune disorders (sales 6.71 billion CHF)
- Herceptin – an antibody for the treatment of breast and gastric cancers (sales 5.89 billion CHF)
- Avastin – an antibody for the treatment of colon, lung, renal, ovarian, and breast cancers (sales 5.76 billion CHF)
- Pegasys – an antiviral drug for the treatment of hepatitis B and C (sales 1.65 billion CHF)
- Xeloda – a small molecule drug for the treatment of metastatic breast and colorectal cancers (sales 1.52 billion CHF).

*Source*: Roche, *2012 Annual Report*, viewed December 18, 2013, http://www.roche.com/investors/annual_reports/annual_reports_2012.htm

***Pfizer, 2012:*** Pfizer is the second largest pharmaceutical company in the world. The corporate headquarters is located in New York, United States.

In 2012, Pfizer spent US$7.87 billion on R&D, and its income for that year was US$58.99 billion. Pfizer employed more than 91,500 people in 2012. The company focuses on five high-priority areas including small and large molecules in immunology and inflammation, oncology, cardiovascular and metabolic diseases, neuroscience and pain, and vaccines. The top five drugs marketed in 2012 by Pfizer were the following:

- Lyrica – a small molecule drug for the management of postherpetic neuralgia, neuropathic pain associated with diabetic peripheral neuropathy, the management of fibromyalgia, neuropathic pain because of spinal cord injury (sales US$4.15 billion)
- Lipitor – a small molecule drug for the treatment of elevated LDL–cholesterol levels in the blood (sales US$3.95 billion)

- Enbrel – an antibody for the treatment of moderate-to-severe rheumatoid arthritis, polyarticular juvenile rheumatoid arthritis, psoriatic arthritis, and plaque psoriasis and ankylosing spondylitis (sales US$3.74 billion)
- Prevenar 13-valent pneumococcal conjugate vaccine – a vaccine for the prevention of various syndromes of pneumococcal disease in infants and young children and in adults (sales US$3.72 billion)
- Celebrex – a small molecule drug for the treatment of osteoarthritis and rheumatoid arthritis and the management of acute pain (sales US$2.72 billion).

*Source*: Pfizer, *2012 Financial Report*, viewed December 18, 2013, http://www.pfizer.com/files/annualreport/2012/financial/financial2012.pdf

***Novartis, 2012:*** Novartis is the third largest pharmaceutical company in the world. The corporate headquarters is located in Basel, Switzerland.

In 2012, Novartis' sales income was US$56.67 billion and R&D expenses $9.3 billion. The company employed a total of 128,000 people. The top five products marketed by the company were the following:

- Gleevec/Glivec – a small molecule tyrosine kinase inhibitor in cancer treatment, in particular, Philadelphia chromosome-positive ($Ph^+$) chronic myelogenous leukemia (sales US$4.68 billion)
- Diovan – a small molecule angiotensin II receptor antagonist for the treatment of hypertension, congestive heart failure, and postmyocardial infarction (sales US$4.42 billion)
- Lucentis – a monoclonal antibody for the treatment of macular degeneration (sales US$2.40 billion world market except United States, which is under Genentech/Roche)
- Sandostatin – a small molecule drug for use in oncology to treat pituitary tumors and growth hormone-producing tumors (sales US$1.51 billion)
- Exforge – a small molecule drug for the treatment of hypertension (sales US$1.35 billion).

Novartis owns Sandoz, the second largest generics company in the world after Teva.

Table 1.7 lists the new drugs approved by FDA and EMA for Roche, Pfizer, and Novartis in 2012.

*Source*: Novartis, *Financial Report*, viewed December 16, 2013, http://www.novartis.com/downloads/investors/financial-results/quarterly-results/q4-2012-media-release_en.pdf

## 1.7 CASE STUDY #1.2

### 1.7.1 Lipitor and Plavix

This case study describes Lipitor and Plavix and their mechanisms of action. Lipitor and Plavix were the top two best-selling drugs in 2010 before their patents expired.

**TABLE 1.7 New Drugs Approved by FDA and EMA for Roche, Pfizer, and Novartis, 2012**

| Company | Product | Indication |
|---|---|---|
| Roche | Erivedge | Advanced basal cell carcinoma |
| | Perjeta | Metastatic HER2+ breast cancer |
| | Zelboraf | BRAF V600 mutation-positive metastatic melanoma |
| | Avastin | Recurrent, platinum-sensitive ovarian cancer |
| Pfizer | Eliquis | Prevention of stroke and systemic embolism in patients with nonvalvular atrial fibrillation |
| | Xeljanz | Treatment of moderate-to-severe active rheumatoid arthritis |
| | Bosulif | Treatment of previously treated chronic myelogenous leukemia |
| | Lyrica | Treatment of neuropathic pain because of spinal cord injury |
| | Elelyso | Treatment of adults with a confirmed diagnosis of type 1 Gaucher's disease |
| | Inlyta | Treatment of advanced renal cell carcinoma after failure of one prior systemic therapy |
| Novartis | Signifor | Cushing's disease |
| | Bexsero | Meningococcal serogroup B (MenB) vaccine |
| | Votubia | Noncancerous kidney tumors associated with tuberous sclerosis complex |
| | Exjade | Chronic iron overload |

*Source*: Data from 1. Roche 2012, *Annual Report*, viewed December 19, 2013, http://www.roche.com/investors/annual_reports/annual_reports_2012.htm 2. Pfizer 2012, *Financial Report*, viewed December 19, 2013, http://www.pfizer.com/files/annualreport/2012/financial/financial2012.pdf 3. Novartis 2012, *Financial Report,* viewed December 19, 2013, http://www.novartis.com/downloads/investors/financial-results/quarterly-results/q4-2012-media-release_en.pdf

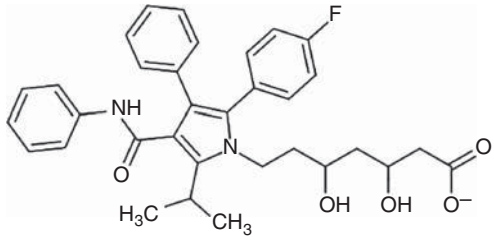
***Lipitor:*** Statins such as Lipitor (atorvastatin) and Zocor (simvastatin) are competitive inhibitors of the enzyme HMG-CoA reductase, which is involved in the biosynthesis of cholesterol. Cholesterol is a fatlike substance (a sterol) that is present in our blood and all the cells of our body. It is synthesized within the body or derived from consumed foods. Cholesterol is an important constituent of cell membranes 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 (refer to 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 the 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.

Lipitor inhibits cholesterol synthesis by increasing the number of LDL receptors to take up the LDL. The active ingredient is atorvastatin calcium. In addition to atovarstatin, Lipitor is formulated with other excipients: calcium carbonate, candelilla wax, croscarmellose sodium, hydroxypropyl cellulose, lactose monohydrate, magnesium stearate, microcrystalline cellulose, Opadry White YS-1-7040 (hypromellose, polyethylene glycol, talc, titanium dioxide), polysorbate 80, and simethicone emulsion.



Atorvastatin

*Source*: Food and Drug Administration 2009, *Lipitor*, viewed Jan 6, 2014, http://www.accessdata.fda.gov/drugsatfda_docs/label/2009/020702s057lbl.pdf

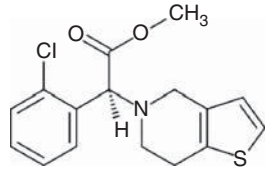
***Plavix:*** Plavix (clopidogrel bisulfate) is used for inhibiting blood clots in coronary artery, cerebrovascular, and peripheral vascular diseases.

In the blood clot formation process, a protein called von Willebrand factor acts as a bridge for the platelets to attach to the collagen via its Ia/Ib glycoprotein surface receptors. This attachment activates the platelets, leading to the secretion of thromboxane $A_2$ ($TXA_2$), adenosine diphosphate (ADP), and serotonin (also known as 5-hydroxytryptamine, 5-HT). The secreted $TXA_2$, ADP, and 5-HT also bind to the surface receptors of platelets and activate the platelets further. This leads to a change in the shape of the glycoprotein IIb/IIIa receptors on the surface of platelets, enabling them to bind to fibrinogen, a plasma protein. The process of aggregation then ensues with fibrinogen linking the receptors of different platelets, culminating in the formation of a platelet mass.

Concomitant with the formation of a platelet mass is the coagulation process whereby soluble fibrinogen is changed into insoluble fibrin to strengthen the platelet mass. A number of clotting factors are transformed from inactivated states into activated states in a series of reactions. The end result is the conversion of prothrombin to thrombin, which then changes fibrinogen to fibrin. Fibrin is a threadlike protein that traps platelets, blood cells, and fluid, leading to the formation of blood clots.

Plavix, through its metabolite, binds to the platelet receptors. It prevents the aggregation of platelets and thus stops the cross-linking process for forming blood clots.

The active ingredient of Plavix is clopidogrel bisulfate, and the tablet is formulated with hydrogenated castor oil, hydroxypropylcellulose, mannitol, microcrystalline cellulose, and polyethylene glycol 6000. The tablet coating consists of ferric oxide, hypromellose 2910, lactose monohydrate, titanium dioxide, triacetin, and Carnauba wax.




Clopidogrel bisulfate

*Source*: Food and Drug Administration 2010, *Plavix*, viewed January 6, 2014, http://www.accessdata.fda.gov/drugsatfda_docs/label/2010/020839s048lbl.pdf

## 1.8 SUMMARY OF IMPORTANT POINTS

1. The process from drug discovery to approval is

   Discovery → Preclinical → Clinical → Marketing Application → Approval

2. The overall process takes 10–12 years and costs above US$ 1 billion.
3. Regulatory supervision is an integral part of the pharmaceutical industry to ensure safety, efficacy, purity, and consistency of drugs for human use.
4. The global pharmaceutical market in 2012 was US$962 billion. Biopharmaceuticals account for more than 10% of the market, with higher growth rate compared to conventional pharmaceuticals.
5. The top selling drug in 2012 was Seretide/Advair from GlaxoSmithKline, and the top biopharmaceutical was Humira from Abbott.
6. The pharmaceutical R&D expenditure, at more than 15% of revenue, is higher than many other technology-based industries.
7. Pharmaceutical companies draw on traditional as well as advances in new technologies to identify and develop new drugs.
8. Pharmaceutical companies rely on patents to protect their intellectual properties.

## 1.9 REVIEW QUESTIONS

1. Provide a definition for the term “drug” as adopted in this book.
2. Describe the process from drug discovery to approval.
3. Describe the role of regulatory bodies such as FDA and EMA. What are their main concerns regarding drugs?

4. Explain the terms GLP, GCP, and GMP. Why are these necessary?
5. Discuss how Humira and Lipitor work in the body.
6. Explain the reason for the high R&D cost for drugs and discuss how the cost can be reduced.
7. Explain why intellectual properties are important to the pharmaceutical companies and how they can be protected. Give examples to illustrate.
8. List some of the approaches for drug discovery.

## 1.10 BRIEF ANSWERS AND EXPLANATIONS

1. Refer to Section 1.1 and Exhibit 1.1 but note that drugs should be used according to the indications and contraindications provided by the manufacturer.
2. This is explained in Section 1.2. The importance of regulatory control is an integral part of the process.
3. FDA is the regulatory agency for drugs in United States whereas EMA is the centralized agency for the EU countries. Refer to Section 7.1 for further explanation. Regulatory agencies are concerned with the safety, efficacy, purity, and consistency of drugs. Their roles are to ensure that drugs are safe and fit for their purpose.
4. GLP stands for Good Laboratory Practice, GCP for Good Clinical Practice, and GMP for Good Manufacturing Practice. Together, these practices ensure there is planning, control, and monitoring of the drug development all the way from preclinical to clinical and manufacturing stages such that procedures are followed, records are kept, and processes are verified and tested.
5. Refer to Exhibit 1.4 and Case Study #1.2.
6. The high R&D cost stems from increasingly stringent regulatory compliance requirements and failures of drugs at later clinical phases mainly because of lack of efficacy and unacceptable adverse events. The introduction of risk-based approach, process analytical technology (refer to Section 9.8), and consolidation of regulatory documents (ICH, refer to Section 7.11) will reduce the regulatory burden. In addition, the development of more specific drugs, better understanding of biochemical pathways, followed by focused evaluation using more representative assays and biomarkers will reduce instances of failure at later stage clinical trials (refer to Section 6.4).
7. Patent rights protect intellectual properties and compensate the high R&D expenditure that pharmaceutical organizations spend on developing drugs. Without the protection of patents it would be difficult for pharmaceutical companies to justify R&D expenditure and continue with innovations. The way forward for the pharmaceutical industry may include the need to review patent law. This is particularly relevant with respect to the exclusivity period and the rules for revoking patent rights under compulsory licensing, whereby a government can force a patent holder to grant rights of the patent to the state or other parties without compensation in royalties.
8. Refer to Section 1.5.

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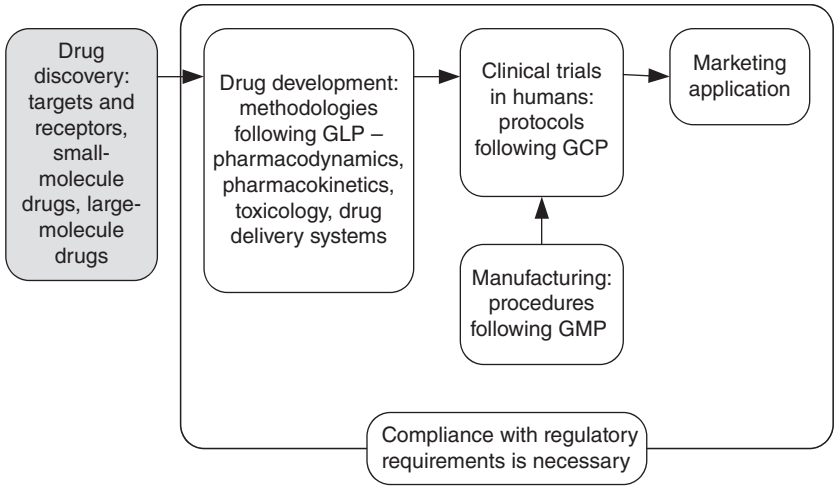
# CHAPTER 2

# DRUG DISCOVERY: TARGETS AND RECEPTORS




## 2.1 DRUG DISCOVERY PROCESSES

For a drug to work, it has to interact with a disease target in the body and intervene in its wayward functions. An analogy is the lock and key comparison, with the lock being

*Drugs: From Discovery to Approval*, Third Edition. Rick Ng.

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 is the irrational approach. It involves scanning 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 likened 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 clumsy. The chances of failure are high, although it should be kept in mind that many drugs on the market today were discovered in this manner.

Further advances in drug discovery led to the rational approach. This approach starts by finding out about the structure of the target and then designing a drug to fit the target and modify its functions. Within the lock and key analogy, this is akin to determining the construction of pin tumblers in the lock first and then designing a key with the appropriate slots and grooves to open the lock.

The latest progress in drug discovery methodology has occurred following contributions from genomics and proteomics research. Here the emphasis is on identifying and validating targets *a priori* to drug discovery. This approach involves finding out the target that causes the disease as the first step in drug discovery. After that, the rational approach would proceed.

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 areas have unmet clinical needs for an effective prophylactic or therapeutic intervention.

The organization then has to evaluate its core competency, technological advantages, competitive barriers, and financial resources before committing to develop a drug to fulfill any given unmet needs. As discussed in Chapter 1, for a monetary outlay that averages around US$1–1.2 billion for each drug development, the organization has to weigh its options carefully. The important factors to consider are the following:

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

The evolving healthcare policies in different countries can strongly impact the market potential for an organization's products. In the early 2010s, healthcare expenditures in the developed markets of North America, Europe, and Japan experienced

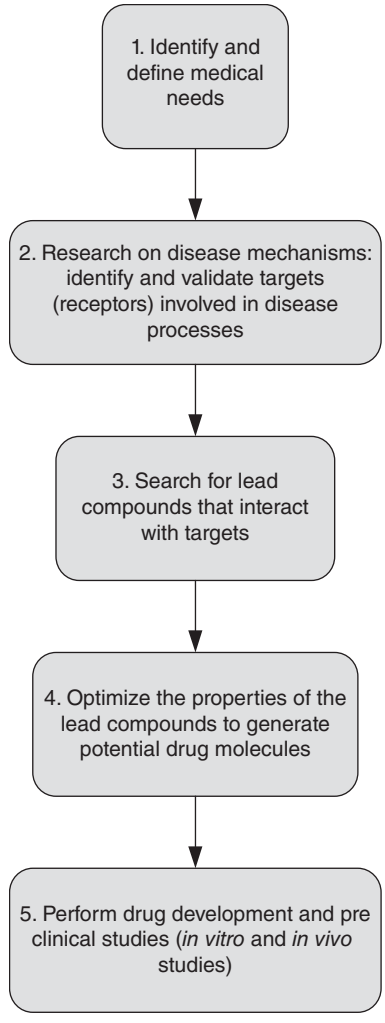



**Figure 2.1** Flow chart of drug discovery processes.

single-digit growth, mainly because of economic and healthcare austerity measures. In contrast, the pharmaceutical emerging countries such as China and India experienced double-digit growth in their healthcare expenditures because of economic growth and healthcare expansions. A projection of the drug expenditure levels in 2017 is presented in Table 2.1. It shows the vast differences in demand for the types of drugs for each therapy class between the developed nations and emerging countries.

In terms of intellectual property, the sales history of Lipitor and Plavix demonstrate the drastic effects of patent expiry. Lipitor was the top selling drug in 2011, but in 2012 it did not rank in the top 20 because of patent expiry. The generic version of Lipitor was ranked 21 in 2012. Similarly, as presented in Chapter 1, Plavix sales plunged after patent expiry in 2012. As new and more effective drugs are introduced, their

**TABLE 2.1 Projected 2017 Drug Expenditures in Developed and Emerging Markets by Therapy Classes**

| Developed Markets | Sales (US$ Billion) | Emerging Markets | Sales (US$ Billion) |
|---|---|---|---|
| Oncology | 74–84 | Pain | 22–25 |
| Diabetes | 34–39 | Other CNS drugs | 20–23 |
| Anti-TNFs | 32–37 | Antibiotics | 18–21 |
| Pain | 31–36 | Oncology | 17–20 |
| Asthma/COPD | 31–36 | Hypertension | 14–17 |
| Other CNS drugs | 26–31 | Diabetes | 10–12 |
| Hypertension | 23–26 | Dermatology | 10–12 |
| Immunostimulants | 22–25 | Antiulcerants | 9–11 |
| HIV antivirals | 22–25 | Cholesterol | 6–8 |
| Dermatology | 22–25 | Asthma/COPD | 3–5 |
| Antibiotics | 18–21 | Antiepileptics | 3–5 |
| Cholesterol | 16–19 | Antivirals excluding HIV | 3–5 |
| Antiepileptics | 15–18 | Immunosuppressants | 3–5 |
| Immunosuppressants | 15–18 | Allergy | 3–5 |
| Antipsychotics | 13–16 | Antidepressants | 3–5 |
| Antiulcerants | 12–14 | Antiplatelet | 3–5 |
| Antidepressants | 10–12 | Antipsychotics | 2–3 |
| Antivirals excluding HIV | 8–10 | Heparins | 1–2 |
| ADHD | 7–9 | Erectile dysfunction | 1–2 |
| Interferons | 6–8 | Immunostimulants | 1–2 |
| Top 20 therapy classes | 71% | Top 20 therapy classes | 45% |
| Others | 29% | Others | 55% |

*Source*: Adapted from IMS Institute for Healthcare Informatics 2013, *The Global Use of Medicines: Outlook Through 2017*, Parsippany, NJ, USA.

ADHD: attention-deficit hyperactivity disorder; CNS: central nervous system; COPD: chronic obstructive pulmonary disease; HIV: human immunodeficiency virus; TNF: tumor necrosis factor.

sales can increase dramatically within a short time span and surpass the sales of more "established" drugs. Overall, the pharmaceutical organization needs to project the expected returns from its drug development investment and assess the competitive factors and barriers, including government regulations, before deciding which drug to develop. Pharmaceutical companies have to be continuously vigilant and forecast the future directions of drug developments and regulatory requirements. They have to use their core technical competencies to deliver a pipeline of products to remain competitive and profitable in the long term.

## 2.3 TARGET IDENTIFICATION

### 2.3.1 Genes and Biochemical Pathways

Most diseases, except in the case of trauma and infectious diseases, have a genetic connection. Genetic makeup and variations (refer to single nucleotide 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, biochemical pathways, and networks helps to pinpoint the cause of the disease. Drugs can be tailor-made to attack the "epicenter" of diseases. In this way, drugs that are more specific (with fewer side effects) and effective (with a high therapeutic index, refer to Section 5.2) can be discovered and manufactured to intervene or restore the cellular or molecular dysfunction.

From the Human Genome Project, we know 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. It is estimated that there are about 20,500 genes that encode proteins. Exhibit 2.1 provides some information about the number of genes and the complexity of life forms.

From these 20,500 genes, many thousands of proteins are produced. Drug targets are normally protein or glycoprotein molecules that make up the enzymes and receptors, with which drugs interact. To date, only about 500 proteins have been identified and targeted by the multitudes of drugs in the market (refer to Case Study #2.1 concerning drug targets). The opportunities opened up by genomics and proteomics research have paved the way for many more targets and new drugs to be discovered.

**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 4,300 genes, and *Saccharomyces cerevisiae* (a fungus commonly known as baker's or brewer's yeast) has 6,000 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 20,500, a similar range to that of mice.

It was initially thought that the number of human genes was of the order of 100,000. The smaller number of 20,500 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 are other factors involved in determining complexity. Each gene may code for more than one protein, to account for human complexity. It is also considered that the variety of cell types present in an organism may be a better indicator for complexity rather than the number of genes.

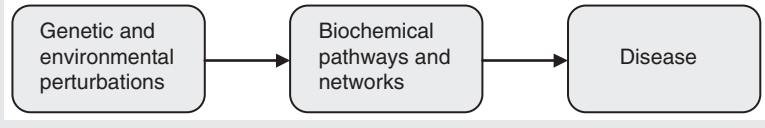
*Source*: Data from Adams, J 2008, 'The complexity of gene expression, protein interaction, and cell differentiation', *Nature Education*, vol. 1, p. 110.

Recent research has shown that diseases and disorders are often not because of aberrations in a single gene alone but multiple genes and complex biochemical pathways and interactions of these pathways forming networks. Our cells receive signals from both outside and inside the body. These signals are relayed via the biochemical pathways and networks. When the biochemical pathways and networks malfunction, our genes, cells, tissues, and organs cannot adjust and regulate properly and disease or diseases can result. Exhibit 2.2 shows the complex interactions of biochemical pathways and networks for some diseases.

**Exhibit 2.2 Biochemical Pathways and Networks**

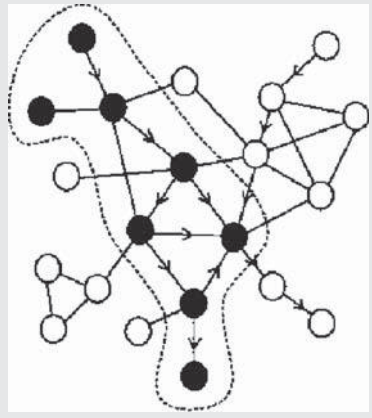
A disease is rarely the result of abnormality in a single gene but usually because of aberrations in multiple genes and a series of biochemical pathways and networks associated with these genes. Environmental effects may also be causal factors in promoting or assisting the development of a disease.

Biochemical pathways are a cascade of interactions among biological molecules that result in certain product/s or changes to the cellular activities. These pathways include those involved in metabolism, gene regulation, signal transmissions, and other cellular activities. Pathways do not function alone. Instead, they occur in complex biochemical networks, which are collections of pathways.




In the network approach to the study of diseases, nodes are used to represent diseases, proteins, and metabolites. The links denote, for example, genes that cause the disease, regulatory mechanisms, protein–protein interactions, or metabolic reactions. A perturbation on a node can affect other surrounding nodes, and consequently the entire network itself. Nodes that have high connectivity are called hubs and their perturbations lead to more significant outcomes. Hubs are considered to be associated with disease genes.

It is postulated that if a gene or molecule is involved in a disease, those genes or molecules in close proximity to it will also be implicated in the disease; this neighborhood of disease state is called a disease module. A disease module represents a group of nodes, and the perturbations (mutations, deletions, copy variations, or expression changes) can be linked to a particular disease, as shown in the figure below as dark nodes enclosed by dotted lines.



Using bioinformatics, networks and hubs can be constructed and disease modules identified. Drugs are designed to target those nodes and linkages implicated in the disease modules. It should be borne in mind that disease modules can overlap and hence treating one module can affect the neighboring modules, and side effects or other interactions may develop.

*Source*: 1. Barabasi, AL, Gulbahce, N and Loscalzo, J 2011, 'Network Medicine: A Network-based Approach to Human Disease', *Nature Reviews Genetics*, vol. 12, pp. 56-68. Reproduced with permission of Macmillan Publishers Ltd. 2. On the basis of data from Wang, J, Zhang, Y, Marian, C and Ressom, HW 2012, 'Identification of aberrant pathways and network activities from high-throughput data', *Briefings in Bioinformatics*, vol. 13, pp. 406-419

Exhibits 2.3, 2.4, and 2.5 provide examples of genetic causes of diseases (cancer, sickle cell anemia, and cystic fibrosis (CF)). It should be noted that although some of these diseases are the result of mutations in a single gene (viz. Huntington's disease and Duchenne muscular dystrophy), most are because of the influence of multiple genes and their effects on biochemical pathways.

### 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 classic method to discover drug targets or receptors (Exhibit 2.6) is to bind the potential receptors with radioligands (Exhibit 2.7) so that targets can be picked out from a pool of other receptors. Bound receptors are then

**Exhibit 2.3 The p53 Protein in Cancer**

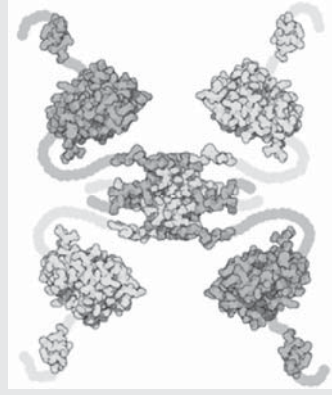
The TP53 gene gives instructions to a cell to make a protein called tumor suppressor protein p53. The tumor suppressor protein, p53, regulates cell division by keeping cells from growing and dividing too fast or in an uncontrolled way. As shown in the picture below, the p53 protein has four identical chains that 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 uncontrolled cell growth.

In the case of cancer, the gene that codes for p53 is mutated. The mechanism for programmed cell death becomes inactivated and no longer functions. Cancer cells then continue growing and dividing at the expense of surrounding cells, thus leading to tumor formation. It is also found that the oncogene – murine double minute (mdm2) – overexpresses the mdm2 protein that binds to the transactivation domain of p53 and blocks p53's transcription process, switching off the cell death program.

*Source*: Goodsell, DS 2000, *p53 Tumor Suppressor*, viewed January 23, 2014, http://www.rcsb.org/pdb/101/motm.do?momID=31. Data from 1. Campbell, MK and Farrell, SO 2011, Biochemistry, 7th edn., Brooks/Cole, Cengage Learning, Belmont, CA; 2. Vousden, KH and Lane, DP 2007, 'p53 in health and disease', *Nature Reviews Molecular Cell Biology*, vol. 8, pp. 275-283; 3. Kirkpatrick, P 2004, 'Unleashing p53', *Nature Reviews Drug Discovery*, vol. 3, p.111.

*p53 molecule*



*Source*: Goodsell, DS 2000, *p53 Tumor Suppressor*, viewed January 23, 2014, http://www.rcsb.org/pdb/101/motm.do?momID=31

**Exhibit 2.4 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 574, is sufficient to cause the red blood cell to deform and constrict blood flow by blocking the capillaries.

*Source*: Data from Campbell, MK and Farrell, SO 2011, *Biochemistry*, 7th edn., Brooks/Cole, Cengage Learning, Belmont, CA.

**Exhibit 2.5 Cystic Fibrosis**

Cystic Fibrosis (CF) is a hereditary disease of abnormal fluid secretion. It affects cells of the exocrine glands, such as the intestine, sweat glands, pancreas, reproductive tract, and especially the respiratory tract. The disease affects about one in 2,500 infants of the Caucasian population in 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*: Data from Karp, G 2013, *Cell and Molecular Biology*, Concepts and Experiments, 7th edn., John Wiley & Sons, Hoboken, NJ.

**Exhibit 2.6 Receptors**

According to the International Union of Pharmacology Committee, a receptor is a cellular molecule, or an assembly of macromolecules, that is concerned directly and specifically in chemical signaling between and within cells. Combination of a hormone, neurotransmitter, drug, or intracellular messenger with its receptor(s) initiates a change in cell function.

*Source*: Data from Neubig, RR, Spedding, M, Kenakin, T and Christopoulos, A 2003, 'International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification. XXXVIII. Update on Terms and Symbols in Quantitative Pharmacology', *Pharmacological Reviews*, 55, pp. 597–606.

**Exhibit 2.7 Radioligands**

Ligands are molecules that bind to a target. They may be endogenous (i.e., 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 cells known to contain the target are 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 on the basis of previously known targets. Targets that do not appear to show homology (similarity) to known ligands and 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 through recombinant technology. In this way, biochemical pathways of the target can be studied in detail, making possible the development of a drug molecule with higher chances of success.

separated from the radioligands, sequenced, and their nucleotide sequence is 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.

***DNA Microarray:*** DNA microarray, also known as DNA or gene chips, is a technology that investigates 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 discover 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. DNA samples extracted 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 2.8 presents a more detailed explanation of microarrays.

***Expressed Sequence tags and In Silico Methods:*** Expressed sequence tags (ESTs) are short nucleotide sequences of cDNA with about 200–500 base pairs. They are parts

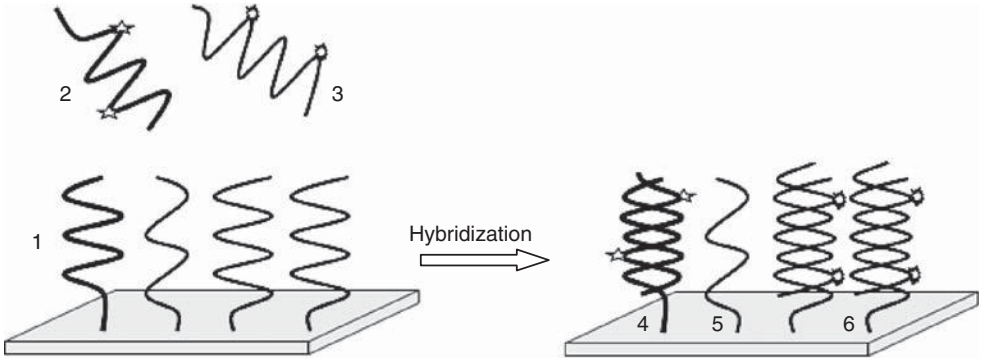



**Figure 2.2** Microarray slides. 1) Thousands of short, single DNA strands containing specific genes are deposited onto glass slide at designated positions, 2) cDNAs from diseased cells are labeled with red fluorescent dye, 3) cDNAs from healthy cells are labeled with green fluorescent dye, 4) Pairing of diseased cDNAs with specific genes on glass slide, 5) Noncomplementary pairing of certain genes, 6) Pairing of healthy cDNAs with other specific genes. Fluorescence image of red and green intensities reveals genes involved in diseased and health cells. Refer to Exhibit 2.8.

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 a tag for each gene on the genome.

The scanning of nucleotide sequences is achieved through *in silico* (computer) methods. The premise is that all proteins, even those with sequences that appear considerably different, can be members of families sharing essentially similar structures and functions.

Scientists carry out searches on databases. Each EST of interest can be compared with sequences in proteins and the degree of match determined. A technique called threading is used, which involves using data on 3D protein structures, coupled with knowledge of the physicochemical properties of amino acids, to determine if the amino acid sequence is likely to fold in the same way as a sequence for which the structure is known. In this way, more information about the putative target protein can be assessed. As of January 2013 there are about 74 million ESTs in public databases.

## 2.4 TARGET VALIDATION

Once a potential disease-causing target has been identified, a process of validation is carried out to confirm the functions and effects of the target. The ultimate target validation is a series of human clinical trials in which the effects of a drug on the target are evaluated. However, this kind of validation is at the other end of the drug discovery spectrum, when much time and commitment have already been expended on the drug. What is required at an early stage is validation of the target identified, to lay the path for developing appropriate drugs aiming at this target. This will ensure that time, resources, and investments are optimized.

Some questions that target validation have to address are:

- What is the function of the target?
- Which disease pathway does the target regulate?
- How important is this disease pathway?
- What is the expected therapeutic index if a drug is to interact with the target?

Validations can be divided into two groups: *in vitro* laboratory tests and *in vivo* disease models using animals.

Typically, *in vitro* tests are cell- or tissue-based experiments. The aim is to study the biological functions of the target as a result of binding to potential ligands (drug candidates). Parameters such as ionic concentrations, enzyme activities, and protein expression profiles are studied.

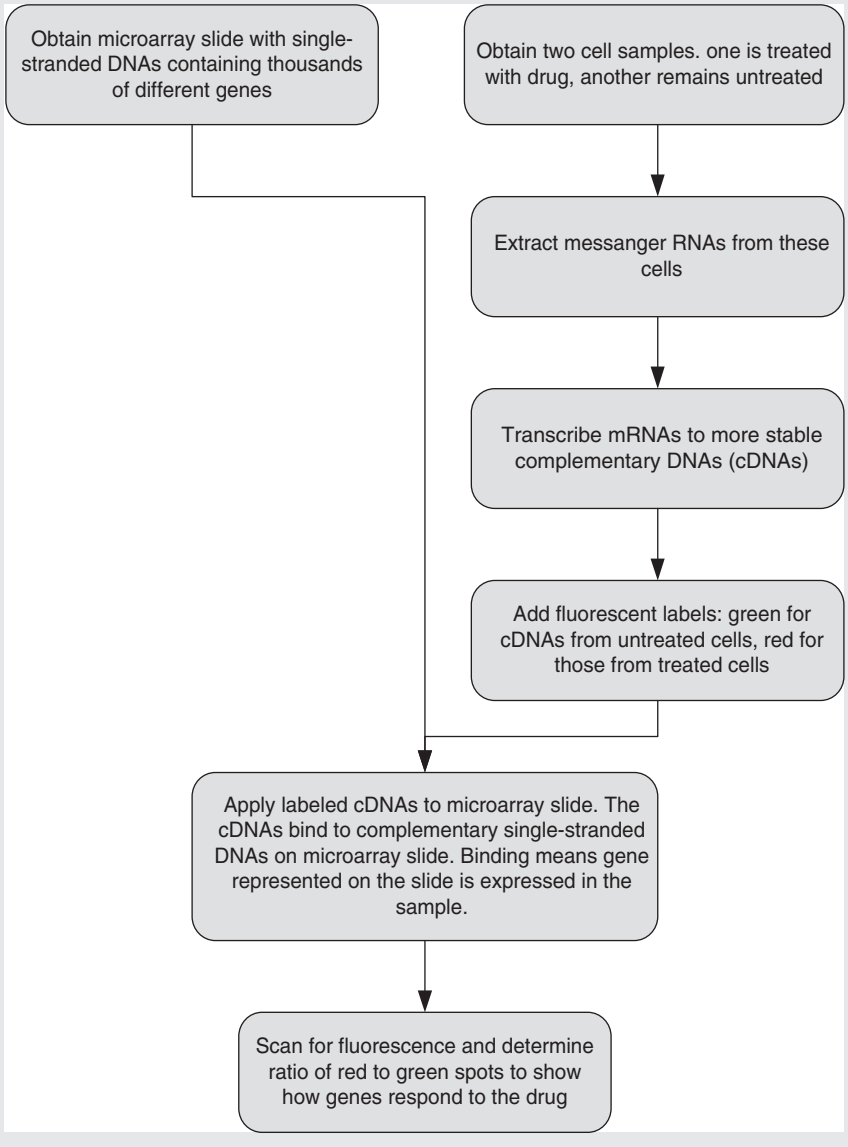
For *in vivo* studies, animal models are set up, and the way the target is involved in the disease is analyzed. One such model is the use of knockin/knockout or transgenic mice (Exhibit 2.9). It should be borne in mind, however, that there are differences between humans and animals in terms of gene expression, functional characteristics, and biochemical reactions. Nevertheless, animal models are important for the evaluation of drug–target interactions in a living system.

**Exhibit 2.8 Microarrays**

To use the microarray, known sequence of short DNA is printed in a regular grid-shaped pattern onto a solid support of a membrane or glass slide. mRNAs are isolated from healthy and diseased cells. The mRNAs are used to generate complementary DNAs (cDNAs). Fluorescent tags are attached to the cDNAs, and the cDNAs are mixed and incubated with the microarray supports (slides).

Through a process called hybridization probing, the genes from the samples pair up with their complementary counterparts on the solid supports. When the hybridization step is completed, a scanner (laser beam and camera) is used to capture the fluorescence image of the array. The expression levels of genes from the diseased and healthy cells can be deciphered by comparing the intensities of red and green fluorescence. For example, if the disease causes some types of genes to be expressed more, more of these genes will hybridize with the DNA on the solid support, providing a greater intensity of red fluorescence than of green. In this way, disease targets can be identified.

This flowchart shows a schematic representation of the use of a microarray for identification of disease genes.




*Source*: Data from Friend, SH and Stoughton, RB 2002, 'The magic of microarrays', *Scientific American*, February, pp. 44–53.

More recently, *in silico* target validation has been used. This is similar to the method discussed for ESTs. The DNA sequence of the putative target is compared with those of known liganded receptors. If homology (similarity) of sequences and structures are determined, they can provide clues to ligands that are likely to interact with the target.

To better identify and validate targets, an unprecedented partnership – the Accelerating Medicines Partnership (AMP) – was set up among Food and Drug Administration (FDA), National Institute of Health (NIH), pharmaceutical companies, and nonprofit organizations in early 2014. The aim was to identify and validate targets for Alzheimer's disease, diabetes mellitus type II, autoimmune disorders, and systemic lupus erythematosus (refer to Exhibit 2.10).

## 2.5 DRUG INTERACTIONS WITH TARGETS OR RECEPTORS

It should be clarified that targets identified using microarrays are mainly the genes that regulate or contribute to diseases. These gene targets give clues to the proteins that are affected. In most situations, it is the proteins or receptors that drug molecules are developed to interact with to provide the therapy. The exceptions are in cases such as antisense and RNA interference drugs and gene therapy, where the nucleotides, RNA, and genes are targeted, respectively (refer to Sections 3.4 and 3.5).

When presented to the target, drug molecules can elicit reactions to switch on, switch off, or alter certain biochemical reactions. The main drug targets in the human body can be classified into three categories:

- *Enzymes:* There are many different types of enzymes in the human body. They are required for a variety of functions. Drugs can interact with enzymes to modulate their enzymatic activities.
- *Intracellular receptors:* These receptors are in the cytoplasm or nucleus. Drugs or endogenous ligand molecules have to pass through the cell membrane (a lipid bilayer) to interact with these receptors. The molecules must be hydrophobic or

**Exhibit 2.9 Knockin and Knockout Mice**

Genetic research, such as microarray, reveals the possible genes that may cause the disease under study. Animals, particularly mice, are bred to develop the disease as study models

Transgenic mice are bred with the putative gene modified: insertion of a gene giving rise to "knockin" mice, or inactivation/deletion of a gene to produce "knockout" mice models. The effects of gene knockins and knockouts are studied in relation to the progress of disease. It is also possible to study drug interactions by treating these mice with potential drug candidates.

*Source*: Data from 1. Gibson, G and Muse, SV 2009, *A Primer of Genome Science*, 3rd edn., Sinauer Associates, Inc., Sunderland, MA; 2. Harris, S 2001, 'Transgenic knockouts as part of high-throughput, evidence-based target selection and validation strategies', *Drug Discovery Today*, vol. 16, pp. 628–636.

**Exhibit 2.10 The Accelerating Medicines Partnership**

The Accelerating Medicines Partnership (AMP) was formed in early 2014 between FDA, NIH, 10 pharmaceutical companies, and several nonprofit organizations "to distinguish biological targets of disease most likely to respond to new therapies and characterize biological indicators of disease, known as biomarkers". The partners will invest US$230 million over 5 years to speed up the identification and validation of disease targets. Another important aspect of the partnership is that data and analyses generated will be made publicly available to the broad biomedical community.

The specific goals are the following:

- Alzheimer's disease
  - Identify biomarkers
  - Conduct large-scale systems biology analyses of brain tissue samples of patients with Alzheimer's disease to validate targets.
- Type II diabetes
  - Build knowledge portal of DNA sequence, genomic information, and clinical data to identify promising targets
  - Use data to predict likelihood of success of drug development aimed at these targets.
- Autoimmune disorders
  - Collect and analyze samples from patients with rheumatoid arthritis and systemic lupus erythematosus (lupus) to provide disease process
  - Identify differences in patient responses to understand disease mechanisms.

*Source*: Data from National Institutes of Health 2014, *NIH industry and non-profits join forces to speed validation of disease targets*, viewed February 4, 2014, http://www.nih.gov/news/health/feb2014/od-04.htm

coupled to a hydrophobic carrier to cross the cell membrane and enter the cytoplasm or nucleus.

- *Cell surface receptors:* These receptors are on cell surface and have an affinity for hydrophilic binding molecules. Signals are transduced from external stimuli to the cytoplasm and affect cellular pathways via these surface receptors. There are three main superfamilies (groups) of cell surface receptors: G-protein coupled receptors (GPCRs), ion channel receptors, and catalytic receptors using enzymatic activities.

Hydrophilic or water-soluble drugs do not cross membranes. They stay in the bloodstream for durations that are normally short, lasting in the order of seconds, and mediate responses of short duration. In contrast, hydrophobic drugs, being water-insoluble, require carrier molecules for transport through the bloodstream. Hydrophobic drugs remain in the bloodstream and can persist for hours and days, providing much longer effects.

When the action of a drug is to activate or switch on a reaction, the drug is termed an "agonist." On the contrary, if a drug switches off the reaction, or inhibits or blocks the binding of other agonist components onto the receptor, it is called an "antagonist." When the interaction is with an enzyme, the terms "inducer" and "inhibitor" are used to denote drugs that activate or deactivate the enzyme.

Appendix 3 lists some selected drugs and their mechanisms of action, showing their roles as agonists or antagonists and inducers or inhibitors. Figures 2.3 and 2.4 provide schematic lock and key representations of the agonist and antagonist actions. It should be noted that the drug molecule (agonist or antagonist), receptor, and cell membrane are in fact complicated three-dimensional structures. The analogy is that only certain keys can be inserted into the lock and activate or deactivate the lock. Some facts about interactions between drug molecules and targets to bear in mind are the following:

- Binding is specific
- Binding occurs at particular sites in the target molecule
- Binding is reversible.

Allosteric binding occurs when two molecules bind to different sites on the target. When the two molecules are identical, it is termed homotropic interaction. If the molecules differ from each other, it is termed heterotropic interaction. Binding is competitive when two different ligand molecules compete for the same site. We discuss ligand binding further in Chapter 5. The specificity of ligand–receptor interaction is illustrated in Exhibit 2.11.

### 2.5.1 Types of Interactions

Binding between drug molecule and receptor or enzyme is critically dependent on the shapes and sizes of the molecules. To deliver therapeutic actions, drug molecules with the right shapes and sizes have to be designed to fit into the binding sites (pockets) of the receptor or enzyme. Another important factor is the nature of the coupling. Before a drug can fit into the binding site, it has to overcome thermal and vibrational motions at the cellular level. The attractive forces must be strong enough for the drug to dock with the binding site. When molecules couple together, the type of bonding can be divided into covalent bonding and electrostatic interactions due to hydrogen bonding or van

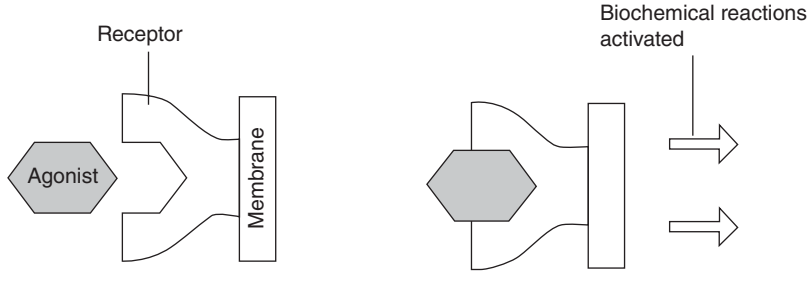



**Figure 2.3** Agonist binding to receptor initiates biochemical reactions.

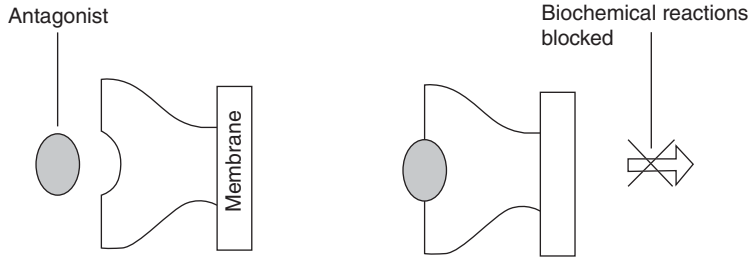



**Figure 2.4** Antagonist binding to receptor blocks biochemical reactions.

der Waals forces. The stronger the coupling between the drug and binding site, the more sustained is the interaction.

Covalent bonds are strong bonds. Actual bonds are formed between the interacting molecules via the sharing of electrons. Hence, this type of interaction is expected to provide long-lasting effects (although not many drug–receptor bonds are of this nature).

Electrostatic forces are due to the ionic charges residing on the molecules, which attract or repel one another. The macromolecular structures of the receptors and enzymes mean there are a number of ionic charges to attract the oppositely charged drug molecules. The forces of electrostatic interactions are weaker than covalent bonding. Electrostatic interactions are more common in drug–receptor interactions. There are two types of electrostatic interactions:

- Hydrogen bonding
- Van der Waals forces.

**Exhibit 2.11 Aspirin (Acetylsalicylic Acid)**

The enzyme prostaglandin $H_2$ synthase-1 (PGHS-1) manufactures prostaglandin $H_2$ ($PGH_2$), which is converted to prostaglandin $E_2$ and causes fever and inflammation. PGHS-1 contains two protein subunits with long channels.

The chemical arachidonic acid enters these channels and becomes converted to prostaglandin $H_2$. Aspirin, with the correct shape and size, enters these channels and blocks entry of arachidonic acid. As a result, the agent for causing fever and inflammation cannot be manufactured. Unfortunately, an undesirable effect of aspirin is that it blocks other types of PGHS, including the types that protect the stomach lining, paving way for stomach bleeding.

Recent advances with other anti-inflammatory drugs, ibuprofen and naproxen, which only work by physically blocking the channel to arachidonic acid, mean that the adverse effect of stomach bleeding can be avoided.

Refer to Case Study #2.2 for more discussion on anti-inflammatory drugs.

*Source*: Data from Garavito, M 1999, 'Aspirin', *Scientific American*, May, p. 108.

Hydrogen bonds are due to the attractive forces between the distorted electron cloud of a hydrogen atom and other more electronegative atoms such as oxygen and nitrogen. The attractive forces are weaker than covalent bonds, but many hydrogen bonds can be formed in macromolecular protein molecules. Van der Waals forces are weaker attractive forces due to the attraction between neutral atoms.

A third type of interaction follows from hydrophobic effects. These are the result of nonelectrostatic domains interacting. This type of interaction occurs mainly with the highly lipid-soluble drugs in the lipid part within the cytoplasm of the cell.

## 2.6 ENZYMES

Enzymes are biological molecules that catalyze biochemical reactions. The thermodynamics of biological enzymatic reactions are described in Exhibit 2.12.

Almost all enzymes are proteins. They provide templates whereby reactants (substrates) can bind and are favorably oriented to react and generate products. The locations where substrates bind are known as "active sites." Because of the specific 3D structures of the active sites, the functions of enzymes are specific, that is, each particular type of enzymes catalyzes specific biochemical reactions. Enzymes speed up reactions, but they are not consumed and do not become part of the products. Enzymes are grouped into six functional classes by the International Union of Biochemists (Table 2.2).

In some cases, enzymes require the assistance of coenzymes (cofactors) to ensure the reactions proceed. Coenzymes are vitamins, metal ions, acids, and bases. They can act as transporters or electron acceptors or be involved in oxidation–reduction reactions. At the completion of the reaction, coenzymes are released, and they do not form part of the products. For some reactions that are energetically unfavorable, an energy source provided by the endogenous compound adenosine triphosphate (ATP) is needed to ensure the reactions proceed, as shown in the following reactions:

$$\mathrm{ATP} + \mathrm{H_2O} \rightarrow \mathrm{ADP} + \mathrm{Ph} + \mathrm{H^+} \quad \Delta G = -30.54\ \mathrm{kJ/mol}$$

$$\mathrm{ATP} + \mathrm{H_2O} \rightarrow \mathrm{AMP} + \mathrm{PhPh} + \mathrm{H^+} \quad \Delta G = -45.60\ \mathrm{kJ/mol}$$

where ADP is adenosine diphosphate, Ph is a phosphate group ($PO_4^{3-}$), PhPh are two phosphate groups, and $\Delta G$ is the energy released. When $\Delta G$ is negative, the reaction is spontaneous.

Enzymatic reactions can be impeded by the addition of exogenous molecules. This is how drugs are used to control biochemical reactions, and most drugs are used for inhibitory functions. Drugs may function as competitive inhibitors or as noncompetitive inhibitors. Competitive inhibitors compete with the substrates for binding to the active sites, whereas noncompetitive inhibitors bind to another location (allosteric site) but affect the active site and its consequential interactions with the substrates. Some drugs used as enzyme inhibitors are the following:

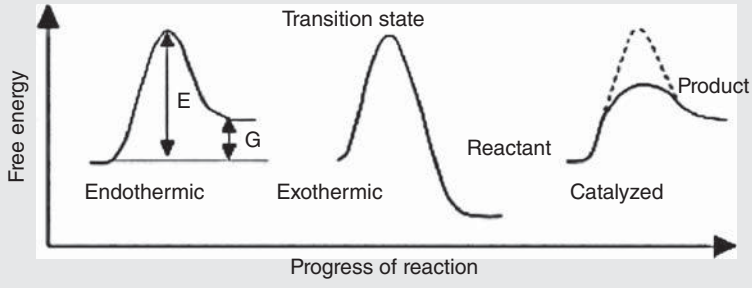
- Esomeprazole (Nexium, AstraZeneca): proton pump inhibitor for the prevention of relapse in reflux esophagitis

**Exhibit 2.12 Thermodynamics of Enzymatic Reactions**

In general, there are two types of biochemical reactions: exothermic and endothermic. Exothermic reactions are those where the energy states (free energy, labeled as G) of the reactants are higher than those of the products – they are energetically favorable. Endothermic reactions are those for which the products have higher energy states than the reactants – they are energetically unfavorable.

Regardless of whether the reaction is favorable or not, the reactants have to come together in close proximity to react. They have to overcome a potential energy barrier that may involve displacing solvating molecules around the reactants and reorientating the reactants. The energy needed to overcome this potential energy barrier is called the activation energy (see figure given below).

Enzymes bind to the reactants and provide an alternative mechanism of lower activation energy for the reaction to proceed. Hence, enzymes speed up biochemical reactions that are otherwise too sluggish to advance.




*Note*: *E* is the potential energy barrier, known as the activation energy. *G* is the free energy. The catalyzed reaction provides an alternative reaction pathway with lower activation energy.

- Captopril (Capoten, Bristol-Myers Squibb): angiotensin-converting enzyme (ACE) inhibitor for the treatment of hypertension
- Propranolol (Innopran XL, GSK): beta blocker for the treatment of hypertension and ischemic heart disease
- Imatinib mesylate (Gleevec, Novartis): tyrosine kinase inhibitor for the treatment of chronic myeloid leukemia (refer to Exhibit 7.3)
- Sertraline (Zoloft, Pfizer): selective serotonin (5-hydroxytryptamine; 5HT) uptake inhibitor for treating major depression and obsessive compulsive disorder
- Atorvastatin (Lipitor, Pfizer) and simvastatin (Zocor, Merck): HMG-Coenzyme A inhibitors for the reduction of cholesterol level in blood (refer to Case Study #1.2).

**TABLE 2.2 Classification of Enzymes**

| Number | Classification | Biochemical Properties |
|---|---|---|
| 1 | Oxidoreductases | Remove or add hydrogen atoms in oxidation or reduction reactions |
| 2 | Transferases | Transfer functional groups from one molecule to another. Kinases are specialized transferases that transfer phosphate from ATP to other molecules |
| 3 | Hydrolases | Hydrolyze various functional groups |
| 4 | Lyases | Add water, ammonia, or carbon dioxide across double bonds, or remove these elements to produce double bonds |
| 5 | Isomerases | Convert between different isomers |
| 6 | Ligases | Form a bond between molecules |

Exhibit 2.13 shows two selected drugs, celecoxib (a COX-2 inhibitor) and orlistat (a lipase inhibitor), and their actions on disease targets.

Drugs are also used to inhibit the enzymatic reactions of foreign pathogens that enter the human body. An example is the use of reverse transcriptase inhibitor and protease inhibitor for combating human immunodeficiency virus (HIV), as shown in Exhibit 2.14. Some new inhibitors are used to block HIV from attaching to the human cell CD4, thus stopping replication and infection of other cells as presented in Exhibit 2.15.

## 2.7 RECEPTORS AND SIGNAL TRANSDUCTION

Cells communicate to coordinate the biological functions within the human body. If the biochemical pathway communication system is interrupted or messages are not conveyed fully, our bodily functions can go haywire. An example of this is discussed in Exhibit 2.2 on biochemical pathways and networks and Exhibit 2.3, which shows that if the p53 protein is mutated, cell growth is unchecked and cancer can form.

There are hundreds of receptors on the cell surface. They act as "antennas" to receive signals from the extracellular environment. These signals may be from endogenous sources within the human body, such as neurotransmitters, cytokines and hormones, or exogenous sources from outside environment, such as viruses and drugs. On receiving the signals, receptors transduce these signals to the cell interior. Within the cell, the signal may cause a cascade of reactions. Figure 2.5 illustrates this signal transduction process.

Signals may be relatively straightforward, as in the case of ion channels for opening and closing of channel gates to control migration of ions. There are also signals that are more complex, involving the binding of ligand to the receptor. A consequence of the binding is a conformational (shape) change in the receptor, which leads to further amplifying processes.

We discuss below a number of receptor classes and analyze how signals are transduced. These receptors are GPCRs, ion channel receptors, tyrosine kinases, and intracellular (nuclear) receptors. A list of selected drugs and target receptors is shown in Tables 2.3, and 2.4 presents the mechanisms of action for several drugs.

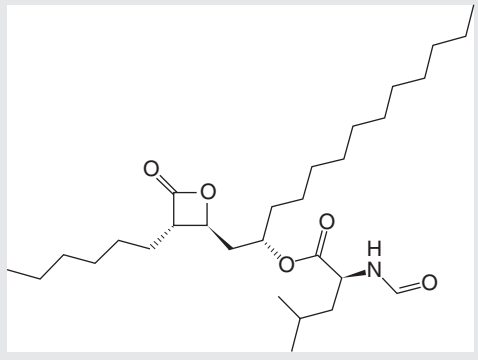
**Exhibit 2.13 Two Selected Drugs**

**COX-2 Inhibitor**

*Celecoxib (Celebrex, Pfizer):* This drug inhibits the enzyme COX-2, which is involved in pain and inflammation, but it has no effect on the COX-1 enzyme, which helps to maintain stomach lining. It is prescribed for the relief of pain and symptoms of osteoarthritis and rheumatoid arthritis. Previously, nonsteroidal anti-inflammatory drugs (NSAIDs) were used. NSAIDs inhibit both COX-1 and COX-2 enzymes and cause stomach bleeding (refer to Case Study #2.2).

**Lipase Inhibitor**

*Orlistat (Xenical, Roche):* This drug is prescribed for the treatment of obesity. It inhibits the gastrointestinal lipase enzymes by binding to the lipase through the serine site and inactivates the enzyme. Fat in the form of triglycerides cannot be hydrolyzed by the lipase and converted to free fatty acids and monoglycerides. Thus, there is no uptake of fat molecules into the cell tissue.




*Source:* Data from Pemble IV, CP, Johnson, LC, Kridel, SJ and Lowther, WT 2007, 'Crystal structure of the thioesterase domain of human fatty acid synthase inhibited by Orlistat', *Nature Structural & Molecular Biology*, 14, pp. 704–709.

### 2.7.1 G-Protein Coupled Receptors

G-Protein Coupled Receptors (GPCRs) represent possibly the most important class of target proteins for drug discovery (refer to Case Study #2.1). They are always involved in signaling from outside the cell to inside. The number of diseases that are caused by a GPCR malfunction is enormous and therefore it is not surprising that most commonly prescribed medicines act on a GPCR. It is estimated that about 27% of drugs are designed to target the GPCR receptors.

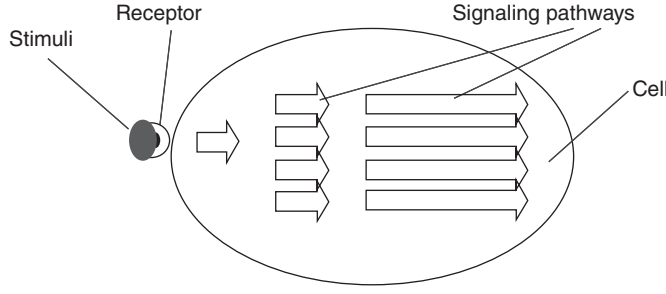



**Figure 2.5** Signal transduction showing cascades of reaction occurring inside the cell.

The common feature of this superfamily of receptors (there are many different families and subtypes of receptors in this group) is that there are seven domains that cross the cell membrane (Figure 2.6). These seven transmembrane receptors are often referred to as serpentine receptors.

The serpentine receptors are coupled to the G-proteins (guanine nucleotide regulatory proteins) inside the cell. There are three subunits that make up the G-proteins: α, β, and γ. When a ligand, such as a drug or neurotransmitter, binds to a receptor on the cell surface, the shape of the receptor changes. This induces an activated change in the trimeric clusters of α, β, and γ subunits within the cell. A phosphorylation (the addition of a phosphate group, such as $PO_3H_2$, to a compound) reaction occurs, in which guanosine diphosphate (GDP) changes to guanosine triphosphate (GTP):

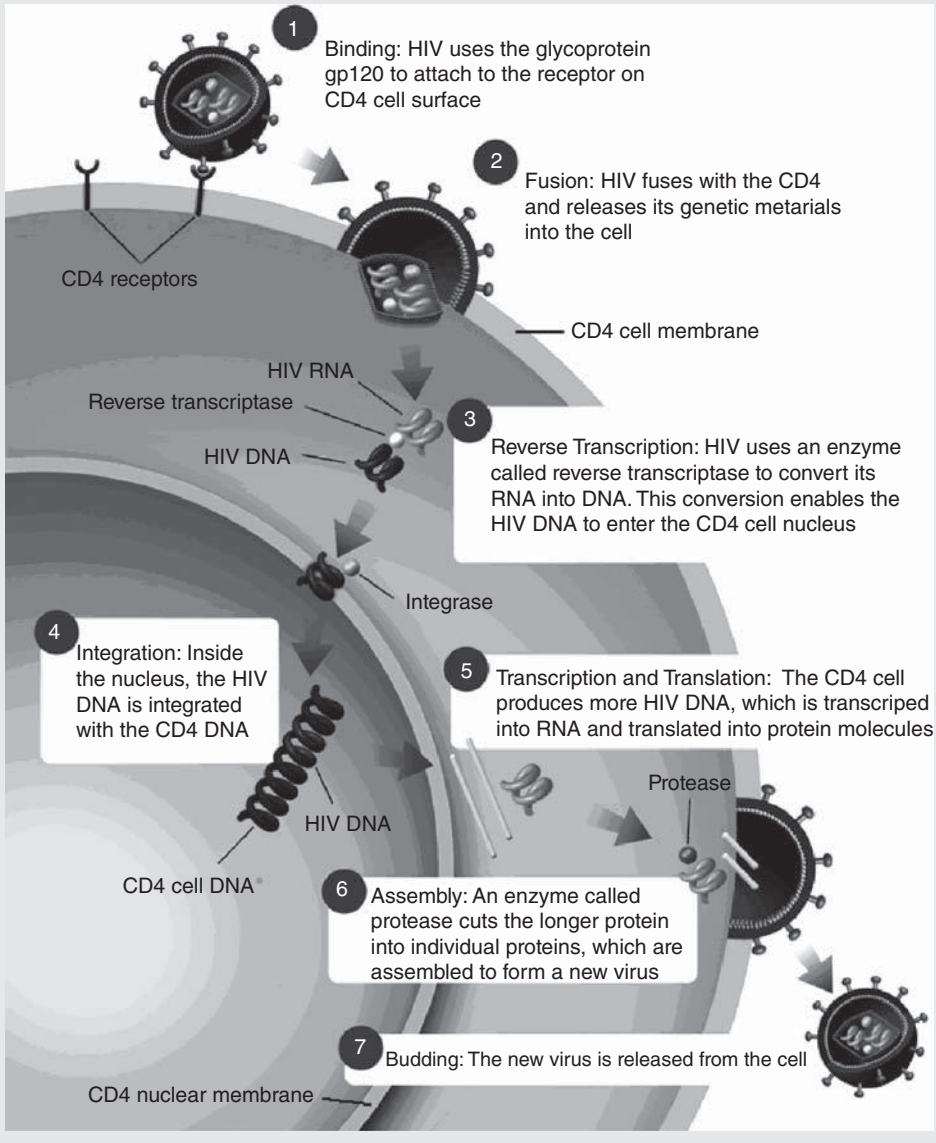
$$\text{GDP} + \text{phosphate} \rightarrow \text{GTP}$$

**Exhibit 2.14 Drugs Against HIV**

The diagram below shows the various stages (1–7) of HIV infection of CD4 cell (refer to Exhibit 4.7).

The drugs used against HIV are the following:

- *Entry Inhibitor*: Selzentry (maraviroc), it binds to the CCR5 coreceptor on the surface of CD4 cell and prevents attachment of HIV gp120 protein.
- *Fusion Inhibitor:* Fuzeon (enfuvirtide), it disrupts the HIV fusion mechanism and prevents CD4 cell from being infected.
- *Reverse Transcriptase Inhibitor* (*RTI*)*:* There are three types: Nucleoside RTI, Nucleotide RTI, and Nonnucleoside RTI. An example is Retrovir (zidovudine, AZT); it is structurally similar to a nucleoside and blocks chain linkages to form HIV DNA.
- *Integrase Inhibitor*: Tivicay (dolutegravir), it binds to the integrase active site and disrupts the HIV replication cycle.
- *Protease Inhibitor*: Reyataz (atazanavir), it inhibits the HIV protease enzyme and prevents formation of mature virions.




*Source*: NIH, AIDSinfo, 2014, *The HIV Life Cycle*, viewed November 3, 2014, http://aidsinfo.nih.gov/education-materials/fact-sheets/19/73/the-hiv-life-cyclehttp://www.chemistry.wustl.edu/~edudev/LabTutorials/CourseTutorials/bb/HIV/DrugStrategies.html

**Exhibit 2.15 Entry Inhibitor HIV Drugs**

Existing treatment for HIV focuses mainly on the use of protease inhibitors and reverse transcriptase inhibitors. This strategy, however, is to inhibit the HIV from replicating after it has invaded the CD4 cell.

A more recent strategy is the use of entry inhibitors, which seek to stop the HIV from entering the CD4 cell. There are two such drugs: enfuvirtide (Fuzeon) by Roche and maraviroc (Selzentry) by Pfizer, approved by FDA in March 2003 and August 2007, respectively.

Fuzeon works by attaching to the glycoprotein gp41 of the HIV and preventing the virus from using the gp41 to attach to the surface proteins of the CD4 cell. Selzentry, on the contrary, targets the CCR5 receptor on the CD4 surface. Once bound, the HIV cannot attach to CD4 as the CCR5 is not available to interact with the HIV.

Research is ongoing on another type of inhibitor, which targets the glycoprotein gp120 on the HIV.

*Source*: Data from Food and Drug Administration 2012, *Fuzeon*, viewed January 23, 2014, http://www.fda.gov/safety/medwatch/safetyinformation/ucm318611.htm; 2. Food and Drug Administration 2014, *Selzentry*, viewed January 23, 2014, http://www.fda.gov/safety/medwatch/safetyinformation/ucm215506.htm

This reaction then switches on the effector molecule, and the signal is relayed along the pathway (refer to Figure 2.7). When the enzyme GTPase hydrolyzes GTP to GDP and removes the phosphate group, the trimeric subunits change back to the inactivated state. This receptor is once again ready to receive and transmit further signals.

GPCRs are involved in a wide range of diseases, including asthma, hypertension, inflammation, cardiovascular disease, cancer, gastrointestinal, and central nervous system (CNS) diseases. From the Human Genome Project, it is estimated that there are about 1,000 GPCRs. Current therapeutic drugs target only about 50 of these GPCRs. There are therefore many possibilities of developing new drugs to target this family of receptors.

### 2.7.2 Ion Channel Receptors

There are two main types of ion channel receptors: ligand-gated and voltage-gated. In addition, some ion channels are regulated through GPCRs or via activation by amino acids.

The ligand-gated family consists of receptors of the so-called cys-loop superfamily (nicotinic receptor, gamma-aminobutyric acid [$GABA_A$ and $GABA_C$] receptors, glycine receptors, 5-hydroxytryptamine [$5\text{-}HT_3$] receptors, and some glutamate activated anionic channels). The common feature is that they are made up of five subunits (designated as two $\alpha$, one $\beta$, one $\gamma$, and one $\delta$ subunits – Figure 2.8). Natural ligands for this family of ion channels are acetylcholine (Ach), GABA, glycine, and aspartic acid.

**TABLE 2.3 Selected Drugs and Target Receptors**

| Drug | Therapeutic Category | Drug Target |
|---|---|---|
| Amlodipine (Norvasc, Pfizer) | Cardiovascular | Ion channel |
| Atorvastatin (Lipitor, Pfizer) | Cardiovascular | Enzyme inhibitor |
| Augmentin/amoxillin plus clavulanic acid (GlaxoSmithKline) | Anti-infective | Enzyme inhibitor |
| Bevacizumab (Avastin, Genentech) | Cancer | Vascular endothelial growth factor inhibitor |
| Celecoxib (Celebrex, Pharmacia) | Musculoskeletal | Enzyme inhibitor |
| Clopidogrel (Plavix, BMS/Sanofi-Aventis) | Hematology | Platelet receptor inhibitor |
| Erythropoietin (Epogen, Amgen) | Hematology | Transmembrane agonist |
| Erythropoietin (Procrit, Ortho Biotech) | Hematology | Transmembrane agonist |
| Esomeprazole (Nexium, AstraZeneca) | Gastrointestinal/metabolism | Ion channel |
| Etanercept (Enbrel, Amgen) | Rheumatoid arthritis | TNFα |
| Fluoxetine (Prozac, Eli Lilly) | Central nervous system | GPCR |
| Lansoprazole (Takepron, Takeda) | Gastrointestinal/metabolism | Ion channel |
| Loratadine (Claritin, Schering) | Respiratory | GPCR |
| Olanzapine (Zyprexa, Eli Lilly) | Central nervous system | GPCR |
| Omeprazole (Losec, AstraZeneca) | Gastrointestinal/metabolism | Ion channel |
| Paroxetine (Seroxat, GlaxoSmithKline) | Central nervous system | GPCR |
| Sertraline (Zoloft, Pfizer) | Central nervous system | GPCR |
| Simvastatin (Zocor, Pfizer) | Cardiovascular | Enzyme inhibitor |
| Trastuzumab (Herceptin, Genentech) | Cancer | Overexpressed HER2 protein |
| Venlafaxine (Effexor, Wyeth) | Central nervous system | Serotonin–norepinephrine reuptake inhibitor |

*Source*: Adapted from Renfrey, S and Featherstone, J 2002, 'From the analyst's couch: Structural proteomics', *Nature Reviews Drug Discovery*, 1, pp. 175–176; 2. Imming, P, Sinning, C and Meyer, A 2006, 'Drugs, their targets and the nature and number of drug targets', *Nature Reviews Drug Discovery*, 5, pp. 821–834.

CNS, central nervous system; GPCR, G-protein coupled receptor.

**TABLE 2.4 Mechanisms of Action for Selected Drugs**

| Drug | Mechanism |
|---|---|
| *Antihypertensive* | |
| Hydrochlorothiazide | Increases sodium and water excretion, decreases blood volume, thereby reduces cardiac output |
| Prazosin | Alpha-adrenergic receptor antagonist, inhibits sympathetic stimulation of arteriolar contraction |
| Atenolol | Beta-adrenergic receptor anatagonist, reduces cardiac output by decreasing heart rate and contraction |
| Captopril | Angiotensin-converting enzyme inhibitor, decreases arterial and venous pressure, reduces cardiac load |
| Verapamil | Calcium channel blocker, relaxes vascular smooth muscle |
| *Hyperlipidemia* | |
| Atorvastatin | HMG-CoA reductase inhibitor, inhibits conversion of HMG-CoA to mevalonic acid for the synthesis of cholesterol |
| *Anticoagulant* | |
| Warfarin | Inhibits synthesis of clotting factors II (prothrombin), VII, IX, and X |
| Heparin | Activates antithrombin III, inhibitor of thrombin, and factor X |
| Clopidogrel | Antiplatelet, inhibits expression of glycoprotein receptors to reduce fibrinogen binding and platelet aggregation |
| *Central nervous system (CNS)* | |
| Benzodiazepines | Acts as sedative-hypnotic, opens ion channels, chloride ion influx, leading to neuronal membrane hyperpolarization |
| Bupivacaine | Anesthetic, binds to sodium channel, decreases sodium permeability, stops action potential from propagating and thus transmission of sensory input becomes inhibited |
| Phenothiazine | Functions as antipsychotic by blocking dopamine and 5-HT receptors |
| Fluoxetine | Reduces neurotransmitter uptake by acting as selective serotonin reuptake inhibitor |
| Albuterol | A bronchodilator that blocks selective $\beta_2$-adrenergic receptor, increases cyclic adenosine monophosphate concentration in smooth muscle, and causes muscle to relax |
| *Antiulcerant* | |
| Cimetidine | Histamine $H_2$ receptor antagonist, reduces volume of gastric acid produced |
| *Antineoplastic (cancer)* | |
| Methotrexate | Inhibits dihydrofolate reductase, enzyme that converts folate to tetrahydrofolate for thymidine and purine synthesis |
| Fluorouracil | Generates two active metabolites: one prevents synthesis of thymidine, the other interferes with RNA function |
| Doxorubicin | Binds to DNA and uncoils DNA |

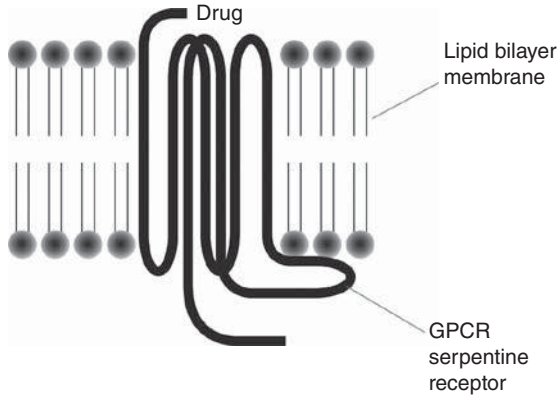



**Figure 2.6** A G-protein coupled receptor.

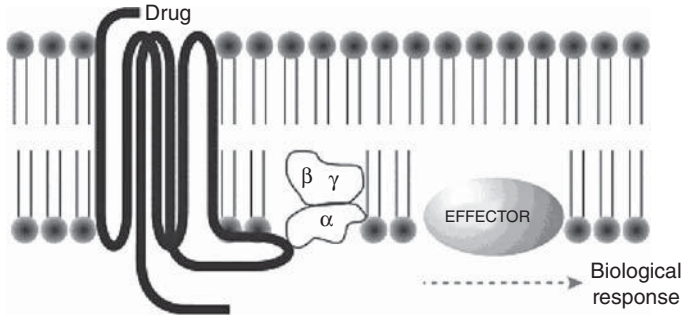



**Figure 2.7** Signal cascade in GPCR.

They are, in general, synaptic transmitters. Almost all drugs that act on the CNS target the ion channel receptors and modify some steps in the synaptic processes.

Normally, in the resting state, the channel is impermeable to ions. When a ligand binds to the receptor, it becomes activated and opens a channel to a diameter of about 6.5 Å ($6.5 \times 10^{-10}$ m). This action allows the migration of, for example, extracellular sodium ions to the interior of the cell. A cascade of further changes then proceeds within the cell to amplify the signal.

Voltage-gated ion channels depend on changes of transmembrane voltage to regulate the opening and closing of channel gates. A common feature of this type of receptor is the presence of four domains, where each domain consists of six membrane-spanning regions. Some of these channels are the sodium, calcium, and potassium channels, and they regulate the influx of these ions into the cell interior to propagate the signal.

Diseases mediated through ion channel receptors include cardiovascular disease, hypertension, and CNS dysfunctions. A voltage-gated ion channel is a key to the treatment of CF (Exhibit 2.5).

Some examples of the effects of neurotransmitters of the CNS binding to the GPCR and ion channel are given in Exhibit 2.16.

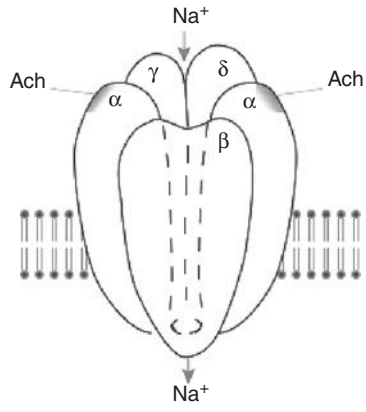



**Figure 2.8** A ligand-gated ion channel receptor. Ach, acetylcholine. The binding of Ach to the α subunits opens the ion channel, allowing $Na^+$ ions to flow through the channel into the cell.

**Exhibit 2.16 Neurotransmitters Binding to GPCR and Ion Channel**

Acetylcholine – binds to cholinergic receptor, of which there are two types: muscarinic and nicotinic

GABA – binds to both GPCR and ion channel receptors

Dopamine – binds to dopamine receptors D1 and D5: activates adenylyl cyclase; binds to receptors D2, D3, and D4; inhibits adenylyl cyclase.

Norepinephrine – binds to α and β-adrenergic receptors: causes vasoconstriction and increases blood pressure for the treatment of hypotension and shock.

Serotonin – binds to 5-HT receptor to act as excitatory and inhibitory neurotransmitter; as inhibitory function treats anxiety and depression; as excitatory function treats antipsychotic.

### 2.7.3 Tyrosine Kinases

This class of receptor transmits signals carried by hormones and growth factors. The structure consists of an extracellular domain for binding ligands and a cytoplasmic enzyme domain. The function of kinases is to enable phosphorylation. (Phosphorylation is the addition of a phosphate group ($PO_4^{3-}$) to a protein or other chemical molecule. Phosphorylation switches enzymes on and off, thereby changing their function and activity). Phosphorylation regulates most aspects of cell life.

When a ligand binds to the receptors, the receptors dimerize and join together. This action activates the enzyme within the cell. As a result, protein molecules are phosphorylated (Figure 2.9).

Other kinase receptors are serine/threonine kinases, protein kinases, and mitogen-activated protein (MAP) kinases. Insulin, transforming growth factor-beta (TGF-β), and platelet-derived growth factor (PDGF) are the natural ligands that interact with kinase receptors.

### 2.7.4 Intracellular Receptors

Intracellular (nuclear) receptors are located inside cells, in the cytoplasm or nucleus. These receptors can bind to DNA and regulate the expression of genes. Endogenous ligands such as hormones and drugs attach to these receptors to either activate (up regulate) or inhibit (down regulate) transcription messages from genes. Hence, these receptors are important in controlling the development of homeostasis (the action of a system to regulate itself to remain stable and constant) and metabolism.

There is a large superfamily of these intracellular receptors. The family's common feature is a single polypeptide chain consisting of three distinct domains:

- Amino terminus: this region in most instances is involved in activating or stimulating transcription
- DNA binding domain: amino acids in this region are responsible for the binding of the receptor to specific sequences of DNA
- Carboxy terminus or ligand-binding domain: this is the region that binds ligands.

So far, 48 intracellular receptors have been identified and they belong to 11 subgroups. Some of the common receptors are estrogen receptors, glucocorticoid receptors, and progesterone receptors. The top selling drug in 2012, Seretide/Advair, targets the intracellular receptor.

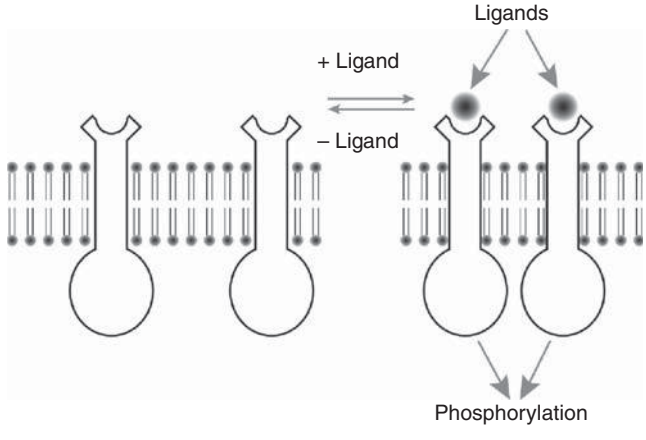



**Figure 2.9** A tyrosine kinase receptor.

## 2.8 ASSAY DEVELOPMENT

To study drug–receptor/enzyme interaction, it is not always convenient or appropriate to use a living system of the target receptor. Instead, biological assays can be devised to mimic the target. Very often, the assays use multicolor luminescence- or fluorescence-based reagents. In this way, the reaction path can be followed in space and time to enable quantitative evaluation of the reaction.

Many parameters can be monitored, such as free-ion concentrations, membrane potentials, activities of specific enzymes, rate of proton generation, transport of signaling molecules, and gene expression.

Primary assays are devised to incorporate physiological or enzymatic targets for screening biological activity of potential drug compounds. The biological assays are then reconfirmed in specific biological and whole cell assays to characterize the target compound interaction.

Exhibit 2.17 shows some current assays used in ligand–receptor studies.

## 2.9 CASE STUDY #2.1

### 2.9.1 Number of Drug Targets

A study in 2006 (refer to the source below) set out to determine the number of targets for approved drugs. It analyzed more than 21,000 drug products approved by FDA. By

**Exhibit 2.17 Reporter Assays and Bioluminescence**

Assays can be prepared with a reporter system containing, for example, the firefly luciferase gene. The reporter cells are coupled to receptor genes. When a ligand binds to the receptor, luminescence glow can be observed. In this way, the effects of the signaling events are evaluated.

There are other reporter gene systems, such as β-galactosidase (a bacterial enzyme), chloramphenicol acetyltransferase (a bacterial enzyme), and aequorin (a jellyfish protein).

*Source*: Naylor, LH 1999, 'Reporter gene technology: The future looks bright', *Biochemical Pharmacology*, 58, pp. 749–757.

Using blue-light photoreceptors from *Bacillus subtilis* and *Pseudomonas putida* that contain light oxygen voltage sensing domains, flavin-mononucleotide-based fluorescent proteins were produced that can be used as fluorescent reporters in both aerobic and anaerobic biological systems.

*Source*: Data from Drepper, T et al. 2007, 'Reporter proteins for in vivo fluorescence without oxygen', *Nature Biotechnology*, vol. 25, pp. 443–445.

**TABLE 2.5 Molecular Targets of FDA-Approved Drugs**

| Class of Drug Target | Species | Number of Molecular Targets |
|---|---|---|
| Targets of approved drugs | Pathogen and human | 324 |
| Human genome targets of approved drugs | Human | 266 |
| Targets of approved small molecule drugs | Pathogen and human | 248 |
| Targets of approved small molecule drugs | Human | 207 |
| Targets of approved oral small molecule drugs | Pathogen and human | 227 |
| Targets of approved oral small molecule drugs | Human | 186 |
| Targets of approved therapeutic antibodies | Hematology | 15 |
| Targets of approved biologics | Pathogen and human | 76 |

*Source*: Adapted from Overington, JP, Al-Lazikani, B and Hopkins, AL 2006, 'How many drug targets are there?', *Nature Reviews Drug Discovery*, vol. 5, pp. 993-996. Reproduced with permission of Macmillan Publishers Ltd.

**TABLE 2.6 Distribution of Drug Targets**

| Drug Targets | Percentage |
|---|---|
| GPCR | 26.8 |
| Nuclear receptor | 13.0 |
| Ligand-gated ion channel | 7.9 |
| Voltage-gated ion channel | 5.5 |
| Others | 46.8 |

*Source*: Adapted from Overington, JP, Al-Lazikani, B and Hopkins, AL 2006, 'How many drug targets are there?', *Nature Reviews Drug Discovery*, vol. 5, pp. 993-996.

removing duplicate active ingredients, salt forms, supplements, vitamins, and imaging agents, it deduced that there are only 1,357 unique drugs. Of these 1,204 are "small molecule drugs" and 166 are biological drugs.

The study determined that there are 324 distinct molecular targets, of which 266 are human genome-derived proteins and the remainders are bacterial, viral, fungal, or other pathogenic organism targets. Small molecule drugs target 248 proteins, whereas biologics are for 76 proteins as shown in Table 2.5.

It also determined that more than 50% of the drug targets belong to the four major families: GPCR, nuclear receptor, ligand-gated ion channels, and voltage-gated ion channels as displayed in Table 2.6.

## 2.10 CASE STUDY #2.2

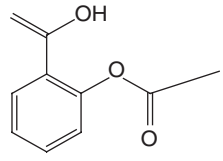
### 2.10.1 Anti-inflammatory Therapy

Since 1898, aspirin (2.1) has been used to treat pain and inflammation. Although it is effective, it causes adverse effects in the forms of gastrointestinal bleeding and ulceration. More potent NSAIDs such as ibuprofen (2.2) and naproxen (2.3) were developed

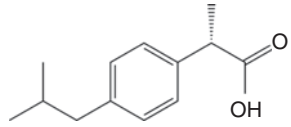
in the 1960s and 1970s. Unfortunately, they also suffer from the same effect in causing varying degrees of bleeding and ulceration with prolonged use.

Studies in the 1970s revealed that the enzyme cyclooxygenase (COX) converts arachidonic acid (2.4) to an intermediate, prostaglandin $H_2$ (2.5), as shown in the following equation:

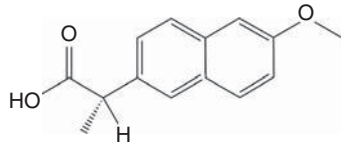
$$\text{Arachidonic acid} \rightarrow \text{Hydroperoxy endoperoxide prostaglandin } G_2(PGG_2) \rightarrow \text{Prostaglandin } H_2(PGH_2)$$




(2.1)




(2.2)

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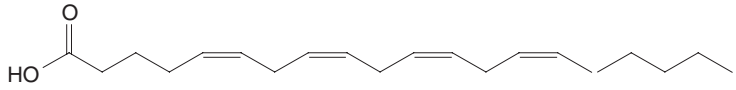


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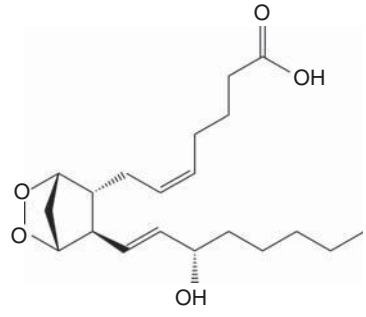



(2.5)

Another enzyme converts prostaglandin $H_2$ to prostaglandin $E_2$ ($PGE_2$):

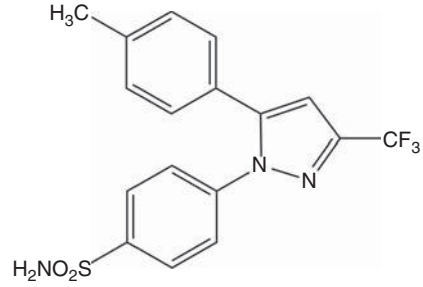
$$\text{Prostaglandin } H_2 \rightarrow \text{Prostaglandin } E_2$$

It is $PGE_2$ that is responsible for mediating pain and inflammation. NSAIDs such as aspirin, ibuprofen, and naproxen block the active site of the COX enzyme, preventing the arachidonic acid from docking to this site and becoming converted to $PGH_2$ (refer to Exhibit 2.11).

In the early 1990s, two isoforms of the COX enzyme were found: COX-1 and COX-2, with about 60% homology between them (there is also a COX-3, but it is a variant of COX-1; there are differences in the two active sites between COX-1 and COX-2, with the latter possessing an additional hydrophilic pocket).

COX-1 is found in healthy individuals and is important in maintaining a balanced physiological role in the kidneys and stomach. COX-2 is induced in the case of inflammation where it mediates the inflammation process. Aspirin, ibuprofen, and naproxen inhibit both COX-1 and COX-2 indiscriminately. Although this reduces the production of $PGE_2$ through the inhibition of COX-2, it upsets the hemostasis function of COX-1, which has a protective function for the mucosal lining, and leads to bleeding and ulceration.

Specific COX-2 inhibitors were developed in the 1990s, culminating in the approval of three drugs in late 1990s and early 2000s: Celecoxib (Celebrex), Rofecoxib (Vioxx), and Valdecoxib (Bextra) (refer to (2.6)–(2.8) and Exhibit 2.13). These drugs work well as anti-inflammatories and have no appreciable side effects with respect to bleeding and ulceration. But through prolonged use and under high dosage, both Vioxx and Bextra were found to have the potential to cause heart attacks and strokes. These two drugs were withdrawn from the market in 2004/2005, although Celebrex is still in the market with a change in labeling to warn of potential cardiovascular problems for chronic use.

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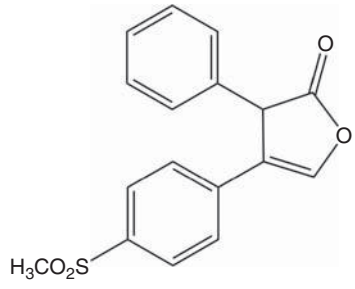


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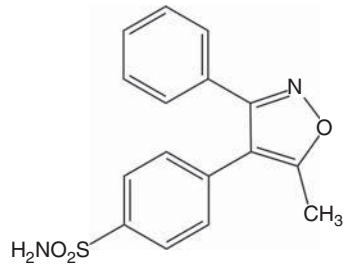


(2.8)

Work in early 2000s showed a more complicated role for the COX-2 enzyme; together with additional enzymes, COX-2 generates a number of prostaglandin compounds besides $PGE_2$, which have other regulatory functions:

$PGH_2$ + enzyme → $PGD_2$ (involves in sleep regulation and allergic reactions)

$PGH_2$ + enzyme → $PGF_2$ (controls contraction of the uterus during birth and menstruation)

$PGH_2$ + enzyme → thromboxane ($TXA_2$, stimulates contraction of blood vessels and induces platelet aggregation [clotting])

$PGH_2$ + enzyme → $PGI_2$ (dilates blood vessels and inhibits platelet aggregation; PGI may protect against artheriosclerosis and damage to stomach lining)

It is postulated that selective inhibition of COX-2 by Vioxx, for example, halts the production of $PGE_2$ as shown below:

$$PGH_2 + \text{enzyme (microsomal prostaglandin E synthase, mPGES)} \rightarrow PGE_2$$

The selective inhibition of COX-2 has no effect on COX-1, which continues to manufacture $PGE_2$ using additional cytosolic prostaglandin E synthase enzyme (cPGES), and hence the integrity of the stomach lining is maintained. But at the same time COX-2 inhibition reduces the production of $PGI_2$, leading to the possibility of cardiovascular problems.

These new findings will undoubtedly pave the way for other more selective drugs to be developed, and one can anticipate better anti-inflammatory drugs to be available to treat pain and inflammation.

*Source*: 1. Michaux, C et al. 2005, 'A new potential cyclooxygenase-2 inhibitor, pyridinic analogue of nimesulide', *European Journal of Medicinal Chemistry*, 40, pp. 1316–1324; 2. Stix, G 2007, 'Better ways to target pain', *Scientific American*, January, pp. 84–88.

## 2.11 SUMMARY OF IMPORTANT POINTS

1. Pharmaceutical companies evaluate the future direction and R&D activities on the basis of medical needs, market size, government policies and regulations, patent protection, and key competencies.
2. Most diseases, apart from trauma and infections, have origins in the genes or the proteins associated with them, as well as the biochemical pathways and networks.
3. Current approach to drug discovery starts with the identification of target or targets that cause or lead to disease.
4. Microarray is a technology used to study gene interactions and control of biochemical pathways.
5. Target validation is necessary to confirm the validity of a target as a representative of a disease model before too much investment and time is expended on it.
6. Once targets are validated, potential drug candidates are designed to bind to and interact with these targets.
7. The main drug targets are enzymes and receptors that are found on the cell surface or reside within the intracellular matrix.
8. Drugs interact with enzymes and receptors mainly through van der Waals forces and hydrogen bonding; they need the correct shapes and sizes to fit into the active sites of the targets.
9. Drugs work in two ways: as agonists and antagonists. Agonists activate the receptors whereas antagonists deactivate, block, or inhibit the receptors.
10. After binding of drug and receptor, a cascade of signal transductions occurs within the cell and is manifested as a variety of effects on the diseased biochemical pathways.
11. The major receptors are GPCRs, intracellular (nuclear) receptors, ion channels, and tyrosine kinases.
12. Assays are devised to test biological systems in laboratories as they are readily available and provide a means to evaluate the effects of potential drug candidates.

## 2.12 REVIEW QUESTIONS

1. Why is target validation an important process?
2. Explain how microarray works.
3. What are the main targets for drugs?
4. Explain how enzymes work and describe the different types of enzymes.
5. What is meant by signal transduction?
6. Describe the different types of receptors and explain how they function.
7. Why do scientists use assays in drug discovery and development?

## 2.13 BRIEF ANSWERS AND EXPLANATIONS

1. The importance of target validation is to confirm the role of the target and its effect on the biological process in altering the disease before a substantial investment is committed to the R&D.
2. Refer to Section 2.3 and Exhibit 2.8.
3. The main drug targets are enzymes, intracellular (nuclear) receptors, and extracellular (cell surface) receptors. Drugs are normally designed to interact with these entities either as agonists or antagonists to achieve control over the disease pathway.
4. Refer to Section 2.6. Explanation should include the blocking action of the drug on the enzyme and provision of examples of drugs in achieving this function.
5. Refer to Section 2.7 and Figure 2.5. It should be noted that the signal transduction process is very dynamic, and there are many cascading pathways. This explains the need to have drugs with specific interactions to reduce other reactions that give rise to adverse events (side effects). Case Study #2.2 shows the effects of drug specificity.
6. The important receptors are GPCRs, ion channels, tyrosine kinases, and intracellular (nuclear) receptors. Refer also to Appendix 3 for specific functions of the drugs in interacting with receptors.
7. Assays provide a means to test the potential drug candidate quickly and in a cost-effective manner. Until such time as the efficacy and safety assays (including preclinicals in animals) are completed and show that the candidate has the potential in becoming a drug, it should never be tested on humans.

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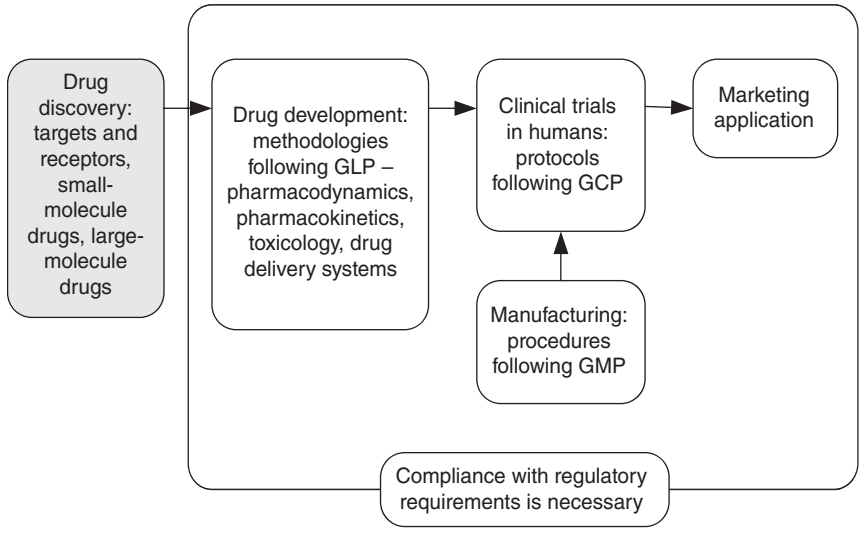
# CHAPTER 3

# DRUG DISCOVERY: SMALL MOLECULE DRUGS




## 3.1 INTRODUCTION

The World Health Organization (WHO) in its report – "WHO Traditional Medicine Strategy 2002–2005" estimated that up to 80% of the African people and a significant percentage of the worldwide population currently practice some form of traditional

*Drugs: From Discovery to Approval*, Third Edition. Rick Ng.

**Exhibit 3.1 Forms of Traditional Medicines**

*Decoctions*: These are liquid extracts of active components and volatile oils from natural products.

*Tinctures*: These are made by steeping fresh or dried herbs in alcohol or vinegar.

*Syrups*: These are made by combining tinctures or medicinal liquors with honey or glycerin.

*Ointments*: Typically, these are prepared by mixing floral or plant ingredients with essential oil and wax, such as beeswax.

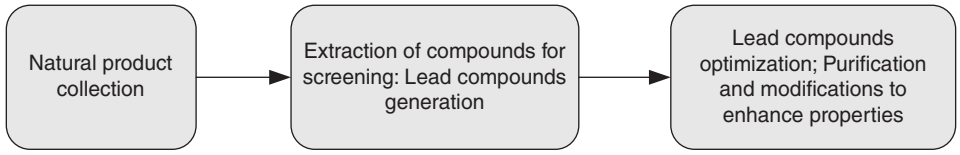



**Figure 3.1** The basic steps of the irrational drug discovery process.

medical treatment. Typically, these treatments are in the forms of decoctions, tinctures, syrups, or ointments with plant or animal products (refer to Exhibit 3.1).

However, for most readers of this book, the drugs that we are familiar with, such as analgesics (paracetamol), antibiotics (penicillin), hormones (insulin), and vaccines (influenza), are not part of the traditional medicinal armory. These drugs are either chemically synthesized (small molecule drugs with typical molecular weights of less than 500 Daltons where Dalton is a unit of mass for atoms and molecules) or produced using recombinant DNA technology (protein-based large molecule drugs with molecular weights in excess of thousands of Daltons). We describe the discovery methodologies for the small molecule drugs in this chapter, and the methodologies for the protein-based large molecule drugs in Chapter 4.

There are two main approaches to discovering small molecule drugs: the irrational approach and the more recent structured rational approach. Other drug discovery methodologies are Antisense, RNA interference (RNAi), and chiral drugs.

## 3.2 IRRATIONAL APPROACH

The basic steps of the irrational or random scanning approach are shown in Figure 3.1.

### 3.2.1 Natural Product Collection

This approach of drug discovery commences with the collection of natural materials from their habitats. Such collections typically gather 1–5 kg of materials, which may consist of the leaves, shoot, bark, and roots of plants. Marine life forms are also collected (refer to Exhibit 11.1). The collection locations are recorded to facilitate further collection of materials should it be necessary.

**Exhibit 3.2 Regulations for Biodiversity Prospecting**

Until 1993, obtaining samples of plants, microorganisms, animals, and marine life forms was straightforward. Normally, a researcher would arrive at the collection site (with permission from the local authority) and collect samples without much restriction.

There are now new rules for biodiversity prospecting regarding the collection of natural products. The 1993 Convention on Biological Diversity (CBD) established sovereign national rights over biological resources and committed member countries to conserve them, develop them sustainably, and share the benefits resulting from their use. Currently, there are 193 member countries in the convention.

CBD requires that permission is obtained before biological samples can be taken. To comply with the CBD, an Access and Benefit-Sharing Agreement (ABA) has to be agreed between the researcher and the source country providing the natural products. The ABA sets out the clauses with respect to the observation, development, and benefit sharing accruing from the use of natural products for medical applications.

With the ABA, the source country must know in advance how the natural products are to be exploited and the benefits can be shared. If the CBD is not observed, the natural products can be treated as being poached and the patent based on these products may be invalidated.

The latest issue being addressed by the Convention is the Nagoya Protocol on Access to Genetic Resources and the fair and equitable sharing of benefits arising from their utilization. More than half the number of international ratifications required has been in place since the end of 2013.

*Source*: Data from 1. Gollin, MA 1999, 'New rules for natural products research', *Nature Biotechnology*, 17, pp. 921–922; 2. Convention on Biodiversity 2013, *Landmark treaty on genetic resources surpasses halfway mark to entry into force*, viewed January 28, 2014, http://www.cbd.int/doc/press/2013/pr-2013-12-20-abs-egypt-en.pdf

An important aspect of natural product collection is to provide biodiversity, that is, products with different and diverse chemical compositions so that many potential variations of chemical compositions and structures can be extracted for testing.

Earlier, collections of natural products (also called bioprospecting) used to be relatively straightforward, with little formality and few encumbrances. Now, however, new rules (Exhibit 3.2) exist to protect the natural habitats.

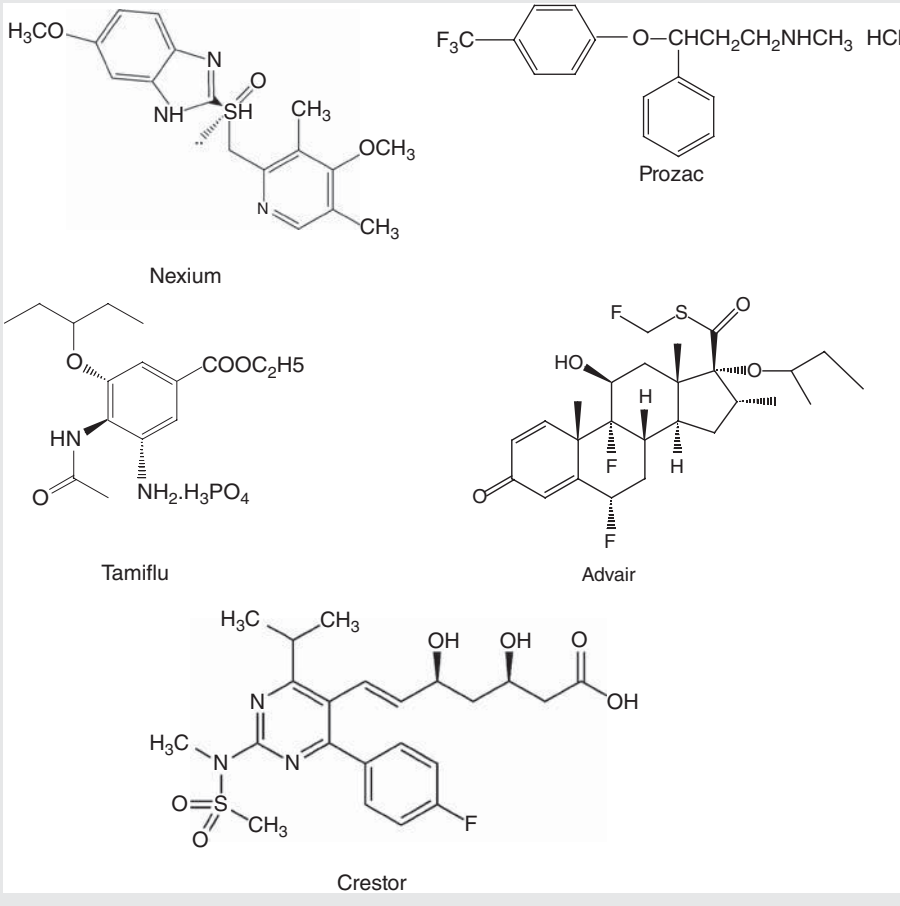
### 3.2.2 Extraction of Potential Drug Compounds

Compounds are extracted from natural materials using organic solvents such as alcohol. Tannins and chlorophylls from plant materials are normally removed using chromatographic columns, as they can interfere with the screening. In some cases, animal products, such as venoms from snakes, are gathered for screening. In other cases, collections of microorganisms are examined. Two examples are provided below:

- One of the first angiotensin-converting enzyme (ACE) inhibitors was teprotide. It is an antihypertensive drug for use after heart attacks. The active ingredient was isolated from the venom of a South American viper snake. Other well-known ACE inhibitors such as captopril and enalapril were developed on the basis of modifications to the venom's chemical structures.
- Tetracyclines are a group of antibiotics derived from bacteria. Chlortetracycline was isolated from *Streptomyces aureofaciens* and oxytetracycline from *Streptomyces rimosus*. Tetracyclines act by binding to receptors on the bacterial ribosome and inhibiting bacterial protein synthesis.

Exhibit 3.3 shows the chemical formulas of some of the well-known drugs.

**Exhibit 3.3 Chemical Formulas of Selected Drugs**



### 3.2.3 Screening Compounds to Find "Hits": Lead Compound Generation

The next step is the screening of thousands of these compounds to find lead compounds or potential drug molecules that bind with receptors and modulate disease pathways. When an interaction happens, it is referred to as a "hit."

In some cases, compounds are purchased from laboratories or other suppliers. Collections of various compounds are called "libraries," and libraries of large pharmaceutical organizations contain between hundreds of thousands and millions of compounds. For example, the Developmental Therapeutics Program of the United States National Cancer Institute has a collection of more than 600,000 synthetic and natural compounds.

Lead compounds are those that have shown some desired biological activities when tested against assays. However, these activities are not optimized. Modifications to the lead compounds are necessary to improve the physicochemical, pharmacokinetic, and toxicological properties for clinical applications.

### 3.2.4 Purification and Modifications to Optimize Lead Compounds

Following "hits," lead compounds are purified using chromatographic techniques, and their chemical compositions are identified via spectroscopic and chemical means. Structures may be elucidated using X-ray crystallography techniques or nuclear magnetic resonance (NMR) methods.

Further tests are carried out to evaluate the potency and specificity of the isolated lead compounds. This is usually followed by modification of the compounds to improve properties; these modifications occur through synthesis of variations to the compounds via chemical processes in the laboratory and frequently involve modifications to the functional groups. The optimized lead compounds go through many iterative processes to keep improving and optimizing the drug interaction properties to achieve improved potency and efficacy.

Only after all these exhaustive tests are a few candidates selected for preclinical *in vivo* studies using animal disease models. The current approach is to perform as many of the tests as possible on tissue cultures or cell-based assays, as they are less costly and provide results more readily. At the end of this long preclinical process, selected drug candidates with sufficient efficacy and safety required for human clinical trial are available.

Drug discovery and development is a tortuous path – there are factors that constrain its development such as government policies and regulations, ethical obligations or considerations and even objections from unexpected quarters, such as animal rights and environmental groups (Exhibit 3.4).

### 3.2.5 High Throughput Screening

The screening of thousands of natural products using wet laboratory chemistry processes is extremely time consuming. To alleviate this, the latest technology in screening is based on laboratory automation and robotics systems. This is termed high throughput screening (HTS) or ultra-HTS (UHTS). These two systems can screen thousands and hundreds of thousands of samples per day, respectively.

The heart of the HTS system is a plate, or tray, featuring tiny wells where assay reagents and samples are deposited and their reactions monitored. The plates have

**Exhibit 3.4 Paclitaxel (Taxol)**

Paclitaxel (Taxol, Bristol-Myers Squibb) is a chemotherapy drug for cancers of the ovaries, breasts, and certain lung cancers. It was discovered by the US National Cancer Institute in the 1960s. Originally, it was extracted from the bark of the North American yew tree (*Taxus brevifolia*). Clinical tests had necessitated the harvesting of the bark, and this method damaged the trees irreversibly.

Environmental groups objected to this practice, and many demonstrations were staged. A solution was eventually found when the needles of the European yew tree (*Taxus baccata*) provided a source for the paclitaxel precursor without destroying the bark.

Paclitaxel was introduced by Bristol-Myers Squibb in 1993. Today, paclitaxel or a precursor can be obtained from cell cultures of *Taxus* media formed by hybridizing *Taxus baccata* and *Taxus cuspidate*.

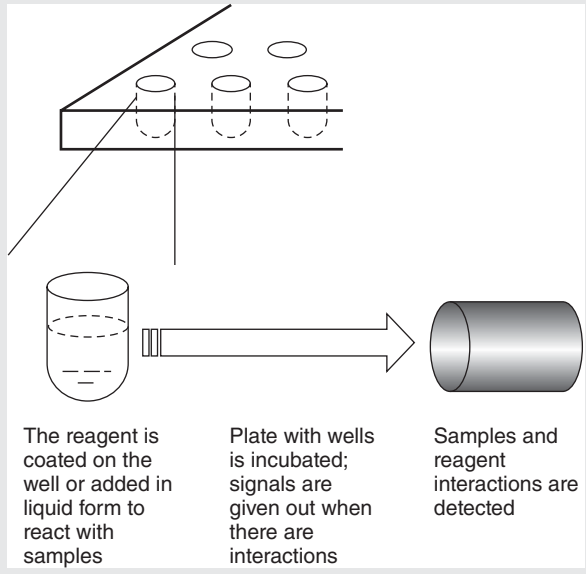
*Source*: Data from Goodman, J and Walsh, V 2001, The Story of Taxol: Nature and Politics in *The Pursuit of an Anti-cancer Drug*, Cambridge University Press, Cambridge, UK.

changed from 96 wells (in a matrix of 8 rows by 12 columns) to 384-well, and now to high-density 1536-well and 3456-well formats, which enable large-scale screening to be undertaken. Assay reagents – such as chemical compounds, cells, or enzymes – may be coated onto the plates or deposited in liquid form together with test samples into the wells. Some wells may remain empty and be used as experimental controls. Both samples and assay reagents may be incubated, and those that interact show signals, which can be detected. A variety of detector systems is used, ranging from radioactive readouts to fluorescence and luminescence. These signals indicate "hits" (positive reactions), and the strengths of the signals indicate the quality of "hits." Lead compounds with good quality "hits" warrant further evaluation as potential candidates for optimization or modification to become drug candidates. Exhibit 3.5 shows a schematic representation of HTS.

The aim of HTS and UHTS is cost-effectiveness and speed of compound scanning. Hence, the robotics system not only has to deliver fast and accurate liquid samples into the wells, it has to be miniaturized to conserve the volumes of the valuable samples and expensive assay reagents required. For the 1536- or 3456-well plates, the liquids being dispensed are in the nanoliter ($10^{-9}$ L) to picoliter ($10^{-12}$ L) range. The ink jet technology provided the technological basis for liquid dispensation in HTS.

The design of assay systems is another particularly important factor for testing the sample compounds. Assays have to be specific and sensitive. The assays used for HTS come in many forms. There are binding assays or enzyme-based or cell-based assays. Cell-based assays have become an important test compared with other *in vitro* assays, as they can provide information about bioavailability, cytotoxicity, and effects on biochemical pathway. Invariably, the enzyme-based and cell-based assay systems consist of receptors (GPCRs, ion channels) or mimetics of receptors (components that mimic active parts of receptors). Normally, the assays are linked to an indicator that

**Exhibit 3.5 High Throughput Screening**




shows the ligand–receptor interaction as some form of signal. Radioligand binding assays were used previously. However, because of the lengthy processing and limited data provided, radioligands binding assays have been superseded by other assays. Scintillation proximity assays (SPAs) and reporter systems such as luciferase (an enzyme in fireflies that gives off light) are common forms of signal generation for assay systems (refer to Section 2.8). The advantage of cell-based assays over biochemical assays is that cell-based assays enable analysis of sample compound activity in an environment that is similar to the one in which a drug would act. It also provides a platform for toxicity studies.

The NIH has set up a consortium called the Molecular Libraries Screening Center Network (MLSCN), which performs HTS on assays provided by the research community. It currently has more than 300,000 chemically diverse compounds. This is an initiative of the Molecular Libraries Roadmap that also has two more components: Cheminformatics and Technology Development. The aim is to generate a comprehensive database of chemical compounds and their bioactivities to enhance the capability for the development of new drug entities.

## 3.3 RATIONAL APPROACH

The premise of the rational approach is that drug discoveries are based on the knowledge of the three-dimensional (3D) structure, and the amino acid sequence of the chosen receptor would reveal potential binding sites for drug molecules. Structural

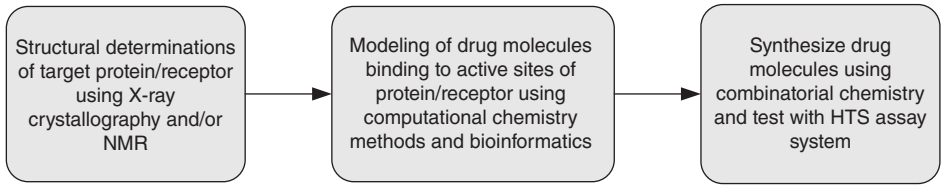



**Figure 3.2** The basic steps of the rational drug discovery process.

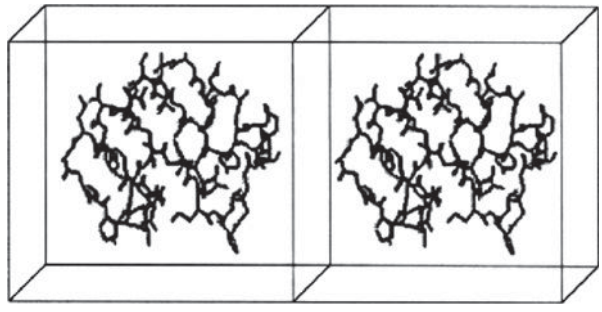


**Figure 3.3** Crystalline molecules in 3D. Unit cells are imaginary blocks used to represent the regular arrangement of molecules in 3D space.

information and modeling simulations will help to design a drug that will fit precisely within the binding site, similar to the lock and key concepts discussed in Chapter 2. Such information about the receptor or its protein structure will eliminate unlikely drug candidates at a very early stage of drug discovery and significantly improve the probability of obtaining a successful drug. The steps in rational drug discovery are summarized in Figure 3.2.

The standard techniques used for 3D structural determinations are X-ray crystallography and NMR spectroscopy. Modeling of drug–receptor interactions is studied using computational chemistry (*in silico*) methods and mining data through bioinformatics. The modeled drug is then synthesized using combinatorial chemistry and screened against assays in HTS system. These enabling technologies are described below.

### 3.3.1 X-Ray Crystallography

To determine the structures of drug compounds or their protein receptor molecules using X-ray crystallography, it is necessary to have these compounds or molecules available in crystalline form. For example, when crystals of protein are formed, the protein molecules are arranged in orderly fashions like tiny imaginary "cubes" stacked on top of one another. Each of these building blocks contains a molecule of protein and is termed a unit cell (Figure 3.3).

For examining atomic structures with bond lengths of 1–2 Å ($1\ \text{Å} = 1 \times 10^{-10}$ m), the interrogating beams ideally should have wavelengths of the same dimensions, to resolve atomic details. X-ray fulfils this criterion because its wavelength, for example, $CuK_{\alpha}$ (X-ray using copper target) is 1.5418 Å, which is similar to atomic dimensions.

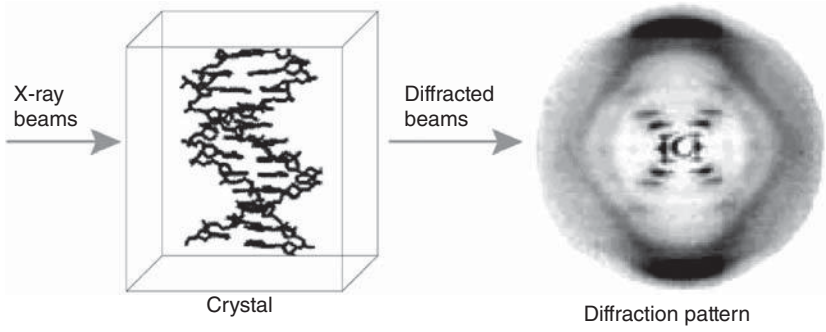



**Figure 3.4** Steps in X-ray structure determination. X-ray scattering by the crystal gives rise to a diffraction pattern. From the diffraction pattern, the molecular structure can be determined using Fourier transformation mathematical calculations. (*Source*: Diffraction photograph from Franklin, RE and Gosling, RG (1953), 'The structure of sodium thymonucleate fibres. I. The influence of water content', *Acta Crystallographica*, 6, pp. 673–677. Available at: http://journals.iucr.org/. Reproduced with permission of the International Union of Crystallography)

When X-ray beams are focused on different orientations of these unit cells, the regular crystal lattice arrangement scatters the X-ray, and a 3D diffraction pattern consisting of thousands of diffraction spots is created. The intensity of each diffraction spot is the summation of constructive interference by the atoms (represented as electron density) in the molecule at a certain orientation, and the dimensions and pattern arrangement are due to the geometric positions of these atoms within the protein molecule. From the intensities and diffraction pattern, together with solving the phase angles (angles in a mathematical sinusoidal function) of the diffracted beams, highly complex mathematical functions are used to determine the protein structure. Figure 3.4 shows a diagrammatic representation of the crystal structure determination process. A more detailed description of X-ray crystallography is presented in Exhibit 3.6.

A major drawback with X-ray crystallography is the requirement to obtain crystals of proteins, which is a difficult process. However, techniques are being improved and many structures of proteins have been solved. The Protein Data Bank, a public database managed by Rutgers (The State University of New Jersey), and the University of California San Diego have close to 100,000 structures in 2014.

The nature of crystallization also means that the protein molecules are "frozen" in space rather than in the natural liquid state as found in the human body. When a ligand is cocrystallized with protein, the active binding site is easily discernable from the structure determined. In situations where it is not possible to insert the ligand, the active site on the protein, which is normally in the form of a clef or pocket on the surface, can be inferred from comparison with other known structures. Once the structure of the active site is known, potential drug molecules can be designed using computational chemistry methods (Exhibit 3.7). Examples of some of the well-known drugs discovered using X-ray crystallography are the human immunodeficiency virus (HIV) protease inhibitor drugs, such as amprenavir (Agenerase – now replaced by a second generation drug called fosamprenavir (Lexiva) and nelfinavir (Viracept)). They were designed by studying the interactions of potential drug compounds using the crystal structure

of HIV protease. The flu drugs zanamivir (Relenza) and oseltamivir (Tamiflu) were developed with extensive modeling of the crystal structure of neuraminidase, as described in Exhibit 3.7.

**Exhibit 3.6 X-Ray Crystallography**

Electrons are the components in atoms that scatter X-rays. The magnitude of scattering is measured in terms of the atomic scattering factor, $f$, which is proportional to the number of electrons in the atom. Hence, heavier atoms have higher atomic scattering factors. For a molecule composed of many atoms, the combined scattering of X-rays by a group of atoms is known as the structure factor, which is the summation of all the atomic scattering factors in space from the unit cell. This is given by the equation:

$$F(hkl) = \sum f_j \cos 2\pi(hx_j + ky_j + lz_j) + \sum f_j \sin 2\pi(hx_j + ky_j + lz_j)$$

where $F$ is the structure factor, $f$ is the atomic scattering factor, $h$, $k$, $l$ are the indices for imaginary diffracting planes, and $x$, $y$, $z$ the position of the scattering atom.

It should be noted that $F$ is a vector quantity with magnitude and direction (phase angle).

The diffraction pattern provides us with intensity and geometric information. The equation for the intensity of each diffracting plane, $I_{hkl}$, is given by:

$$I(hkl) = | F(hkl)|^2.\mathrm{LP}.A$$

where LP is a combined geometry and polarization factor, and $A$ is the absorption correction factor.

To determine the structure, we have to locate the atoms, which are given by the electron density equation:

$$\rho(xyz) = \frac{1}{V}\sum_h \sum_k \sum_l F(hkl) \exp(-2\pi i(hx + ky + lz))$$

where $V$ is the volume of the unit cell.

Note that we need $F$, a complex quantity, to find the electron density. However, from the intensity we can only derive the amplitude of $F$. The lack of phase information requires specific methods for solution, which is beyond the scope of this book.

From the intensity, the value for the observed $F$ is obtained. This is substituted into the electron density equation for locating the atoms that determine the structure of the protein. Through an iterative process, the observed and calculated $F$ values are compared to determine the "goodness of fit" and hence the quality of the structure.

The quality function is given by the crystallographic reliability factor, $R_f$:

$$R_f = \sum | F_o - F_c | / F_o$$

where $F_o$ is the observed structure amplitude from the real molecule, and $F_c$ is the structure factor calculated from the derived structure.

A perfect match has $R_f = 0$. Most protein structures have $R_f$ values of 0.05–0.10, and a completely random structure gives an $R_f$ of 0.59.

### 3.3.2 Nuclear Magnetic Resonance Spectroscopy

NMR is another powerful tool for determining the 3D structures of compounds. In contrast to X-ray crystallography, NMR requires that the compound be in solution, rather than crystalline. It provides information about the number and types of atoms in the molecule and the electronic environment around these atoms.

The principle behind NMR is that, when a molecule is placed in a strong external magnetic field, certain nuclei of atoms within the molecule, such as $^{1}H$, $^{13}C$, $^{15}N$, $^{19}F$,

**Exhibit 3.7 Development of Zanamivir (Relenza) and Oseltamivir (Tamiflu)**

Flu virus has two types of spikes on the surface: neuraminidase and hemagglutinin. The neuraminidase and hemagglutinin undergo mutations, and these mutations account for the different types of flu viruses (refer to Exhibit 4.2).

The virus uses its hemagglutinin to bind to human cells by interacting with the sialic acid on the human cell surface. The cell then takes up the virus. The virus eventually enters the nucleus, where it replicates to produce many new virus genes. The virus genes combine to become multiple copies of viruses, which are released from the cell. When viruses are released, there is a coating of sialic acid on the hemagglutinin, rendering it unable to bind to new cell surface. But the neuraminidase of the virus is able to cleave the sialic acid, thereby letting the hemagglutinin loose to attach and infect other cells.

There is a conserved part on neuraminidase, and this does not mutate or bind to sialic acid. X-ray crystallography revealed that this conserved part is a cleft with four parts. Drug molecules were designed to fit into this cleft and jam the neuraminidase, so that it is not available to cleave the sialic acid. When the sialic acid remains intact on the hemagglutinin, the virus is unable to attach to new cells and propagate the infection.

Two drugs were designed: zanamivir (Relenza) by GSK and oseltamivir (Tamiflu) by Roche. Zanamivir is a powder that has to be inhaled, and oseltamivir is an oral drug.

*Source*: Data from Laver, WG, Bischofberger, N and Webster, RG 1999, 'Disarming flu viruses', *Scientific American*, January, pp. 78–87.

and $^{31}$P, will resonate as they absorb energy at specific frequencies that are characteristic of their electronic environment. Because most drug and protein molecules are composed of hydrogen, carbon, nitrogen, fluorine, and phosphorus, NMR is ideally suited to unravel structural information of drugs and proteins.

An NMR spectrum shows the types of environment around the nuclei (atoms) and the ratios of these nuclei. Compared with X-ray crystallography, NMR has the advantage of being carried out in concentrated solutions rather than requiring crystal samples. The solution states are more representative of the native environment of receptor proteins. NMR can be used to study ligand–receptor interactions. A receptor protein is labeled with isotopes such as $^{13}$C or $^{15}$N, and changes in their spectra when bound with ligands can be monitored.

However, NMR is limited to molecules with molecular weights of less than 35 kDa. When both techniques are combined, X-ray crystallography and NMR can provide invaluable information for drug design. With the precise binding site topologies derived from X-ray crystallography and dynamic properties obtained from NMR, tailor-made drug molecules can be designed to fit in the binding sites.

Exhibit 3.8 presents a more detailed description of NMR, and Exhibit 3.9 illustrates the use of NMR in drug discovery.

### 3.3.3 Bioinformatics

Bioinformatics is the use of information technology for the collation and analysis of biological data. This field of study began in the 1980s; it was initially set up by the US Department of Energy for the storage and retrieval of short sequences of DNA. The resultant database is called GenBank. Now GenBank has been transferred to the National Institutes of Health's National Center for Biotechnology Information (NCBI). Many more databases, both public and private, have been set up to enable scientists to deposit, revise, retrieve, and analyze biological information. The Human Genome Project is a prime example of bioinformatics. Terabytes ($2^{40}$ bytes) of capacity are used to store the sequence information of billions of DNA base pairs.

As bioinformatics evolves and matures, more and more information beyond sequences of DNAs and amino acids is added to the database. The amount of data that can be generated is phenomenal. It is reported that the growth in bioinformatics data exceeded even the well-known Moore's Law for electronics, which states that the number of transistors on a chip doubles every 18 months. The Internet has played a central role in the growth of bioinformatics. It provides a comprehensive and easily accessible means for information storage, retrieval, and analysis.

A process called data mining is used to extract the ever-expanding valuable information from databases. Data mining consists of complex computer algorithms and mathematical functions with testable hypotheses for a range of analyses. The analyses are homology comparison, gene identification, RNA transcription, and protein translation. Some of these are discussed in Chapter 2 under microarrays and expressed sequence tags for target identification. Newly sequenced DNA can be compared with previously sequenced DNA segments of model organisms. Sequences that match or closely resemble model systems enable scientists to predict the likely proteins being produced. Bioinformatics also assists scientists to assess the probable

**Exhibit 3.8 Principles of NMR**

It is a fundamental property of atomic particles, such as electrons, protons, and neutrons, to have spins. Spins can be classified as $+½$ or $-½$ spin. For example, a deuterium atom, $^{2}H$, has one unpaired electron, one unpaired proton, and one unpaired neutron. The total nuclear spin = ½ (from the proton) + ½ (from the neutron) = 1. Hence, the nuclear spins are paired and result in no net spin for the nucleus. For atoms such as $^{1}H$, $^{13}C$, $^{15}N$, $^{19}F$, and $^{31}P$, the nuclei consist of protons with unpaired spins.

In the presence of an external magnetic field, the spin of the nucleus can align in two energy states: with or against the field. When energy is applied at the right frequency, resonance occurs and the spin flips from one energy state to another according to the formula:

$$\nu = \gamma B$$

where $\nu$ is the frequency, $\gamma$ is the gyromagnetic ratio, and $B$ is the external field strength.

Nuclei are affected by the microenvironment around them. Electrons around the nuclei shield the magnetic field experienced by the nuclei. If the electrons are withdrawn, the nuclei will experience a stronger magnetic field and require more energy (higher resonance frequency) to flip the spins and vice versa. For $^{1}H$ NMR, the hydrogen nuclei of a compound can resonate downfield (higher frequency) or upfield (lower frequency) relative to a standard called tetramethylsilane (TMS).

The NMR spectrum also provides information about the number of nuclei under each distinct environment. This is given by the area under each resonant peak representing the relative number of nuclei of each type. Furthermore, the surrounding nuclei also cause a splitting pattern. For example, a H surrounded by "$n$" other H neighbors will have its resonance peak split into "$n + 1$" peaks.

*Source*: Data from Teng, Q 2004, *Structural Biology: Practical NMR Applications*, Springer, New York, NY.

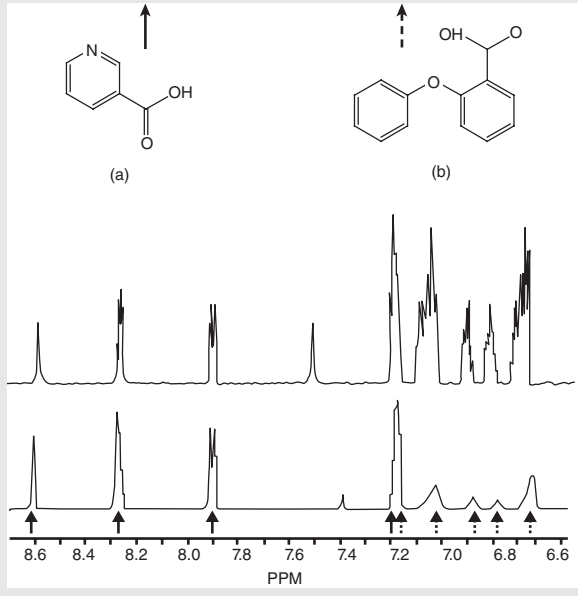
**Exhibit 3.9 NMR in Drug Discovery**

In addition to being used for structural determinations of protein targets, NMR is increasingly being used to examine the dynamic interactions of ligand–receptor binding. Two NMR properties are particularly important: chemical shift and nuclear spin relaxation.

When a ligand binds to a protein receptor, it perturbs the microchemical environment of the protein nuclei through bond formation, hydrogen bonding, and/or van der Waals forces. The shifting of the resonance frequency reflects the strengths of the interaction.

The nucleus absorbs the magnetic energy and flips to another spin state. After a finite time, the spin state reverts to the original state through an equilibrium process. Generally, small molecules with fast rotational motions have slow relaxation rates. When a ligand binds to a target, the interaction slows the rotational motions. The relaxation time thus changes. This is observed via the Nuclear Overhauser Effects (NOEs), which are a measure of Brownian motions (rotational motions of molecules). A negative NOE for a small molecule is indicative of binding to the protein target.

An example of the use of NMR to design inhibitors of the protein kinase p38 is shown below. The first NMR spectrum shows the resonance peaks of nicotinic acid (a) and 2-phenoxy benzoic acid (b) in the absence of a target enzyme. When a target enzyme is added, in this case the p38 MAP kinase, binding of the ligand and the enzyme causes line broadening and attenuation of the resonance peaks. This is shown by the second NMR spectrum, in which the affected peaks are those of the 2-phenoxy benzoic acid (from 7.2 to 6.6 ppm), indicating the interactions between p38 MAP kinase and 2-phenoxy benzoic acid.




*Source*: 1. Peng, JW, Lepre, CA, Pejzo, J, Abdul-Manan, N and Moore, JM 2001, 'Nuclear Magnetic Resonance-Based Approaches for Lead Generation in Drug Discovery', in James, TL (ed.), *Nuclear Magnetic Resonance of Biological Macromolecules*, Vol. 338, Academic Press, San Diego, California. Reproduction with permission of Elsevier. 2. Data from Pellecchia, M, Sem, DS and Wuthrich, K 2002, 'NMR in drug discovery', *Nature Reviews Drug Discovery*, 1, pp. 211–219.

3D structures of proteins. Bioinformatics, in conjunction with structural data from X-ray crystallography and NMR, helps scientists focus on the likely target and the binding sites for drugs to be designed.

In addition, bioinformatics databases have been expanded to integrate data on the absorption, distribution, metabolism, excretion, and toxicity of drugs. Through these comprehensive sets of data, scientists have powerful means to relate disease targets and their cellular functions to physiological and pathological processes.

Another application of bioinformatics is the use of pharmacogenomics. There are some diseases, such as sickle cell anemia (Exhibit 2.4), in which the difference of one amino acid group can have drastic consequences. These differences in nucleotides are termed single nucleotide polymorphisms (SNPs). SNPs, whether because of genetic origins or environmental factors, translate to individual differences. By understanding these SNPs using bioinformatics, more personalized medicines with better efficacy and less adverse effects can be prescribed (refer to Section 11.5).

In essence, bioinformatics is applied as below:

- Scan the DNA sequences to determine locations of genes
- Analyze transcription and translations of genetic codes to proteins (refer to Appendix 2.3)
- Determine possible functions and structures for proteins
- Predict binding sites for drug interactions and modulations of causative effects of disease pathway
- Provide information for drugs to be designed to fit binding sites
- Analyze SNPs for tailored prescriptions to individuals.

A simplified bioinformatics process for provision of information is shown in Figure 3.5.

### 3.3.4 Computational Chemistry

Computational chemistry is an *in silico* method (computational approach) that is used to determine the structure–activity relationships (SARs) of ligand–protein receptor binding. It encompasses a number of techniques, such as computer assisted drug design, computer aided molecular design, and computer assisted molecular modeling. There are many software algorithms written for computational chemistry, with different emphasis on modeling and SAR functions.

When a ligand (drug molecule) interacts with a protein, the protein binds with the drug and undergoes varying degrees of conformational change (structural

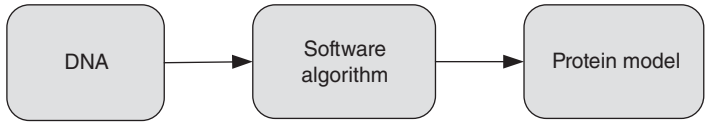



**Figure 3.5** Bioinformatics flow of information.

rearrangement) to accommodate the drug. As a result, the biological activity regulated by the protein is modified, as shown by the equation below, relating SAR as a function of the interaction:

$$\text{SAR} = f(\text{ligand–protein bonds and conformational change})$$

The aim of computational chemistry is to perform virtual screening using computer-generated ligands via a *de novo* drug design method – the design of drug compounds by incremental construction of a ligand model within a model of the receptor or enzyme active site. Libraries of virtual ligands are generated using computer on the basis of certain building blocks or frameworks (scaffolds) of chemical compounds. The method uses a genetic algorithm that simulates the genetic evolutionary process to produce "generations" of virtual compounds. The new structures may have improved ability to bind to the receptor protein, similar to the concept of "survival of the fittest" in the biological process.

To maximize the chances of success, it is necessary to build in "drug-like" properties. One of the "drug-like" criteria adopted is the "Lipinski Rule of 5" – so named because of its emphasis on the number 5 and multiples of 5. The rule states that potential drug candidates are likely to have poor absorption and permeability if they have:

- Greater than 5 hydrogen bond donors (the sum of –OH and $–NH_2$ groups)
- Molecular weight greater than 500
- Log *P* (the octanol/water partition coefficient, which indicates lipophilicity) greater than 5, or
- Greater than 10 hydrogen bond acceptors (the sum of nitrogen and oxygen atoms).

Using information about the 3D shape of a protein receptor active site, which is derived from X-ray crystallography or NMR, ligands from the virtual library can be selected and fitted into the site. This is a modeling process known as docking simulation (Figure 3.6).

Ligands are selected on the basis of their "drug-like" properties, shapes, and the orientations and distributions of chemical functional groups complementary to those of the protein. For example, a hydrogen bond donor of the ligand matches with a hydrogen bond acceptor of the protein, where positive electrostatic charge aligns with negative electrostatic charge and so on. Constituent side chains or functional groups of the ligands are varied to provide many different configurations for the docking analysis, with a view to optimize the best ligands as potential drug candidates.

Docking simulation is distinct from wet laboratory chemistry, where chemical reactions are performed using real rather than virtual compounds. The docking approach is more cost effective and efficient than the conventional chemical synthesis route. It allows a large database of virtual compounds to be screened and matched up with the binding site of the targeted protein.

Scoring systems are set up to quantitatively calculate how well a ligand docks with an active site in terms of alignment, hydrogen bonding, van der Waals forces, and electrostatic and hydrophobic interactions. In addition, flexibilities of both the ligands and protein in the binding process have to be considered as they accommodate each other.

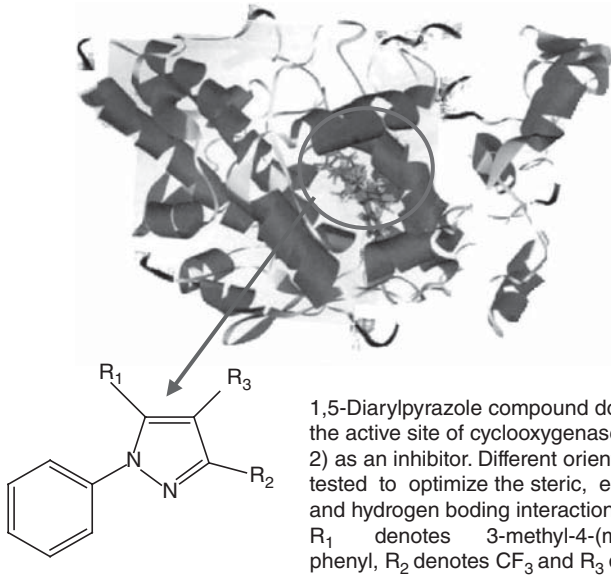



**Figure 3.6** Docking simulations. (*Source*: Liu, H, Huang, X, Shen, J, et al. 2002, 'Inhibitory mode of 1,5-diarylpyrazole derivatives against cyclooxygenase-2 and cyclooxygenase-1: Molecular docking and 3D QSAR analyses', *Journal of Medicinal Chemistry*, 45, pp. 4816–4827. Reproduced with permission of the American Chemical Society).

The affinity of interactions can be calculated in a number of ways. One example is the force field method, used to calculate the free energy of binding for the ligand–protein system before and after the docking, as given by the equation:

$$\Delta G = T\Delta S_{\mathrm{rt}} + n_{\mathrm{r}}E_{\mathrm{r}} + \Sigma n_{\mathrm{x}}E_{\mathrm{x}}$$

where $\Delta G$ is the free energy of binding, $T\Delta S_{\mathrm{rt}}$ is the loss of overall rotational and translational entropy on binding, $n_{\mathrm{r}}$ is the number of internal degrees of conformational freedom lost on binding, $E_{\mathrm{r}}$ is the energy equivalent of the entropy loss, $n_{\mathrm{x}}$ is the number of functional groups in the ligand, and $E_{\mathrm{x}}$ is the binding energy associated with each ligand functional group.

The energy calculations are the rotational and translational changes and torsional angular effects of the ligands and protein, as well as solvation and desolvation energies because ligands have to displace water molecules normally residing in the active site. An analogy is fitting a hand into a rubber glove – the fingers have to be extended and the glove stretched to accommodate the fit, similar to the rotational, translational, and torsional changes required for a good fit. Entrapped air inside the glove has to be expelled, much like the ligand replacing the water molecules at the active site. A schematic view is shown in Figure 3.7.

There are other scoring functions for rating the docking of ligands to the protein binding site. These functions are *ab initio* (from first principle) quantum mechanical calculations, which take into account the electronic populations of the entire

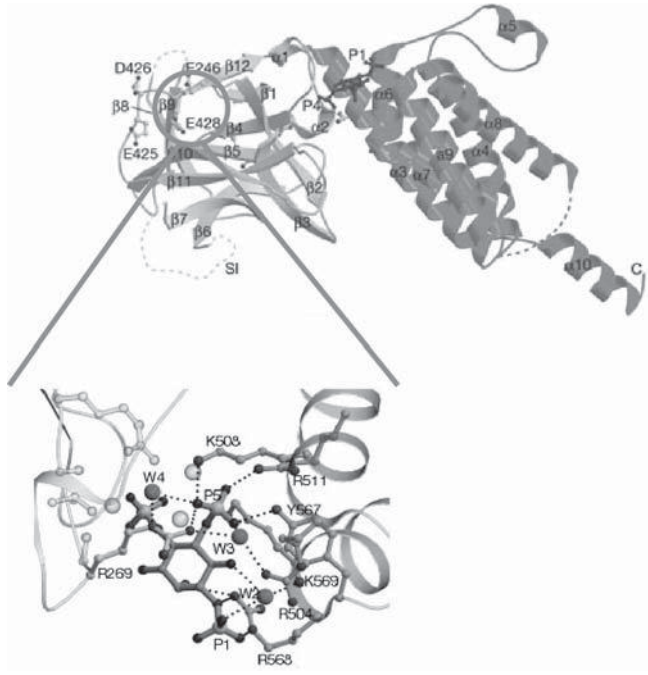



**Figure 3.7** A ligand fitting into a binding site. Binding of inositol 1,4,5-trisphosphate (InsP3) with its receptor. The InsP3 receptor plays a key role in cellular and physiological processes. (*Source*: Bosanac, I, Alattia, JR, Mal, TK, et al. 2002, 'Structure of the inositol 1,4,5-trisphosphate receptor binding core in complex with its ligand', *Nature*, 420, pp. 696–700. Reproduced with permission of Macmillan Publishers Ltd.)

ligand–protein system and the bonding scheme, and molecular Monte Carlo iterative processes (computational algorithms based on repeated random sampling to obtain numerical results), which consider thermodynamic properties, minimum energy structures, and kinetic coefficients.

The results of computational chemistry are some potential drug candidates. These can be synthesized using combinatorial or wet laboratory techniques and then tested with assays. Screening an array of ligands virtually is cost effective and compresses the discovery timeline. Exhibit 3.10 shows a typical workflow process for virtual screening.

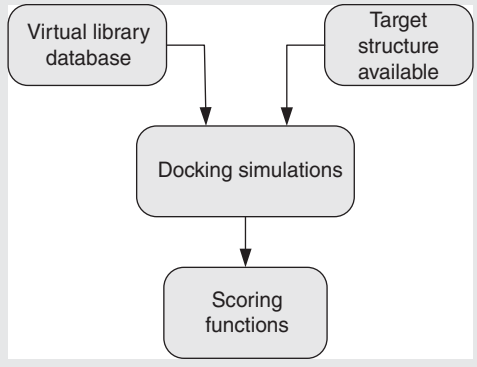
### 3.3.5 Combinatorial Chemistry

Combinatorial chemistry is a laboratory chemistry technique to synthesize a diverse range of compounds through methodical combinations of building block components. These building blocks (reagents) are added to reaction vessels, and the reactions proceed simultaneously to generate an almost infinite array of compounds, limited only by the imaginations of the scientists. This technique is in contrast to traditional methods, in which compounds are synthesized sequentially by mixing one reagent with another and

**Exhibit 3.10 Virtual Screening Process**

Presented below is a pictorial description of the workflow of a virtual screening run against a specific target. The typical workflow consists of a preparation of the virtual library database and the target. Docking simulations are taken next, and various scoring functions are used to rate the "goodness of fit" for the potential candidate to the target.

There are a number of docking programs used, such as DOCK, GOLD, FlexX, GLIDE, and ICM. The most common docking function as a guide for "goodness of fit" is the root-mean-square distance (RMSD) that defines the percentage of ligands that are within a defined distance, commonly 2 Å, of the binding site.




*Source*: Data from Verdonk, ML et al. 2011, 'Docking Performance of Fragments and Druglike Compounds', *Journal of Medicinal Chemistry*, vol. 54, pp. 5422–5431.

with further reagents to build up the compound. By using combinatorial chemistry techniques, large libraries of many thousands of compounds can be prepared very quickly, unlike the laborious collection of natural compounds from the field. However, there is a debate as to which method – combinatorial chemistry or natural collection – would provide more diverse range of compounds and biodiversity to be tested.

The selection of building blocks is based on information derived from, for example, computational chemistry, where potential virtual ligand molecules are modeled to fit a receptor protein binding site. Combinatorial chemistry commences with a scaffold or framework to which additional groups are added to improve the binding affinity. Compounds are prepared and later screened using HTS. In this way, many compounds are tested within a short timeframe to speed up drug discovery.

There are two basic combinatorial chemistry techniques: (i) parallel synthesis and (ii) split and mix methods. They are illustrated below.

*Parallel synthesis:* We start the reaction by using two sets of building blocks, amines (A) and carboxylic acids (B). The amines are first attached to solid supports, normally

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A1 B1 | A1 B2 | A1 B3 | A1 B4 | A1 B5 | A1 B6 | A1 B7 | A1 B8 | A1 B9 | A1 B10 | A1 B11 | A1 B12 |
| A2 B1 | A2 B2 | A2 B3 | A2 B4 | A2 B5 | A2 B6 | A2 B7 | A2 B8 | A2 B9 | A2 B10 | A2 B11 | A2 B12 |
| A3 B1 | A3 B2 | A3 B3 | A3 B4 | A3 B5 | A3 B6 | A3 B7 | A3 B8 | A3 B9 | A3 B10 | A3 B11 | A3 B12 |
| A4 B1 | A4 B2 | A4 B3 | A4 B4 | A4 B5 | A4 B6 | A4 B7 | A4 B8 | A4 B9 | A4 B10 | A4 B11 | A4 B12 |
| A5 B1 | A5 B2 | A5 B3 | A5 B4 | A5 B5 | A5 B6 | A5 B7 | A5 B8 | A5 B9 | A5 B10 | A5 B11 | A5 B12 |
| A6 B1 | A6 B2 | A6 B3 | A6 B4 | A6 B5 | A6 B6 | A6 B7 | A6 B8 | A6 B9 | A6 B10 | A6 B11 | A6 B12 |
| A7 B1 | A7 B2 | A7 B3 | A7 B4 | A7 B5 | A7 B6 | A7 B7 | A7 B8 | A7 B9 | A7 B10 | A7 B11 | A7 B12 |
| A8 B1 | A8 B2 | A8 B3 | A8 B4 | A8 B5 | A8 B6 | A8 B7 | A8 B8 | A8 B9 | A8 B10 | A8 B11 | A8 B12 |

**Figure 3.8** Additions of amines (A) and carboxylic acids (B) in a 96-well plate.

polystyrene beads coated with linking groups, in a separate reaction vessel for each amine. After the amines have been attached, excess unreacted amines are washed off. Next, the carboxylic acids are added to the amines to form the desired amides. We illustrate these steps in Figure 3.8. Assuming there are eight amines to react with 12 carboxylic acids in a 96-well plate with eight rows and 12 columns of tiny wells, the amines (A1–A8) are added across the rows to each well containing the polystyrene beads. Different types of carboxylic acids (B1–B12) are added to the wells in each column.

After the reactions, the compounds are separated from the beads (for instance, by using UV light), which severs the linking groups. Purification steps are applied to separate the enantiomeric compounds (refer to Section 3.6). From a mere 20 reagents, 8 amines, plus 12 carboxylic acids, we end up with 96 different compounds. By using different types of reagents, for example, X, Y, and Z, we generate X x Y x Z compounds. Hence, very large libraries are obtained through such combinations.

*Split and mix:* Here we use eight amines and eight carboxylic acids as our example. The amines are added to eight different reaction vessels and attached to polystyrene beads. Next, the amines bound to polystyrene are taken and mixed in one reaction vessel. The mixed amines are then split into eight vessels of equal portions. Each of these vessels contains amines of A1–A8 bound to the beads. Carboxylic acids are separately added to each vessel: B1 to vessel 1, B2 to vessel 2, and so on. The compounds prepared would be as follow:

Vessel 1: A1B1, A2B1, A3B1, … A8B1
Vessel 2: A1B2, A2B2, A3B2, … A8B2
.
.
Vessel 8: A1B8, A2B8, A3B8, … A8B8

Compounds can be tagged via "coding" groups on the polystyrene beads. The coding can be performed for each reaction step. At the completion of the reactions, each compound can be uniquely identified through a decoding process. All the compounds are screened, tested against target assays, and the potent ones ("hits") are selected for analysis, which may include further synthesis to refine the "hits" and optimization to yield lead compounds.

Exhibit 3.11 gives a synopsis of the development of Gleevec using the rational approach.

### 3.3.6 Genomics and Proteomics

Genomics is the use of genetics and molecular biology to study an organism's entire genome. From the sequence of the genome derived, patterns of gene expressions in cells under various conditions, healthy or diseased, can be discerned.

DNA sequencing includes the following steps:

Identify region of genome of interest → generate clones of the region
→ purify DNA from clones → sequence purified DNA

The Human Genome Project (refer to Appendix 2, A2.3.3) was completed in 2006. One method it used to improve the speed and quality of the sequencing was by capillary array electrophoresis (CAE). This technique, however, is now superseded by the "massively parallel DNA sequencing method" (Exhibit 3.12).

Proteomics, on the contrary, is the application of molecular biology, biochemistry, and genetics to study the structures and functions of proteins expressed by cells. Unlike the genome, which is reasonably static, the proteome changes constantly in response to intra- and extracellular signals. As proteins are vital to our cells and their biochemical pathways, an in-depth understanding of proteomics helps to elucidate processes of disease and devise means to counteract errant cells and processes.

It is estimated that there are more than two million different proteins in our body. Proteins are involved in a whole host of functions vital to our well-being:

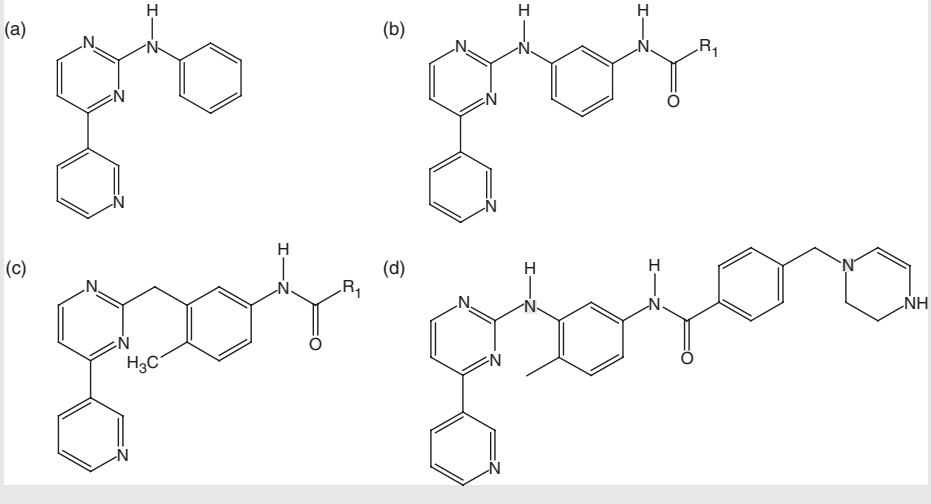
- As enzymes responsible for catalytic reactions
- As messengers for signaling and transmission
- As defense systems against microorganisms
- As components for oxygen transport and blood clotting
- As controls to regulate growth
- As ingredients of tissues and muscles.

When a disease happens, defective proteins are often involved. Most drugs also target receptors and enzymes, which are themselves proteins. Through an understanding of proteins and their functions, better and more specific drugs can be developed (refer to Appendix 2, A2.3, for information about proteins and Exhibit 3.13 for information about protein extraction and studies).

**Exhibit 3.11 A Rational Approach to the Development of Imatinib Mesylate (Gleevec)**

Imatinib mesylate (Gleevec, Novartis; Glivec in countries other than the United States) is a drug for the treatment of chronic myeloid leukemia (CML). CML is a result of a chromosomal problem and gives rise to high levels of white blood cells. An enzyme called *BCR-ABL* is involved. The *BCR-ABL* gene encodes a protein with elevated tyrosine kinase activity (See Exhibit 7.3).

The lead compound for Gleevec was identified in the screening of a combinatorial library. This compound is a phenylaminopyrimidine derivative that inhibits protein kinase C (PKC). It is a signal transduction inhibitor. Using docking studies and X-ray crystallography, different groups were introduced into the basic phenylaminopyrimidine template. Stronger PKC inhibition was obtained with a 3′-pyridyl group, compound (a). An amide group provided an inhibitory effect on *BCR-ABL* tyrosine kinase, compound (b). Compound (c) lost PKC activity but improved tyrosine kinase inhibition. Solubility and bioavailability were studied, and finally a methylpiperazine compound (d), code name ST1571, was selected for clinical trial.




*Source*: Capdeville, R, Buchdunger, E, Zimmermann, J and Matter A 2002, 'Glivec (ST1571, Imatinib), a rationally developed, targeted anticancer drug', *Nature Reviews Drug Discovery*, 1, pp. 493–502. Reproduction with permission of Macmillan Publishers Ltd.

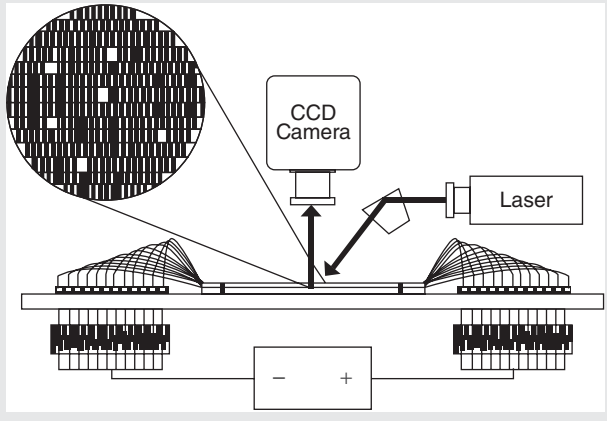
### 3.3.7 Metabolomics

The NIH refers to the term "metabolomics" as a means to "identify, measure and interpret the complex, time-related concentration, activity and flux of endogenous metabolites in cells, tissues and other biosamples such as blood, urine, and saliva".

**Exhibit 3.12 Capillary Array Electrophoresis**

DNA samples are introduced into the 96-capillary array. When the samples are separated through the capillaries, the fragments are irradiated with laser light. A charged coupled device measures the fluorescence and acts as a multichannel detector. The bases are identified in order in accordance with the time required for them to reach the laser-detector region.

Now, the current technology, called massively parallel DNA sequencing platform or next generation sequencing, is several orders of magnitude faster than CAE. This is a high throughput DNA sequencing technique and it significantly reduces time and cost significantly compared to CAE.




*Source*: 1. Huang, XC, Quesada, MA and Mathies, RA 1992, 'DNA Sequencing Using Capillary Array Electrophoresis', *Analytical Chemistry*, 64, pp. 2149–2154. Reproduced with permission from the American Chemical Society; 2. Based on data from Shendure, J and Ji, H 2008, 'Next-generation DNA sequencing', *Nature Biotechnology*, vol. 26, pp. 1135–1145.

Together with genomics and proteomics, metabolomics – by tracking the changes to the metabolites – helps to study the multivariate ways in the interactions between cells, tissues, and organs via many complex biochemical pathways. The quantitative studies of the substrates, intermediates, and products from biochemical reactions can yield useful data about healthy and diseased states and allow for the effects of potential drug candidates to be assessed.

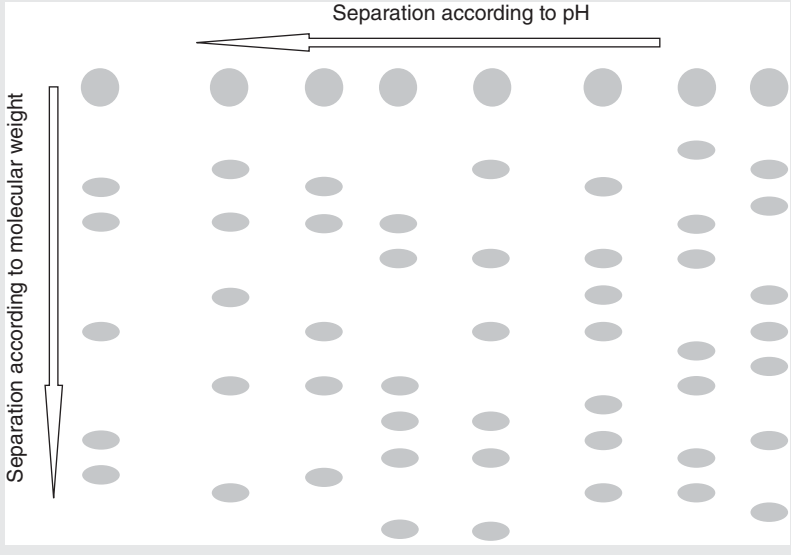
Focusing on the metabolic targets for cancer is one example of using metabolomics. It is known that metabolism changes between normal and malignant cells. The metabolic changes due to cancer lead to activation of protooncogenes (a gene that regulates normal cell growth but converted to an oncogene by mutation) and inactivation of tumor suppressor genes. Further, the accumulation of certain metabolites, such as succinate and fumarate, are known to promote oncogenesis (generation of tumors). Biochemical pathways for the maintenance of normal activities are disturbed and diverted by

**Exhibit 3.13 Protein Extraction and Studies**

To study protein from a particular cell type, the cells are grown in nutrients. After a few days, millions of cells are collected and detergents are added to rupture the cell membranes, thus enabling proteins to be released into solution. The proteins are separated from the cell debris by centrifugation where the proteins remain in solution and cell debris settle to the bottom of the vessel.

Proteins can be separated using the 2D electrophoresis method. The first dimension is the separation according to the pH of the proteins. The proteins are placed on a gel strip in a buffer solution. An electrical current is applied and the proteins separate and migrate to their isoelectric points (pI).

Next, the proteins are separated according to size. A detergent solution is added to the proteins gel strip to confer a negative charge to the proteins. Then the gel strip is placed on a precast gel where a voltage is applied, and the proteins separate in accordance with their sizes, the larger ones moving through the gel at slower rates than the smaller ones.




At this stage each spot on the gel may contain up to several proteins. The spot of interest is removed by cutting the gel and dissolving it with an enzyme. The cleaved proteins are studied by using chromatography and mass spectroscopy techniques to determine the amino acids in the proteins and their sequences. The results are compared with a database and the identities of proteins are revealed.

malignant cells. This results in the up-regulation of some biochemical pathways and down-regulations of others. Selected examples of promising metabolic targets for cancer therapy are listed in Table 3.1

### 3.3.8 Systems Biology

The advances of drug discovery technologies have been spectacular in the past two decades, yet they failed to improve the discovery of more disease-ameliorating molecules. The productivity of new drugs did not match the concomitant increased investment and technological efforts.

Critical to the success of drug discovery is the understanding of complex biological and disease systems. Systems biology starts from the premise to relate the complex biological systems down to the level of organs, tissues, cells, and their molecular pathways and regulatory networks. It is perceived that biological systems are robust against various perturbations such as mutations, toxins, and environmental changes, but are ill-equipped to deal with perturbations for which they are not optimized.

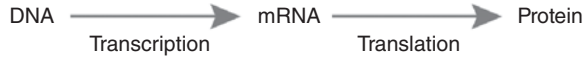
Systems biology uses computational analysis to integrate genomics, proteomics, and metabolomics data with disease physiology information. A knowledge of the type of genes, protein expression levels, and metabolite production aids in identifying the specific molecular pathways switched on during certain disease states. This provides an *in silico* framework for constructing testable biological systems.

Cell, tissue, and organ type of models consisting of networks of signaling pathways, nodes, and regulatory points can be probed with putative drug molecules (refer to Exhibit 2.2). It is postulated that through understanding of the interactions between robustness of biological systems, diseases, and drug effects, systems biology could provide a better discovery approach for drugs with the desired efficacy and less adverse effects.

Exhibit 3.14 provides a diagrammatic visualization of the approaches to tackle this new field of study.

## 3.4 ANTISENSE APPROACH

Genetic information is transcribed from the genes in the DNA to mRNA. The information is then translated from the mRNA to synthesize the requisite protein (refer to Appendix 2). This process is depicted below:




The aim of antisense therapy is to identify the genes that are involved in disease pathogenesis. Short lengths of oligonucleotides of complementary sense (hence "antisense") are bound to DNA or mRNA (Figure 3.9). These antisense drugs are therefore used to block expression activity of the gene. Information (the sense) from either the gene (DNA) or the mRNA is blocked from being processed (transcribed or translated), and the manufacture of protein is thus terminated. This technology differs

**TABLE 3.1 Selected Examples of Promising Metabolic Targets for Cancer Therapy**

| Targets | Pathways | Drug Agents or Approaches | Development Stage | Observations |
|---|---|---|---|---|
| *Bioenergetic metabolism* | | | | |
| Glucose transporter 1 (GLU1) | Glycolysis | WZB117, RNAi* | Preclinical data | Pharmacological or genetic inhibition of glucose transporter 1 exerts antineoplastic effects, both *in vitro* and *in vivo* |
| Glutaminase 1 (GLS1) | Glutamine metabolism | 968, BPTES, RNAi | Preclinical data | Malignant cells expressing mutant isocitrate dehydrogenase may be particularly sensitive to GLS1-targeting agents |
| Monocarboxylate transporter 1 (MCT1) | Krebs cycle | C117977, AZD3965, CHC, RNAi | AZD3965 is in clinical development | AZD3965 is currently being tested in a Phase I clinical trial enrolling patients with advanced solid tumors; these agents may be incompatible with the use of MCT1-transported drugs such as 3-B |
| *Anabolic metabolism* | | | | |
| Choline kinase | Lipid biosynthesis | CK37, TCD-717, RNAi | TCD-717 is in clinical development | The safety and therapeutic profile of TCD-717 is currently being tested in patients with advanced solid tumors |
| Phosphoglycerate mutase 1 (PGAM1) | Pentose phosphate pathway | PGMI-004A, RNAi | Preclinical data | Pharmacological or genetic inhibition of PGAM1 attenuates tumor growth *in vitro* and *in vivo*, presumably due to inhibition of the pentose phosphate pathway |

(*continued*)

**TABLE 3.1 (*Continued*)**

| Targets | Pathways | Drug Agents or Approaches | Development Stage | Observations |
|---|---|---|---|---|
| *Other metabolisms* | | | | |
| Indoleamine-2,3-dioxygenase (IDO) | Tryptophan metabolism | RNAi | Preclinical data | IDO-derived kynurenine promotes tumor progression via cell-intrinsic and cell-extrinsic mechanisms |
| Mammalian target of rapamycin (mTOR) | Cell growth autophagy | Rapalogues, Torins | Rapalogues are prescription drugs for the treatment of graft rejection and several tumors | Although mTOR inhibitors may limit tumor growth, they may also favor chemoresistance or neocarcinogenesis |

*Source*: Galluzzi, L, Kepp, O, Heiden, MGV and Kroemer, G 2013, 'Metabolic targets for cancer therapy', *Nature Reviews Drug Discovery*, 12, pp. 829–846. Reproduction with permission of Macmillan Publishers Ltd.
*RNAi = RNA interference (refer to Section 3.5).

from conventional drugs whereby the drugs interfere with the disease-causing protein, rather than stopping its production. It is also perceived that antisense drugs have high specificity as they can match their targets by countering their genetic codes.

A strategy for antisense therapy is based on the binding of oligodeoxyribonucleotides to the double helix DNA. This stops gene expression either by restricting the unwinding of the DNA or by preventing the binding of transcription factor complexes to the gene promoter. Another strategy centers on the mRNA. Oligoribonucleotides form a hybrid with the mRNA. Such a duplex formation ties up the mRNA, preventing the encoded translation message from being processed to form the protein.

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

Currently, there are only two antisense drugs in the market – Vitravene (active ingredient: fomivirsen) for the treatment of cytomegalovirus (CMV)-induced retinitis (inflammation of the retina) in AIDS patients and Kynamro (active ingredient: mipomersen sodium), which is an oligonucleotide inhibitor of apolipoprotein B-100 synthesis to treat patients with a rare type of high cholesterol called homozygous familial hypercholesterolemia (HoFH). Fomivirsen has 21 nucleotides complementary

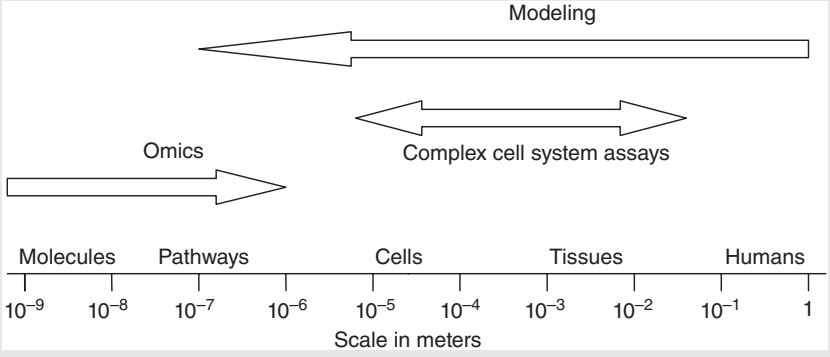
**Exhibit 3.14 Systems Biology**

Several approaches are utilized to study systems biology.

The bottom-up approach starts from the molecular level, the "omics," to identify and evaluate the genomic and proteomic basis of diseases.

The top-down approach attempts to integrate human physiology and diseases to provide models to understand disease pathways at organ levels.

Another way is the intermediate method to bridge the two approaches above. This method is to develop a set of biologically multiplexed activity profile data. It integrates biological complexity at multiple levels: pathways, signal transductions, and environmental factors.




*Source*: Butcher, EC, Berg, EL and Kunkel, EJ 2004, 'Systems biology in drug discovery', *Nature Biotechnology*, 22, pp. 1253–1259. Reproduction with permission of Macmillan Publishers Ltd.

to a CMV mRNA sequence, which is necessary for the production of infectious virus. Mipomersen has 20 nucleotides and binds to the coding region of mRNA for apolipoprotein B-100.

An example of a potential antisense drug is provided in Exhibit 3.15, while Table 3.2 lists other antisense drugs in clinical phase.

## 3.5 RNA INTERFERENCE APPROACH

RNAi is a cellular defense mechanism through which double stranded RNAs (dsRNAs) are processed into short lengths of small interfering RNAs (siRNAs) of 20–25 nucleotides by an enzyme called Dicer (Figure 3.10).

The siRNAs assemble into a complex called RNA-induced silencing complex (RISC) and unwind in the process. The single stranded siRNAs then attach to complementary RNA molecules, thus targeting these RNAs for destruction – a process called gene knockdown.

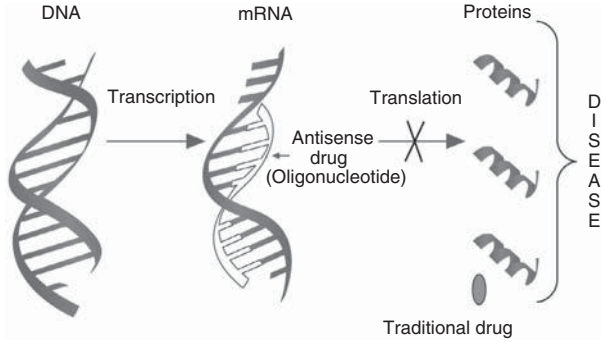



**Figure 3.9** Mode of action for antisense drugs. An example is Fomivirsen (Vitravene, Isis Pharmaceuticals), which is a 21-nucleotide phosphorothioate that binds to the complementary mRNA of cytomegalovirus and blocks the translation process. Cytomegalovirus is a virus that belongs to the herpes group. (*Source*: Adapted from Chang, YT 2002, *Keyword of the Post Genomic Era—Library*, New York University, New York, NY.)

**Exhibit 3.15 Potential Antisense Drug**

*Bcl-2*: B cell lymphoma protein 2 (Bcl-2) is a family of proteins that regulates apoptosis (programmed cell death). Apoptosis is a necessary process whereby aged or damaged cells are replaced by new cells. Dysfunction of the apoptosis process results in disease: inhibition of apoptosis results in cancer, autoimmune disorder, and viral infection, whereas increased apoptosis gives rise to neurodegenerative disorders, myelodysplastic syndromes, ischemic injury, and toxin-induced liver disease.

Some lymphomas, for example, are related to overexpression of Bcl-2. Antisense oligonucleotides are specially designed to target the overexpression of Bcl-2. Oblimersen (Genasense) is an antisense drug by Genta to block Bcl-2 production and enhance the efficacy of other standard chemotherapy drugs such as paclitaxel, fludarabine, irinotecan, and cyclophosphamide. The FDA has refused to approve Genasense on the grounds of insufficient evidence to demonstrate its efficacy, and further confirmatory clinical trial data are necessary.

*Source*: Data from Drugs.com 2014, *Genasense – FDA Appeal Decision Indicates that Genasense Approval Will Require Confirmatory Trial*, viewed January 29, 2014, http://www.drugs.com/nda/genasense_090309.html

Using these principles, it is postulated that when genes causing disease pathways are identified, therapeutic siRNAs in the forms of small drug molecules can be introduced into cells. Through these the siRNAs have high specificity and target only those errant genes and knockdown the disease pathways.

**TABLE 3.2 Selected Antisense Therapies That Have Reached Phase III Trials**

| Drug (Developer) | Target | Indications | Trial Name (Estimated Completion Date) |
|---|---|---|---|
| Aganirsen (Gene Signal) | IRS1 inhibitor | Corneal neovascularization | I-GRAFT (April 2013) |
| Belagenpumatucel-L (NovaRx) | TGB2 inhibitor | Non-small-cell lung cancer | STOP (June 2013) |
| Drisapersen (GSK/Prosensa Therapeutics) | DMD modulator | Duchenne muscular dystrophy | DMD114044 (July 2013) |
| Custirsen (OncoGenex Pharmaceuticals) | CLU inhibitor | Castration-resistant prostate cancer and non-small-cell lung cancer | SYNERGY (December 2013) |
| DIMS-0150/Kappaproct (InDex Pharmaceuticals) | NFKB1 inhibitor and TLR9 modulator | Refractory ulcerative colitis | COLLECT (March 2014) |
| Trabedersen (Antisense Pharma) | TNFSF13 inhibitor and TGF$\beta$R2 antagonist | Refractory or recurrent anaplastic astrocytoma | SAPPHIRE (halted owing to slow patient recruitment) |
| Alicaforsen (Atlantic Healthcare) | ICAM1 inhibitor | Ulcerative colitis and pouchitis | Unknown |

*Source*: Tze, MT 2013, 'Antisense approval provides boost to the field', *Nature Reviews Drug Discovery*, 12, p. 179. Reproduction with permission of Macmillan Publishers Ltd.

CLU, clusterin gene; DMD, dystrophin gene; ICAM1, intercellular adhesion molecule 1 gene; IRS1, insulin receptor substrate 1; NFKB1, nuclear factor-$\kappa$B1 gene; TGFB2, transforming growth factor $\beta$2 gene; TGF$\beta$R2, TGF$\beta$ receptor 2; TLR9, Toll-like receptor 9; TNFSF13, tumor necrosis factor ligand superfamily member 13 gene.

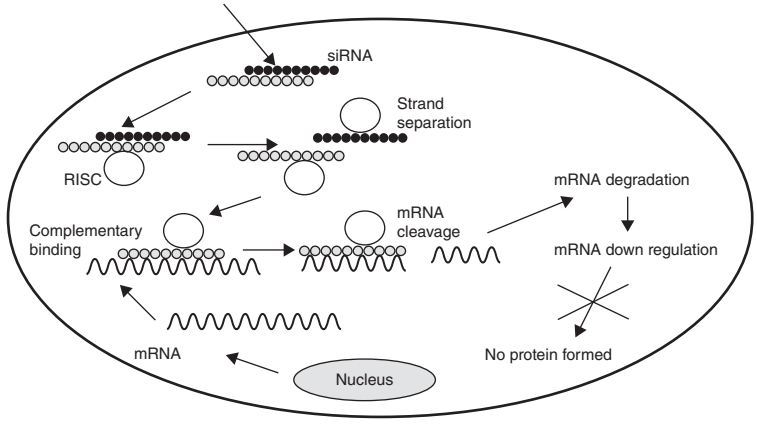



**Figure 3.10** siRNA Cellular Mechanism.

Although this technique has its appeal, there are several challenges. Delivery of the siRNA to the cells has to be devised. One method is the use of plasmid as delivery vehicle. Research is also in progress to use nano particles (1–100 nm) to help deliver the siRNA to the targeted site. Another challenge is to overcome the possibility of invoking the innate immune response. To alleviate this problem, the use of microRNA rather than siRNA has been proposed.

A first in human clinical trial of an RNAi drug that targets vascular endothelial growth factor (VEGF) and the kinesin spindle protein (KSP) in cancer patients has shown that the drug is safe. It mediated cleavage of mRNA in the liver and down-regulated tumor activity, leading to complete regression of liver metastasis (Tabernero, 2013).

## 3.6 CHIRAL DRUGS

Most drugs and biological molecules are chiral. "Chirality" means "handedness," that is, left- and right-hand mirror images. This is because of the existence of chiral centers within the molecules. For example, a carbon atom attached to four different groups can be oriented in such a way that two different molecules that are mirror images are obtained (Figure 3.11).

The two forms of mirror images are enantiomers and stereoisomers. All amino acids in proteins are "left-handed," and all sugars in DNA and RNA are "right-handed." When synthesized without special separation steps in the reaction process, drug molecules with chiral centers result in 50/50 mixtures of both the left- and right-handed forms. The mixture is often referred to as a racemic mixture.

As we can imagine, putting the right hand into a left-handed glove will not give a good fit. Similarly, the presentation of a racemic mixture of drug to a chiral binding site in a protein will not result in effective therapeutic treatment. One drug isomer is the actual effective component, while the other isomer may have varying degree of activity. The other isomer may have little or no net effect, or it may nullify the activity of the active isomer. Worse still, it may even cause an adverse reaction.

Before the 1980s, most drugs were manufactured in racemic mixtures. These drugs are being "rediscovered" so that only the active isomers are synthesized; the active isomer is more effective in the absence of its mirror image, or it can be prescribed in higher dose without the adverse reaction due to the inactive isomer. Another reason is that

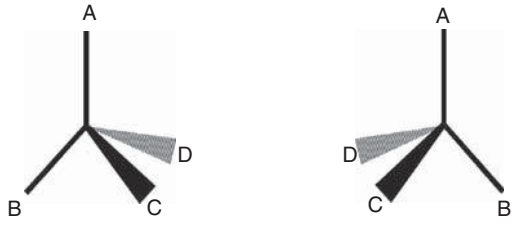
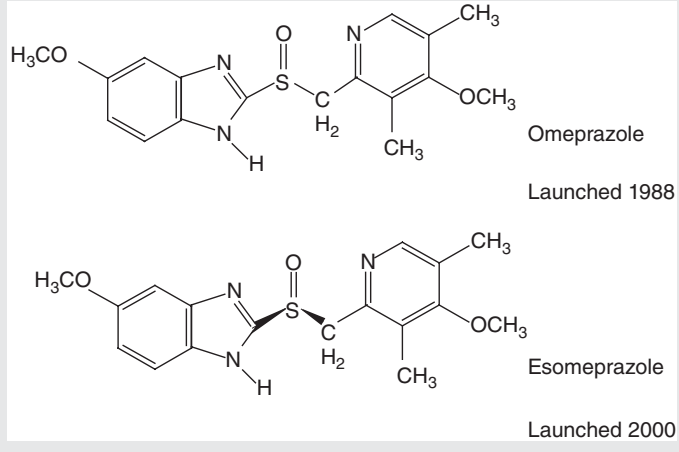



**Figure 3.11** Chiral molecules. Black triangular bond projects out of the page, gray triangular bond projects into the page.

**Exhibit 3.16 Omeprazole and Esomeprazole**

AstraZeneca launched omeprazole in 1988. It is a safe and effective drug for acid reflux, functioning as a proton pump inhibitor. However, the patent has expired, and AstraZeneca now has to compete against generics. The company developed the active isomer and called it esomeprazole. It was approved by the Mutual Recognition process in Europe in July 2000 and by the US Food and Drug Administration in February 2001. The chemical formulas for omeprazole and esomeprazole are shown below.

It was reported that healing of reflux esophagitis with 40 mg per day of Esomeprazole is effective in 78% of patients after 4 weeks of treatment and in 93% of patients after 8 weeks, compared to 65% and 87% of patients treated with 20 mg per day of Omeprazole.




*Note:* The two "S" atoms are chiral centers.

*Source*: Agranat, I, Caner, H and Caldwell, J 2002, 'Putting chirality to work: The strategy of chiral switches of drug molecules', *Nature Reviews Drug Discovery*, 1, pp. 753–768. Reproduction with permission of Macmillan Publishers Ltd.

pharmaceutical companies are rediscovering the active isomer to extend the lifecycle of blockbuster drugs. This is illustrated in Exhibit 3.16.

## 3.7 CLOSING REMARKS

Drug discovery is extremely challenging and demanding. The attrition rate for failures is very high. Although the above approaches for drug discovery afford a higher probability of success, the astute observations and inventiveness of scientists are critical

**Exhibit 3.17 Importance of Observation in Drug Discovery**

Alexander Fleming was studying bacteria. In 1928, he noticed that the bacterial cultures he was growing were ruined when a mold formed in the culture. The mould turned out to be *Penicillium*, which produces a substance called penicillin. This was found to be very effective in killing a variety of bacteria.

**Exhibit 3.18 Discovery of Viagra**

The long, tortuous path from drug discovery to commercialization is amply demonstrated by the sildenafil (Viagra) story.

Scientists at the Pfizer laboratory set out to discover an antihypertensive drug. The mechanism to lower blood pressure is:

- Atrial natriuretic peptide binds to the GPCR receptor.
- This binding activates the enzyme guanylate cyclase.
- Guanylate cyclase converts guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cyclicGMP).
- CyclicGMP lowers intracellular calcium, leading to (i) release of sodium in kidney cells or (ii) relaxation of smooth muscle in blood vessels.

The enzyme phosphodiesterase (PDE) converts cyclicGMP to GMP. The Pfizer scientists wanted to develop a drug to inhibit PDE so that the level of cyclicGMP remained high and point 4 could proceed.

Sildenafil was developed. However, there are different types of PDEs (nine are known today). As discussed previously, a potential drug has to be specific. Sildenafil inhibits PDE-5, which is absent in the kidney, although sildenafil's effect on smooth muscle relaxation was confirmed. The direction of the drug changed to treating angina instead, as sildenafil relaxes the vascular muscle of the heart.

At clinical trials, sildenafil did not work well as a treatment for angina. Instead, it was observed that it overcomes erectile dysfunction. Later, it was found that cyclicGMP also increases the level of nitric oxide, which is needed in penile erections.

Hence, we can see how the focus of treatment for sildenafil changed from antihypertensive to angina treatment to overcoming erectile dysfunction, giving rise to the drug called Viagra.

ingredients for success. Exhibit 3.17 shows how careful observation by Fleming gave rise to one of history's most effective drugs.

Drugs are often discovered through persistent work and continual optimization and many tests and trials. This is exemplified by the history behind the discovery of sildenafil

**Exhibit 3.19 Retovir, an AIDS Vaccine**

Zidovudine (Retrovir, also known as AZT), was the first drug approved for the treatment of AIDS. The drug was first studied in 1964 as an anticancer drug, but it showed little promise. It was not until the 1980s, when desperate searches began for a way to treat victims of HIV, that scientists at Burroughs Wellcome Co., of Research Triangle Park, NC, took another look at zidovudine. After it showed very positive results in human testing, it was approved by the FDA in March 1987 for AIDS treatment.

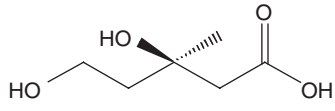
(Viagra, Pfizer), a drug for the treatment of erectile dysfunction, and the AIDS vaccine, zidovidine (Retovir, GlaxoSmithKline) (Exhibits 3.18 and 3.19).

## 3.8 CASE STUDY #3.1

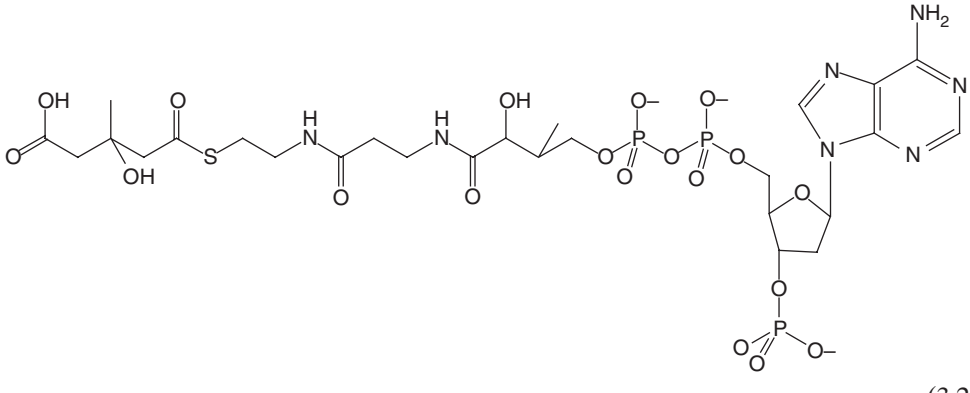
### 3.8.1 Lipitor

Lipitor (atorvastatin calcium) is a synthetic lipid-lowering drug. The chemical name for Lipitor is [R-(R*, R*)]-2-(4-fluorophenyl)-$\beta$, $\delta$-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate. The molecular weight is 1209.42. It is a white to off-white powder. The tablet is formulated in 10, 20, 40, and 80 mg dosages. The excipients used are calcium carbonate, candelilla wax, croscarmellose sodium, hydroxypropyl cellulose, lactose monohydrate, magnesium stearate, microcrystalline cellulose, Opadry white YS-1-7040, polysorbate 80, and simethicone emulsion.

In our bodies cholesterol is manufactured from mevalonate (3.1), which in turn is derived from (S)HMG-CoA (3.2).

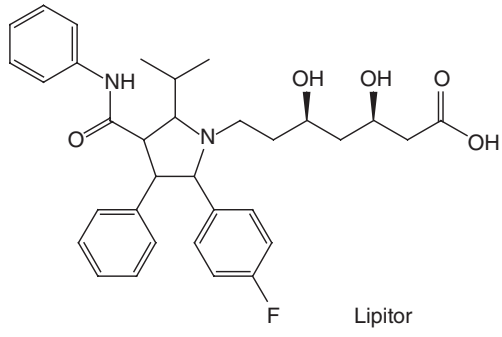



(3.1)



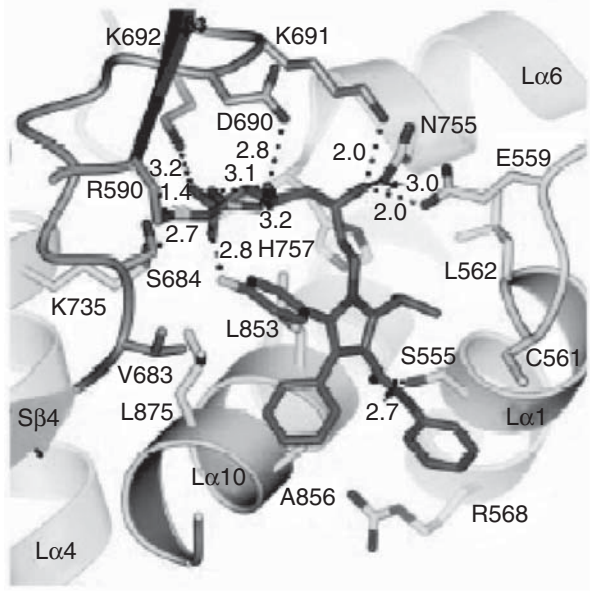

(3.2)

This cholesterol formation reaction is catalyzed by the enzyme HMG-CoA Reductase. One means to stop or reduce the production of cholesterol is to interfere with the supply of mevalonate. This is the function of Lipitor, which acts as an inhibitor of HMG-CoA Reductase.




Lipitor

Lipitor enters the active site of the enzyme, blocking the entry of HMG-CoA, and hence preventing it from being reduced and converted to mevalonate. The diagram below depicts the Lipitor molecule at the active site.




*Source*: Reprinted with permission from Istvan, ES and Deisenhofer, J 2001, 'Structural Mechanism for Statin Inhibition of HMG – CoA Reductase', *Science*, 292, pp. 1160–1160.

A more recent approach is a Phase III study to manage cholesterol by the use of monoclonal antibody inhibition (refer to Section 4.3) together with statin to reduce

low-density lipoprotein cholesterol (LDL-C). The antibody, evolocumab, inhibits proprotein convertase subtilisin/kexin type 9 (PCSK9), a protein that reduces the liver's ability to remove LDL-C from the blood. Positive end point results were reported in early 2014.

*Source*: Fierce Biotech 2014, *Amgen Announces Positive Top-Line Results From Phase 3 LAPLACE-2 Trial Of Evolocumab (AMG 145) In Combination With Statins In Patients With High Cholesterol*, viewed Jan 31, 2014, http://www.fiercebiotech.com/press-releases/amgen-announces-positive-top-line-results-phase-3-laplace-2-trial-evolocuma#ixzz2rwMn0Kqv

## 3.9 CASE STUDY #3.2

### 3.9.1 Early Drug Discovery and Development Guidelines

The NIH has published a document – *Early Drug Discovery and Development Guidelines: For Academic Researchers, Collaborators, and Start-up Companies* – with the aim "to assist academic investigators in advancing new therapies from the discovery phase into early drug development". For novel, new chemical entities, it sets up the following steps and decision points:

*Decision Point #1 – Target Identification*

Criteria:

- Previously published (peer-reviewed) data on a particular disease target pathway or target, or
- Evidence of new biology that modulates a disease pathway or target.

*Decision Point #2 – Target Validation*

Criteria:

- Known molecules modulate target
- Type of target has a history of success (e.g., Ion channel, GCPR, nuclear receptor, transcription factor, cell cycle, enzyme, etc.)
- Genetic confirmation (e.g., Knock-out, siRNA, shRNA, SNP, known mutations, etc.)
- Availability of known animal models
- Low-throughput target validation assay that represents biology
- Intellectual property of the target
- Determine marketability of the target

*Decision Point #3 – Identification of Actives*

Criteria:

- Acquisition of screening reagents
- Primary HTS assay development and validation
- Compound library available to screen
- Active criteria defined
- Performing high throughput screen

*Decision Point #4 – Confirmation of Hits*

Criteria:

- Confirmation on the basis of repeat assay, concentration response curve (refer to Section 5.2)
- Secondary assays for specificity, selectivity, and mechanisms
- Confirmed identity and purity
- Cell-based assay confirmation of biochemical assay when appropriate
- Drugability of chemical class (reactivity, stability, synthetic feasibility, solubility)
- Chemical Intellectual Property (IP)

*Decision Point #5 – Identification of Chemical Lead*

Criteria:

- SAR defined
- Drugability (preliminary toxicity, hERG, Ames – refer to Section 5.4)
- Synthetic feasibility
- Select mechanistic assays
- *In vitro* assessment of drug resistance and efflux potential
- Evidence of *in vivo* efficacy of chemical class
- PK/Toxicity of chemical class known on the basis of preliminary toxicity or *in silico* studies (refer to Section 5.5)

*Decision Point #6 – Selection of Optimized Chemical Lead (refer to Chapter 5)*

Criteria:

- Acceptable *in vivo* PK and toxicity
- Feasible formulation
- *In vivo* preclinical efficacy (properly powered)
- Dose range finding (DRF) pilot toxicology
- Process chemistry assessment of scale-up feasibility

*Decision Point #7 – Selection of a Development Candidate (refer to Chapter 5)*

Criteria:

- Acceptable PK (with a validated bioanalytical method)
- Demonstrated *in vivo* efficacy/activity
- Acceptable safety margin (toxicity in rodents or dogs when appropriate)
- Feasibility of GMP manufacture
- Acceptable drug interaction profile

*Decision Point #8 – Pre-IND Meeting with FDA (refer to Chapter 8)*

Criteria:

- Prepare pre-IND meeting request to FDA, including specific questions.
- Prepare pre-IND meeting package, which includes adequate information for FDA to address the specific questions.
- Prepare the team for the pre-IND meeting.

- Conduct Pre-IND meeting with FDA.
- Adjust project plan to address FDA comments.

*Decision Point #9 – Preparation and Submission of an IND Application (refer to Chapter 8)*

Criteria:

- Acceptable clinical dosage form
- Acceptable preclinical drug safety profile
- Clear IND regulatory path
- Human Proof of Concept (HPOC)/Clinical Proof of Concept (CPOC) plan is acceptable to regulatory agency (Pre-IND meeting)

*Decision Point #10 – Human Proof of Concept (refer to Chapter 6)*

Criteria:

- IND clearance
- Acceptable maximum tolerated dose (MTD)
- Acceptable dose response (DR)
- Evidence of human pharmacology
- Healthy volunteer relevance

*Decision Point #11 – Clinical Proof of Concept (refer to Chapter 6)*

Criteria:

- Meeting the IND objectives
- Acceptable human PK profile
- Evidence of human pharmacology
- Safety and tolerance assessments

*Source*: Hughes, M et al. 2012, 'Early Drug Discovery and Development Guidelines: For Academic Researchers, Collaborators, and Start-up Companies', *Assay Guidance Manual [Internet]*, viewed Jan 27, 2014, http://www.ncbi.nlm.nih.gov/books/NBK92015/pdf/drugdiscovery.pdf

## 3.10 SUMMARY OF IMPORTANT POINTS

1. The discovery of small molecule drugs can be separated into the irrational approach and rational approach.
2. The irrational approach relies on the screening of many compounds in the hope of finding a "hit" with the disease target. Compounds screened are microorganisms, plants, and marine life-forms. It is important to recognize conservation legislation and the sovereignty of the country of origin of these compounds. Extraction and purification are important steps to obtain the potential compound. High throughput screening is a necessary method to evaluate the potential use of the compounds in an efficient manner.
3. The rational approach commences with an understanding of the disease targets. The structures of the targets, including the active sites, are studied using X-ray crystallography and NMR.

4. Bioinformatics, genomics, and proteomics provide information about genes, proteins, and their functions on diseases. Combinatorial chemistry is used to generate different combinations of chemical compounds to test for their possible interactions with their putative disease targets. Structure–activity relationships of interactions are evaluated to find the potential drug candidates for further study. Metabolomics and systems biology are newer techniques in drug discovery.
5. Antisense and RNA interference techniques aim to utilize drug molecules to interfere with the transcription and translation process and stop diseases from progressing at the "source".
6. Chiral drug development aims to provide more effective drugs and extend the product lifecycle of drugs for longer periods.

## 3.11 REVIEW QUESTIONS

1. Describe the irrational approach to drug discovery and provide examples of drugs discovered by this approach.
2. Explain the Access and Benefit-Sharing Agreement for biodiversity prospecting and discuss the pros and cons of this agreement.
3. Which are the techniques used for the rational approach to discover new drugs? Describe combinatorial chemistry and computational chemistry in drug discovery.
4. Give an example of a drug discovered under the rational approach and describe the process undertaken to optimize the drug's effectiveness.
5. Discuss the pros and cons of X-ray crystallography and NMR for structural studies.
6. What are the reasons for the development of new methods, such as metabolomics and systems biology, to aid in the discovery of new drugs?
7. What are the mechanisms of action of antisense and RNA interference drugs in the treatment of diseases?
8. Describe chirality and explain why chiral drug is important.
9. Describe the process for separating proteins in cells.

## 3.12 BRIEF ANSWERS AND EXPLANATIONS

1. Refer to Section 3.2 and associated exhibits.
2. Refer to Exhibit 3.2. The pros are that both parties, the prospectors and the source country, understand the obligations, whereas the cons are the negotiations may be protracted and delay prospecting activities that may yield beneficial compounds.
3. Refer to Section 3.3. Computational chemistry and combinatorial chemistry are presented in Sections 3.3.4 and 3.3.5.
4. Exhibits 3.7 (Relenza and Tamiflu) and 3.11 (Gleevec) are good examples of drugs discovered using the rational approach. The important criteria are (i) finding and validating the target, (ii) determining the active site that can affect disease pathway

and (iii) designing drug candidates using computational chemistry. Once the drug candidates are designed, produce these compounds using combinatorial chemistry and test these using the high throughput system with tailored-made assays.

5. X-ray crystallography can provide very detailed information about the structure of target molecules, but the technique requires good quality crystals to be grown, and the structure determination process can be reasonably time consuming; the information provided is that of a static nature. In contrast, NMR can provide dynamic information about the target interactions with drug candidates. However, the structural information is limited and the technique is not applicable for molecules greater than 35 kDa. Hence a combination of information from X-ray crystallography and NMR is needed to provide integrated information to enable more effective drug discovery.
6. Metabolomics and systems biology are new fields of study to better understand diseases and disease pathways. These new studies may help to discover and develop more effective drugs with fewer adverse reactions in shorter time spans.
7. Refer to Sections 3.4 and 3.5.
8. Refer to Section 3.6. Chiral drugs are more effective than racemic mixtures as they can better interact with active sites to alter disease progression. An important example is the case of Omeprazole and Esomeprazole (Exhibit 3.16). It is also strategically important for pharmaceutical companies to work on chiral drugs to extend the product lifecycle and compete with generics.
9. Refer to Exhibit 3.13.

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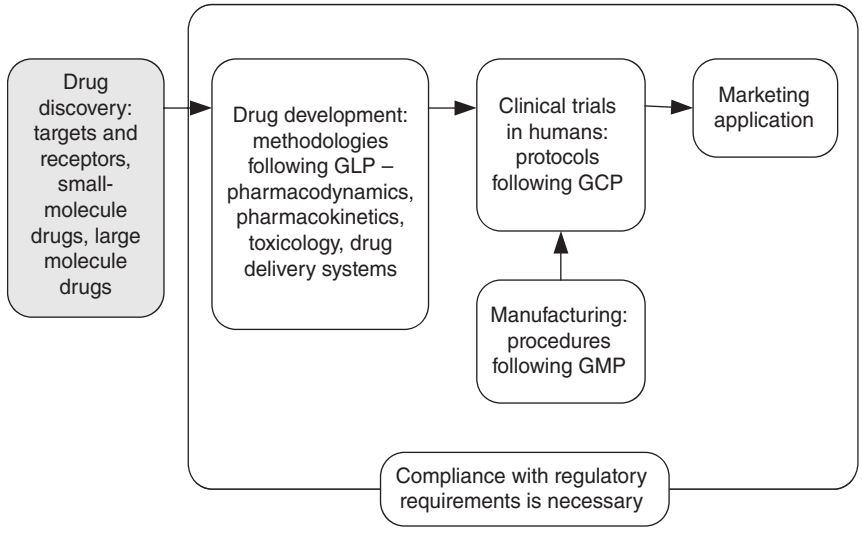
# CHAPTER 4

# DRUG DISCOVERY: LARGE MOLECULE DRUGS




## 4.1 INTRODUCTION

Unlike the small molecule drugs (pharmaceuticals) described in Chapter 3, large molecule drugs (biopharmaceuticals) are mainly protein based. Another distinction is

*Drugs: From Discovery to Approval*, Third Edition. Rick Ng.

that these protein-based drugs are, in the main, similar to natural biological compounds found in the human body; in other words, they are fragments that mimic the active part of natural compounds.

As discussed in Chapter 3, the discovery of pharmaceuticals commences with the scanning of hundreds of compounds, whether with actual materials (irrational approach) or virtual simulations (rational approach). To discover biopharmaceuticals, however, we have to examine the compounds within us, for example, hormones or other biological response modifiers, and determine how they affect the biological processes. In some cases, we study pathogens such as the influenza virus or bacteria to derive the vaccines. In other cases, we copy these biological response modifiers and use them as replacement therapy.

New pharmaceuticals are called new chemical entities (NCEs), and they are produced (synthesized) in manufacturing plants using techniques on the basis of chemical reactions of reactants. New biopharmaceuticals, sometimes known as new biological entities (NBEs), are made using totally different methods. These protein-based drugs are "manufactured" in biological systems such as living cells, producing the desired protein molecules in large reaction vessels as the living cells grow, or by extraction from animal serum. The term new molecular entities (NMEs) can be applied to both NCEs and NBEs.

Biopharmaceuticals are becoming increasingly important. The reason is that they are more potent and specific, as they are similar to the proteins within the body, and hence are more effective in treating our diseases. There are three major areas in which biopharmaceuticals are used: as prophylactic (preventive, as in the case of vaccines), therapeutic (antibodies, enzymes), and replacement (hormones, growth factors) therapy. Exhibit 4.1 presents selected statistics for biopharmaceuticals.

Another term that is used for protein-based drugs is biologics. The FDA definition for biologics is:

> A biological product subject to licensure under the Public Health Service Act is any virus, therapeutic serum, toxin, antitoxin, vaccine, blood, blood component or derivative, allergenic product, or analogous product, applicable to the prevention, treatment, or cure of diseases or injuries to humans. Biological products include, but are not limited to, bacterial and viral vaccines, human blood and plasma and their derivatives, and certain products produced by biotechnology, such as interferons and erythropoietins. Biologics encompass many different protein-based drugs and include blood products such as clotting factors extracted from blood.

Table 4.1 shows the major uses of therapeutic biologics, other than vaccines, in the treatment of patients suffering from various conditions.

In this chapter, we discuss the following topics, but exclude blood products (pharmaceuticals derived from blood or blood components, e.g., clotting factors):

- Vaccines
- Antibodies
- Cytokines
- Hormones

**Exhibit 4.1 Biopharmaceuticals**

There are at present more than 200 biopharmaceutical drugs approved for marketing in the United States and European Union. Biopharmaceuticals are Monoclonal Antibodies (MAbs), Vaccines, Erythropoietin (EPO), Recombinant Human Insulin, Granulocyte-Colony Stimulating Factor (G-CSF), Interferons (IFN), Human Growth Hormones (HGH), and others.

The biopharmaceutical market is around US$200 billion in 2013 and is projected to reach US$500 billion by 2020, growing at 13.5% compound annual growth rate between 2010 and 2020. Among different product segments, MAb is the largest category of biopharmaceuticals. The most common target is cancer and MAb, and vaccines have the most number of R&D activities.

The top 10 biopharmaceuticals in 2013 are listed in Table 1.3, with Humira, Enbrel, and Remicade occupying the top three positions. These three products are all MAbs, and they target the tumor necrosis factor alpha (TNF$\alpha$) to down-regulate the inflammatory reactions associated with autoimmune disease.

*Source*: Data from 1. Walsh, G 2010, 'Biopharmaceutical benchmarks – 2010', *Nature Biotechnology*, 28, pp. 917–924; 2. Industry Experts 2013, *Biopharmaceuticals – A Global Market Overview*, viewed January 29, 2014, http://www.scribd.com/doc/153893824/Biopharmaceuticals-%E2%80%93-A-Global-Market-Overview

- Gene therapy
- Stem cells.

We have included gene therapy and stem cells to present a more comprehensive perspective on medical treatments, although they are not drugs by our conventional definitions.

## 4.2 VACCINES

Most of us are vaccinated soon after we are born. As we grow up and go through different stages of life, we are further vaccinated against other diseases. The basis of vaccination is that administering a small quantity of a vaccine (an antigen that has been treated) stimulates our immune system and causes antibodies to be secreted to react against the foreign antigen. Later in life, when we encounter the same antigen, our immune system will evoke a "memory" response and activate the defense mechanisms by generating antibodies to combat the invading antigen.

A vaccine formulation contains antigenic components that are obtained from or derived from the pathogen. These pathogens include mainly viruses, bacteria, parasites, and fungi. Research has shown that the part of the pathogen, which causes disease, termed virulence, can be decoupled from the protective part, so-called immunity. Vaccine development focuses on means to reduce the virulence factor while retaining the

**TABLE 4.1 Some Examples of Biologics and Their Uses**

| Type of Biologic | Treatment Use | Example |
|---|---|---|
| Antiviral | Preventing respiratory syncytial virus infections in premature infants | Synagis |
| Cancer | Metastatic cancers, lymphoma, leukemia, melanoma | Avastin, Erbitux, Elspar, Gazyva, Herceptin, Kadcyla, Perjeta, Rituxan, Vectibix, Xgeva, Yervoy, Zaltrap, Zevalin |
| Enzyme replacement | Gaucher's disease, Fabry's disease, Mucopolycharidosis, Morquio syndrome | Aldurazyme, Cerezyme, Fabrazyme, Vimizim |
| Erythropoietins | Anemia in kidney and cancer patients | Aranesp, Epogen, Eprex, Mircera, NeoRecormon, Procrit |
| Follicle stimulating hormones | Infertility | Gonal-f, Puregon |
| G-CSF (granulocyte-colony stimulating factors) | Neutropenia (low level of neutrophils leading to susceptibility to infections) | Neulasta, Neupogen |
| Human growth hormone | Natural growth hormone deficiency | Genotropin, Humatrope, Norditropin, Nutropin, Saizen |
| Insulin and insulin Analogs | Diabetes | Humalog, Lantus, Lemevir, Myalept, Novolin, Novolog |
| Interferon $\alpha$ | Hepatitis B and C | Pegasys, Peg-Intron, Roferon-A |
| Interferon $\beta$ | Multiple sclerosis | Avonex, Betaseron, Rebif, Tysabri, |
| Recombinant coagulation factors | Bleeding episodes or surgical bleeding in hemophiliacs | Alprolix, Benefix, Kogenate, NovoSeven, Refacto |
| TNF blockers | Rheumatoid arthritis, Crohn's disease, psoriasis | Cimzia, Enbrel, Humira, Orencia, Remicade, Rituxan, Simponi |
| Others | Acute myocardial infarction, stroke | Activase |
| | Anthrax | Raxibacumab |
| | Asthma | Xolair |
| | Cryopyrin-associated periodic syndrome | Arcalyst, Ilaris |
| | Cystic Fibrosis | Pulmozyme |
| | Dupuytren's contracture | Xiaflex |
| | Glycogen storage disease type II | Myozyme |

**TABLE 4.1 (*Continued*)**

| Type of Biologic | Treatment Use | Example |
|---|---|---|
| | Gout | Krystexxa |
| | Hereditary angioedema | Kalbitor |
| | Hunter syndrome | Elaprase |
| | Macular degeneration (wet) | Lucentis, Eylea |
| | Osteoporosis | Forteo, Prolia |
| | Paroxysmal nocturnal hemoglobinuria | Soliris |
| | Psoriasis | Raptiva |
| | Systemic lupus erythematosus | Benlysta |
| | Vitreomacular adhesion | Jetrea |

immunity stimulation. Administration of vaccines may be oral or parenteral (introduction of medication via injection, infusion, or implantation). After the initial vaccination, booster doses may be needed to maximize the immunological effects.

The number of antigens in a vaccine is called valency. A single antigen vaccine (monovalent) provides immunity against one type of pathogen, whereas a multivalent or polyvalent vaccine with multiple antigens is designed to immunize against multiple strains of the same pathogen or multiple pathogens. Case Study #4.1 describes a 13-valent pneumococcal vaccine and a tetravalent (4-valent) meningococcal vaccine.

### 4.2.1 Traditional Vaccines

Traditionally, vaccines are prepared in a number of ways:

- Attenuated vaccines
- Killed or inactivated vaccines
- Toxoids.

***Attenuated Vaccines:*** The virulence of a pathogen can be reduced in a number of ways: by chemical treatment, by temperature adaptation, or by growing the pathogen in species other than the natural host (a process called "passaging").

The advantages of attenuated vaccines are (i) the cost of preparation is low, (ii) they elicit the desired immunological response, and (iii) normally a single dose is sufficient. The disadvantages are (i) the potential to revert to virulence and (ii) limited shelf life.

Examples of attenuated vaccines are *Bacillus Calmette-Guerin* (BCG) for immunization against tuberculosis, Sabin vaccine for poliomyelitis, attenuated *Paramyxovirus parotitidus* against mumps, and attenuated measles mumps rubella vaccine (MMR) to induce immunity against measles, mumps, and rubella.

***Killed or Inactivated Vaccines:*** Chemical and temperature treatment are normally used to kill or inactivate the pathogen. Formaldehyde treatment is one of the more

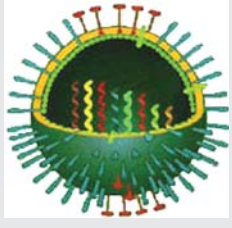
common methods. Other chemicals used are phenol and acetone. Another method is to irradiate the pathogen to render it inactive.

The advantages are (i) nonreversal to virulence and (ii) relatively stable shelf life. The disadvantages are (i) the cost of production is high, (ii) more control is required for production to ensure reliable processes for complete inactivation, and (iii) there is a possibility of reduced immunological response because of the treatment processes, so multiple booster vaccinations may be required.

Examples of killed or inactivated vaccines are cholera vaccine containing dead strains of *Vibrio cholerae*, hepatitis A vaccine with inactivated hepatitis A virus, pertussis vaccine with killed strains of *Bordetella pertussis*, typhoid vaccine with killed *Salmonella typhi*, and influenza vaccine with various strains of inactivated influenza viruses (refer to Exhibit 4.2 for a discussion on influenza viruses and vaccines and Exhibit 4.3 on avian influenza H5N1).

**Exhibit 4.2 Influenza Viruses and Vaccines**

Influenza is caused by the influenza (Orthomyxoviruses) viruses. There are three types of influenza viruses, Influenza A, B, and C (on the basis of their protein matrix, influenza A and B have eight RNA fragments, C has seven). Influenza A can infect humans and other animals, while influenza B and C mainly infect humans only. Unlike influenza A and B, influenza C virus causes very mild illness and does not cause epidemics. Influenza A is categorized into subtypes on the basis of its surface antigens: hemagglutinin and neuraminidase (see Exhibit 3.7). There are no subtype classifications for influenza B. Influenza virus undergoes frequent mutations as it replicates, with Influenza A changing more rapidly than B, causing antigenic shifts in the hemagglutinin and neuraminidase.



*Source*: Cann, AJ 2007, *Influenza Virus Haemagglutination*, viewed January 29, 2014, http://www.microbiologybytes.com/LabWork/haem/haem1.htm. Reproduced with permission of Dr AJ Cann, Leicester University.

The nomenclature for classifying influenza virus is:

Type/Site Isolated/Isolate No./Year, e.g., A/New Caledonia/20/99(H3N2), B/Hong Kong/330/2001.

There have been three influenza pandemics in the twentieth century: in 1918 (Spanish Flu, H5N1), 1957 (Asian Flu, H2N2), and 1968 (Hong Kong Flu, H3N2).

The pandemic in 1918 killed more than 20 million people worldwide, and the other two took the lives of 1.5 million people combined. In 1997, the first avian (chicken) flu was transmitted to humans in Hong Kong. It was caused by Influenza A H5N1 (see Exhibit 4.3 for more information about H5N1).

Antibodies responsive to influenza antigens are specific to the subtype and strain. To have an effective influenza vaccine, it is a requirement that there is an accurate prediction of the subtypes and strains that are expected to circulate in the influenza season months before the season begins. When the antigenic match between vaccine and circulating viruses is close, influenza vaccine is 70–90% effective.

The World Health Organization (WHO) has a network of more than 120 centers worldwide that monitor influenza activity and ensure virus isolates and information are sent to WHO for strain identification and action. Each February (for the Northern Hemisphere winter) and September (for the Southern Hemisphere winter) the WHO provides advanced recommendations for the composition of the influenza vaccine to be manufactured. Similarly, the FDA CBER recommends trivalent influenza vaccine to be prepared for the United States.

For the 2013–2014 winter season in the Northern Hemisphere, the recommended trivalent vaccine by both WHO and FDA is:

- An A/California/7/2009 (H1N1)pdm09-like virus.
- An A(H3N2) virus antigenically like the cell-propagated prototype virus A/Victoria/361/.
- A B/Massachusetts/2/2012-like virus.

*Source*: Data from World Health Organization 2013, *Recommended Composition of Influenza Virus Vaccines for Use in the 2013-14 Northern Hemisphere Influenza Season*, viewed January 31, 2014, http://www.who.int/influenza/vaccines/virus/recommendations/2013_14_north/en/

***Toxoids:*** Toxoids are derived from the toxins secreted by a pathogen. The advantages and disadvantages are similar to those for killed or inactivated vaccines.

Examples are diphtheria and tetanus vaccines. Diphtheria vaccine is produced by formaldehyde treatment of the toxin secreted by *Corynebacterium diptheriae*. Similarly, tetanus vaccine is obtained from toxins of cultured *Clostridium tetani* that has been treated with formaldehyde.

### 4.2.2 New Vaccines

Advances in genomics, molecular biology, and recombinant technology have provided new directions for the discovery, development, and manufacture of vaccines. One of the current approaches is a minimalist strategy to decouple the virulence and immunity functions. The aim is to use only the immunity part to confer protection, so that the vaccine is safe to be administered. The approach can be divided into subunit, vector-based, DNA, and peptide vaccines.

**Exhibit 4.3 Avian Influenza H5N1**

Avian influenza H5N1 is an infectious disease of birds. It can cause two distinct forms of disease: one is mild while the other is deadly. The virus is thought to be spread by migratory birds. Animals, especially farm poultry/animals, that lie under the migratory paths of the birds can become infected. To date culling is the most effective means of controlling the spread of avian influenza in domestic poultry/animals.

There is concern that the virus can infect humans living in close proximity to the infected poultry/animals. Till December 2013, there have been 649 cases of humans infected, with 385 fatalities. Most of the cases were in Indonesia, Vietnam, Egypt, Thailand, and China.

A further fear of this deadly infection is that it may cause a pandemic through two mechanisms: reassortment where the genetic material is exchanged between human and virus, or gradual adaptive mutation where the virus changes to a more potent form. In March 2013, a new strain of avian influenza (H7N9) not previously seen in humans was reported in China. Within several months there were 44 deaths among the 135 cases reported.

There are many different strains of avian influenza: 16 H subtypes and 9 N subtypes. Only those labeled as H5, H7, and H10 have caused deaths in humans. The WHO and member countries through the International Health Regulations (IHR, see Case Study #7.1) are working together to control this infectious disease.

*Source*: Data from World Health Organization 2014, *Avian Influenza in humans*, viewed February 9, 2014, http://www.who.int/ith/updates/20140123/en/

***Subunit Vaccines:*** Subunit vaccines use only a part of the bacteria or virus instead of the entire pathogen. Normally, the part is derived from the outside envelope protein of the pathogen. Discovery of the relevant envelope protein requires knowledge of the genome sequence of the pathogen by identifying open reading frames (ORFs, refer to Exhibit 4.4) that potentially encode novel antigenic surface proteins known as epitopes (Exhibit 4.4), which bind to antibodies. When identified, the ORFs are cloned to express protein epitopes using self-replicating plasmids (refer to Exhibit 10.11 and Appendix 4). The binding properties of the epitopes can be studied using enzyme-linked immunosorbent assay (ELISA, Exhibit 4.4) or a fluorescent-activated cell sorter (FACS, Exhibit 4.4). After laboratory testing, the leading candidates of epitopes are injected into animals to determine whether they elicit any antibody response. Those that provoke a response are selected and optimized to become vaccine candidates with further tests before human clinical trials. Researchers are also working on multiple epitope subunit vaccines, which can provide different antigenic binding sites.

Gardasil is a subunit recombinant vaccine. It is a tetravalent vaccine against human papillomavirus (HPV) implicated in cervical cancer; refer to Exhibit 4.5 for more details.

**Exhibit 4.4 Important Concepts Related to Subunit Vaccines**

An *open reading frame (ORF)* is a sequence of nucleotide in the RNA or DNA that has the potential to encode protein. The start triplet is ATG. It is followed by a string of triplets that code for amino acids. The stop triplet is TAA, TAG, or TGA (see Exhibit A2.3).

An *epitope* is an antigenic determinant of the pathogen. It consists of certain chemical groups that are antigenic, which means that it will elicit a specific immune response by binding to antibodies.

The *enzyme linked immunosorbent assay (ELISA)* is a method for determining the presence of antigen-specific antibodies. Antigens are first solubilized and coated onto solid support, such as 96-well plates. Test samples containing antibodies are added, and the antibodies bind to the antigens on the plate. Excess unbound sample is washed off, and the antigen–antibody complex is incubated with a second antibody linked to an enzyme (e.g., alkaline phosphatase, horseradish peroxidase). The labeling with the enzyme catalyzes certain biochemical reactions and provides a readout (color) to show the presence or absence of the specific antibodies. The process can also be used for detecting antigens. In this case, the antibodies are coated onto the substrate, followed by antigen attachment and conjugation to an enzyme.

*A fluorescence-activated cell sorter (FACS)* is a flow cytometry instrument used to separate and identify cells in a heterogeneous population. Cell mixtures to be sorted are first bound to fluorescent dyes such as fluorescein or phycoerythrin. The labeled cells are then pumped through the instrument and are excited by a laser beam. Cells that fluoresce are detected, and an electrostatic charge is applied. The charged cells are separated using voltage deflection.

**Exhibit 4.5 Gardasil**

Gardasil is a noninfectious recombinant vaccine consisting of capsid proteins from four different human papillomavirus (HPV) of types 6, 11, 16, and 18. HPV causes squamous cell cervical cancer and cervical adenocarcinoma, as well as 35–50% of vulvar and vaginal cancers.

The four antigens are produced in a fermentation process using the yeast *S. cerevisiae* grown in chemically defined media. The purified antigens are formulated in aluminum-containing adjuvant in sterile liquid suspension.

In June 2006, FDA approved the use of Gardasil to vaccinate females from ages 9 to 26.

*Source*: Data from Food and Drug Administration 2009, *Gardasil (Human Papillomavirus Quadrivalent Types 6, 11, 16, 18) Recombinant Vaccine*, viewed February 9, 2014, http://www.fda.gov/safety/medwatch/safetyinformation/ucm176846.htm

***Vector-Based Vaccines:*** Viruses and bacteria are detoxified and used as vehicles to carry vaccines. For example, subunit vaccines are delivered by carrier vehicles to elicit the immune response. An example is the use of canarypox (a virus that infects birds, but not humans) to carry envelope proteins for HIV treatment. Multiple types of envelope proteins can be delivered with this method. Clinical trials with this type of vector-based vaccines are being investigated.

***Conjugate Vaccines:*** Some bacteria, such as those that cause meningitis and pneumonia, have an outer layer of polysaccharide that protects the bacteria's antigens. This polysaccharide coating provides a barrier and renders detection by the human immune system, particularly in children, ineffective. To circumvent this problem, a conjugate vaccine can be designed by joining the antigen to a protein molecule. This combination of the conjugate vaccine can be recognized by the human immune system and hence a more effective response to the invading pathogen can be achieved. Case Study #4.1 describes in further details the pneumococcal vaccine Prevnar 13 and meningococcal vaccines Nimenrix and Bexsero.

***DNA Vaccines:*** DNA vaccines are sometimes called nucleic vaccines or genetic immunization. The host (patient) is directly injected with selected viral genes, which contain engineered DNA sequences that code for antigens. The host's own cells take up these genes and express the antigens, which are then presented to the immune cells and activate the immune response.

***Peptide Vaccines:*** Peptide vaccines are chemically synthesized and normally consist of 8–24 amino acids. In comparison with protein molecules, peptide vaccines are relatively small. They are also known as peptidomimetic vaccines, as they mimic the epitopes. Complex structures of cyclic components, branched chains, or other configurations can be built into the peptide chain. In this way, they possess conformations similar to the epitopes and can be recognized by immune cells. An *in silico* vaccine design approach has been used to find potential epitopes. A critical aspect of peptide vaccines is to produce 3D structures similar to the native epitopes of the pathogen.

### 4.2.3 Adjuvants

Very often, vaccines are formulated with certain substances to enhance the immune response. These substances are called adjuvants (from the Latin *adjuvare*, which means "to help"). The most common adjuvants for human use are aluminum hydroxide, aluminum phosphate, and calcium phosphate. Other adjuvants being used are bacteria and cholesterol. Mineral oil emulsions are normally the adjuvants used in animal studies. The adjuvant known as Freund's Complete Adjuvant consists of killed *tubercle bacilli* dispersed in oil then emulsified in water, and Freund's Incomplete Adjuvant is without the killed *bacilli*. Both these adjuvants are effective in stimulating an immune response, but they cause unacceptable side effects in humans (refer to Table 4.2).

There are three basic mechanisms by which adjuvants assist in improving immune response. Firstly, adjuvants help the immune response by forming reservoirs of antigens that provide a sustained release of antigens over a long period. Secondly, adjuvants act

**TABLE 4.2 Some Common Adjuvants and Their Mechanisms of Action**

| Adjuvant | Composition | Mechanism of Action |
|---|---|---|
| Alum (aluminum hydroxide or aluminum phosphate) | Aluminum hydroxide gel | Enhanced uptake of antigen by APC; delayed release of antigen |
| Alum with a mycobacterial-derived dipeptide | Aluminum hydroxide gel with muramyl dipeptide | Enhanced uptake of antigen by APC; delayed release of antigen; induction of costimulatory molecules on APCs |
| Alum with *Bordetella pertussis* | Aluminum hydroxide gel with killed *Bordetella pertussis* | Enhanced uptake of antigen by APC; delayed release of antigen; induction of costimulatory molecules on APCs |
| Freund's complete adjuvant | Oil in water with killed *tubercle bacilli* | Enhanced uptake of antigen by APC; delayed release of antigen; induction of costimulatory molecules on APCs |
| Freund's incomplete adjuvant | Oil in water | Enhanced uptake of antigen by APC; delayed release of antigen |
| Immune stimulatory complexes | Open cage-like structures containing cholesterol and a mixture of saponins | Delivery of antigen to cytosol, allowing induction of cytotoxic T cell responses |

*Source*: Coico, R, Sunshine, G and Benjamini, E 2003, *Immunology*, 5th edn., Wiley-Liss, New Jersey. Reproduced with permission of John Wiley and Sons Inc.

*Note*: APC, antigen-presenting cell

as nonspecific mediators of immune cell function by stimulating or modulating immune cells. Thirdly, adjuvants can serve as vehicles to deliver the antigen to the spleen and/or lymph nodes, where immune response is initiated.

Another aspect concerning adjuvants is the safety issue. Potentially, adjuvants can cause local and systemic reactions. Local reactions are injection pain, injection site redness, swelling, abscess, and regional lymphadenopathy (abnormal enlargement of lymph nodes). Systemic reactions are anaphylaxis, allergic reactions, fever, nausea, and toxicity. The FDA regulation on adjuvants mandates that "an adjuvant shall not be introduced into a vaccine product unless there is satisfactory evidence that it does not affect adversely the safety or potency of the product." Some recently approved adjuvants are MF59 (oil-in-water emulsion of squalene oil and surfactant) and MPL (Monophosphoryl lipid A, derived from lipid A in the membrane of Gram-negative bacteria).

Edible food sources have been tested to deliver vaccines orally; for example, transgenic potato tuber-based vaccines have been developed. Other food sources, such as bananas, tomatoes, and corn, are being tested in laboratories. Mucosal vaccines utilizing genetically modified enterotoxins (toxic substances produced by microorganisms and cause gastrointestinal symptoms) are a method to deliver vaccines intranasally. Research in this area has to ensure the safety aspect of using enterotoxins.

### 4.2.4 Recent Vaccine Research and Clinical Activities

The field of vaccine research is very active, in particular, in immunotherapy. Unlike conventional vaccine that is used to stimulate our immune system against infections from pathogens such as viruses and bacteria, immunotherapy aims to activate or suppress the immune system as treatment options for diseases, for example, cancers, organ transplant, and allergies (refer to Exhibit 11.3). Exhibit 4.6 summarizes examples of some selected vaccines. Appendix 5 shows a table of the production methods for selected vaccines. Appendix 6 is a list of FDA approved vaccines.

**Exhibit 4.6 Selected Vaccines**

*Cervical Cancer*: See Exhibit 4.5.

*Avian Influenza*: See Exhibit 4.3.

*Alzheimer's Disease*: The vaccine being tested contains a small protein called $\beta$-amyloid (A$\beta$). This protein forms abnormal deposits, or "plaques," in the brains of people with Alzheimer's disease. Researchers believe that A$\beta$ deposition causes loss of mental function by killing the brain neurons. The strategy of A$\beta$ vaccination is to stimulate the immune system to clean up plaques and prevent further A$\beta$ deposits. A Phase I, double-blind, placebo-controlled, 52-week study in two centers in Sweden was conducted. Participants, aged 50–80 years, with mild-to-moderate Alzheimer's disease were recruited to randomly receive the vaccine CAD106 or placebo. The findings suggest that CAD106 has a favorable safety profile and acceptable antibody response in patients with Alzheimer's disease. Larger trials with additional dose investigations are needed to confirm the safety and establish the efficacy of CAD106.

*Source*: Data from Winblad, B 2012, 'Safety, tolerability, and antibody response of active A$\beta$ immunotherapy with CAD106 in patients with Alzheimer's disease: randomised, double-blind, placebo-controlled, first-in-human study', *Lancet Neurology*, 11, pp. 597–604.

*Pneumococcal*: See Case Study #4.1.

*Cancer*: In cancer, the immune system does not recognize the changes in cancer cells. Cancer vaccines seek to mimic cancer-specific changes by using synthetic peptides to challenge the immune system. When these peptides are taken up by T cells (Exhibit 4.7), the immune system is activated. The T cells search for cancer cells with specific markers and proceed to kill them. Some vaccines being tested are (i) a peptide called $\beta$-defensin 2, which activates the immune system against tumor activity, and (ii) an outer coat protein of the human papillomavirus to act as a vaccine against cervical cancer.

*Source*: Data from National Cancer Institute 2011, *Cancer Vaccine*, viewed February 9, 2014, http://www.cancer.gov/cancertopics/factsheet/Therapy/cancer-vaccines

*AIDS (see Exhibit 2.14)*: AIDS is caused by HIV infection. HIV belongs to a large family of retroviruses, the Lentiviridae. The HIV genome is within the RNA. Following infection in humans, the RNA genome of HIV is reverse-transcribed into DNA and integrated within the human cell. HIV undergoes frequent mutation and therefore is highly variable. One technique for producing an AIDS vaccine is to reproduce, using recombinant technology, the surface proteins on the HIV. There are two particular envelope proteins being investigated: gp120 and gp41. The protein gp120 is trimeric and is held together by three transmembrane gp41 proteins. Laboratory studies have shown that vaccines based on these proteins can induce antibody responses to different strains of HIV. Other AIDS treatments are the use of (i) antiviral (AZT, a reverse transcriptase inhibitor) drugs, (ii) drugs (indinavir) that target and inhibit the production of HIV protease, an enzyme required to assemble new virus particles, and (iii) gene therapy – control of viral genome expression through the use of synthetic oligonucleotides.

*Malaria*: Malaria is a major disease in tropical countries. According to the WHO, 300–500 million individuals are infected with malaria. The death tolls are 1.5–3.5 million yearly. There are four species of malaria parasites that infect humans: *Plasmodium falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*. Of these, *P. falciparum*, the predominant malarial parasite found in Africa, is the most virulent. There are four stages in the *P. falciparum* life cycle: (i) sporozoite (3–5 min when it is injected into the blood stream by mosquito), (ii) liver stage (1–2 weeks after the parasite enters the liver, during which it matures; no symptoms are shown in stages (i) and (ii), (iii) blood stage (2 or more days/cycle during which red blood cells are invaded and parasites rupture out of red blood cells; fevers and chills are manifested), and (iv) sexual stage (10–14 days during which parasites mature into the sexual form, ready to be picked up by a mosquito to infect the next person). Vaccine strategies are of three types: preerythrocytic (stages i and ii), blood stage (stage iii), and transmission-blocking (stage iv).

A new malaria vaccine, RTS S/AS02D, from GSK has shown very promising results in a Phase III randomized, controlled, double-blind trial conducted at 11 centers in seven African countries . It reported reduction in incidence of both clinical and severe malaria in children 5–17 months by approximately 50%.

*Source*: Data from 1. Malaria Vaccine Initiative 2014, *Malaria Vaccine Technology Roadmap Update Includes New Targets*, viewed February 9, 2014, http://www.malariavaccine.org/; 2. Albert Schweitzer Hospital et al. 2012, 'A Phase 3 Trial of RTS,S/AS01 Malaria Vaccine in African Infants', *The New England Journal of Medicine*, Dec, pp. 2284–2295.

*Chicken pox*: Chicken pox is a highly contagious viral infection that causes rash-like blisters on the skin surface and mucous membranes. It is generally mild and not normally life-threatening. For adults, the symptoms are more serious and uncomfortable than for children. The disease can also be deadly for some people, such as pregnant women, people with leukemia, or immunosuppressed patients. Varivax (varicella virus vaccine live) from Merck & Co. was tested on about 11,000 children and adults and was approved by the FDA in March 1995 as a chicken pox vaccine.

*Smallpox*: Smallpox is a very contagious disease with a mortality rate as high as 30–35%. It is estimated that smallpox was responsible for 300–500 million deaths in the twentieth century. Fortunately, it had been eradicated in 1979 through strict regimes of vaccination.

ACAM2000 is a smallpox vaccine using live vaccinia virus for active immunization of high risk individuals. Vaccinia virus has the same taxonomic group (classification) as smallpox virus (variola), but it cannot cause smallpox to be developed. The vaccinia virus causes localized virus infection and stimulates production of neutralizing antibodies that cross-protect against smallpox virus. ACAM2000 is supplied in a lyophilized form and reconstituted into a liquid before vaccination.

*Source*: Food and Drug Administration 2013, ACAM2000, *(Smallpox Vaccine) Questions and Answers*, viewed February 9, 2014, http://www.fda.gov/biologicsbloodvaccines/vaccines/questionsaboutvaccines/ucm078041.htm

*Herpes Zoster (Shingles)*: Zostavax is a live attenuated varicella-zoster (VZV) virus vaccine for the prevention of herpes zoster in individuals 60 years or older. It is supplied in frozen lyophilized form and reconstituted before vaccination. The vaccine boosts VZV-specific immunity and protects individuals against zosters and its complications.

*Source*: Food and Drug Administration 2013, *Zostavax (Herpes Zoster Vaccine) Questions and Answers*, viewed February 9, 2014, http://google2.fda.gov/search?q=zostavax&client=FDAgov&site=FDAgov&lr=&proxystylesheet=FDAgov&requiredfields=-archive%3AYes&output=xml_no_dtd&getfields=*

## 4.3 ANTIBODIES

Antibodies are produced by the B cells of the immune system (Exhibit 4.7). They are like weapons of our defense system and can be described as "homing devices or magic bullets" that target antigens and destroy them. Antibodies are immunoglobulins (proteins with immune functions) and are categorized into five different classes: immunoglobulin G and D (IgG and IgD, ~75%), immunoglobulin A (IgA ~15%), immunoglobulin M (IgM ~15%), and immunoglobulin E (IgE <1%). They differ from one another in size, charge, carbohydrate content, and amino acid composition. Within each class, there are subclasses that show slight differences in structure and function from other members of the class. Figure 4.1 shows the different classes of antibodies.

**Exhibit 4.7 Human Immune System**

The human immune system is a remarkable system for combating against foreign substances that invade the body. It protects us from infections by pathogens such as viruses, bacteria, parasite, and fungi. An important aspect of the immune system is the self–non-self-recognition function, by means of markers present on a protein called the major histocompatability complex (MHC). Substances without such markers are discerned and targeted for destruction. Although in most cases the immune system functions properly, at times it breaks down. For some people, the immune system lacks the normal discrimination capability and reverts to attack and destroy their own body cells as if they are foreign. This gives rise to autoimmune diseases such as rheumatoid arthritis, diabetes, multiple sclerosis, and systemic lupus erythematosus. There are also occasions when the immune system responds with undue sensitivities to innocuous substances such as airborne pollen, leading to allergies, as in the case of asthma and hay fever.

Immune responses are mediated through the lymphocytes called B cells and T cells. Lymphocytes are a particular type of white blood cell. White blood cells (leukocytes) are divided into granulocytes (neutrophils, 55–70%; eosinophils, 1–3%; and basophils, 0.5–1%) and agranulocytes (lymphocytes [B and T cells], 20–40%; and monocytes, 1–6%). There are 5,000–10,000 white blood cells per milliliter of blood, compared with 5 million red blood cells in the same volume.

When pathogens enter the human body, cells called macrophages (meaning "big eaters") engulf and ingest the pathogens (antigens). The antigens are processed by the macrophages, and parts of the antigens are displayed on the surface in the form of short peptide chains bound to the MHC protein. These antigen-presenting

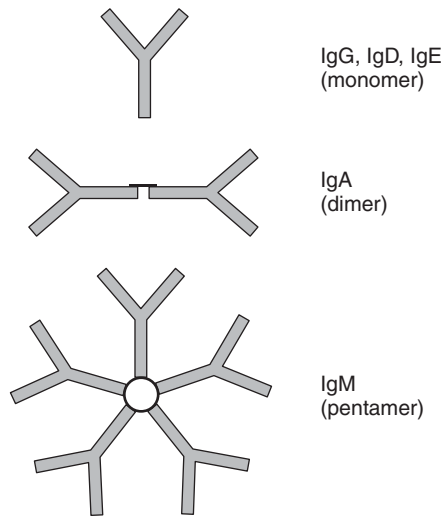



**Figure 4.1** Different classes of antibodies. IgG, IgD, and IgE are monomeric antibodies. IgA and IgM are polymeric antibodies.

cells (APCs) of macrophages and dendritic cells activate the immune response by sensitizing the B and T cells.

B cells are produced by the bone marrow. In response to the activation of CD4+ T helper cells (see below), B cells proliferate and produce antibodies (The term CD stands for "cluster of differentiation". They are proteins coating cell surfaces. Altogether there are more than 160 different types of CDs). The antibodies produced by B cells circulate in the bloodstream and bind to antigens. Once bound, other cells are in turn activated to destroy the antigens.

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

It is estimated that every B and T cell has about 100,000 protein molecules on the surface. There are many variations to these surface molecules, which act as receptors for antigens. As many as $10^{18}$ different surface receptors can be produced, thus giving rise to a vast probability for the B and T cells to recognize and bind to a vast array of antigens.

*Note*: CD4 is a receptor for HIV. Hence, people infected with HIV have suppressed immune response and develop AIDS because the CD4 cannot function normally.

### 4.3.1 Antibody Structure

The structure of an antibody is normally depicted as a capital letter "Y" configuration. IgG is the most predominant antibody. It is a tetrameric molecule consisting of two identical heavy (H) polypeptide chains of about 440 amino acids and two identical light (L) polypeptide chains of about 220 amino acids (Figure 4.2). The four chains are held together by disulfide bonds and noncovalent interactions.

Within the light and heavy chains are domains, which consist of about 110 amino acids. The domains that have similar polypeptide sequence are termed constant domains. These are the $C_H1$, $C_H2$, and $C_H3$ domains of the heavy chain and the $C_L$ domain of the light chain. Where the sequence is variable, the domains are called variable domains, one each on the heavy and light chain: $V_H$ and $V_L$. The variability is confined to particular regions of the variable domain, called the complementarity-determining regions. These regions have the appropriate 3D structure to bind to antigens.

An antibody can be cleaved by enzymes such as papain and pepsin into different fragments (Figure 4.3).

These different fragments are:

- *Variable fragment (Fv)*: The tips of the two "Y" arms vary greatly from one antibody to another. They are the regions that bind to epitopes of antigens and bring them to the natural killer cells and macrophages for destruction.
- *Antigen-binding fragments (Fab), Fab′ and F(ab′)$_2$*: various parts that contain the variable fragment.
- *Constant fragment (Fc)*: This is the stem of the letter "Y." It is the part that is identical for all antibodies of the same class; for example, all IgGs have the same Fc. The Fc fragment is the part that links the antibody to other receptors and triggers immune response and antigen destruction.

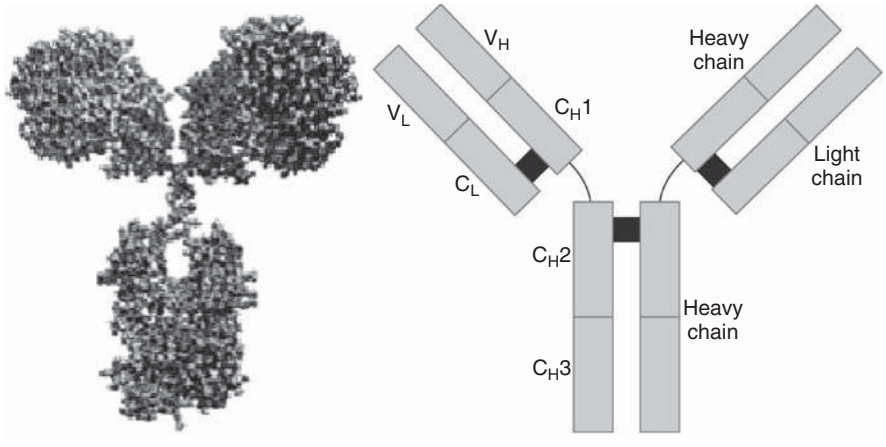



**Figure 4.2** IgG antibody molecule.

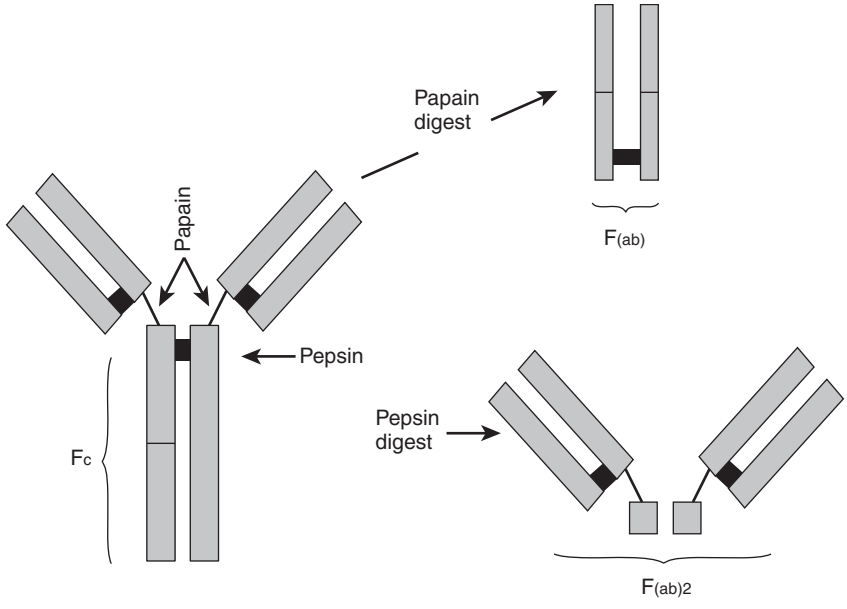



**Figure 4.3** Different fragments of the antibody molecule.

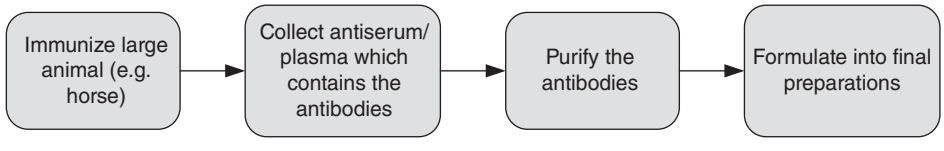



**Figure 4.4** Production of polyclonal antibodies from horse antisera. (*Source*: Data from Walsh, G 2003, *Biopharmaceuticals: Biochemistry and Biotechnology*, 2nd edn., John Wiley & Sons, Chichester, West Sussex.)

### 4.3.2 Traditional Antibodies

Several decades ago, antibodies were obtained by extraction from blood samples (antisera) of immunized animals or human donors. These are polyclonal antibodies, because several different types of antibodies are obtained through this method, although IgG is normally the predominant component. The steps for obtaining polyclonal antibodies are illustrated in Figure 4.4.

Examples of some of the polyclonal antibodies are:

- *Antibodies derived from horse antisera*: botulism antitoxin, diphtheria antitoxin, scorpion venom antisera, snake venom antisera, spider antivenins, and tetanus antitoxin.
- *Antibodies derived from human donors*: hepatitis A and B immunoglobulins, measles immunoglobulins, rabies immunoglobulin, and tetanus immunoglobulin.

Although polyclonal antibodies have been used for passive immunization and therapeutic treatments, there are cases when hypersensitivities are induced. The reason is that polyclonal antibodies contain not only the specific antibody that binds to the desired antigen, but also other antibodies that our immune system will treat as foreign substances and act against.

### 4.3.3 Monoclonal Antibodies

The next development was the production of monoclonal antibodies (MAbs) in the mid-1970s. This uses hybridoma technology, which involves the fusion of antibody-producing B cells to immortal myeloma cells. Figure 4.5 shows the preparation of MAbs using hybridoma techniques. A more detailed discussion of biopharmaceuticals production is presented in Section 10.5.

MAbs are specific in binding to epitopes of antigens. Because MAbs are produced using murine (mouse) spleen cells, human immune system can react against these murine MAbs. The allergic reaction is caused by human antimouse antibodies (HAMA), and they can neutralize the effect of the MAbs, or even induce rashes, swelling, and kidney problems; it may even be life-threatening. In other cases, the murine MAbs may not be as effective as human antibodies because of their murine origin.

### 4.3.4 Humanization of Antibodies

As discussed above, murine antibodies have limitations. The next phase of development is to make these murine MAbs more like human antibodies, by using genetic engineering techniques. A recent approach is to "humanize" the antibodies to reduce HAMA and improve the avidity of the MAbs (avidity is a measure of the affinity or interaction of the binding of an antibody to an antigen). Several strategies have been adopted. They include replacing certain fragments of the antibodies.

***Chimeric Antibodies:*** The first generation is the chimeric antibodies (chimeric comes from the word Chimera, a beast, in Greek mythology, made of three animals: lion, snake, and goat). This type of antibody consists of both murine and human parts. The murine Fv fragments are retained and linked to the Fc fragment of human IgG. An example of the chimeric antibody is ReoPro, which prevents blood clots by binding to a receptor on platelets.

***Humanized Antibodies:*** To further improve the avidity and reduce antigenicity, only the specific antigen-binding region is derived from mouse, while the remainder of the antibody is constructed using human proteins. These are the humanized antibodies and include the breast cancer targeting MAb called Herceptin (refer to Case Study #4.2).

***Fully Human Antibodies:*** Fully human antibodies are the current engineered antibodies. Several techniques are used to construct these antibodies. One method is to fuse human B cells to myeloma cells. These hybridomas will produce fully human MAbs. Another method is to genetically alter mice in the laboratory to contain human

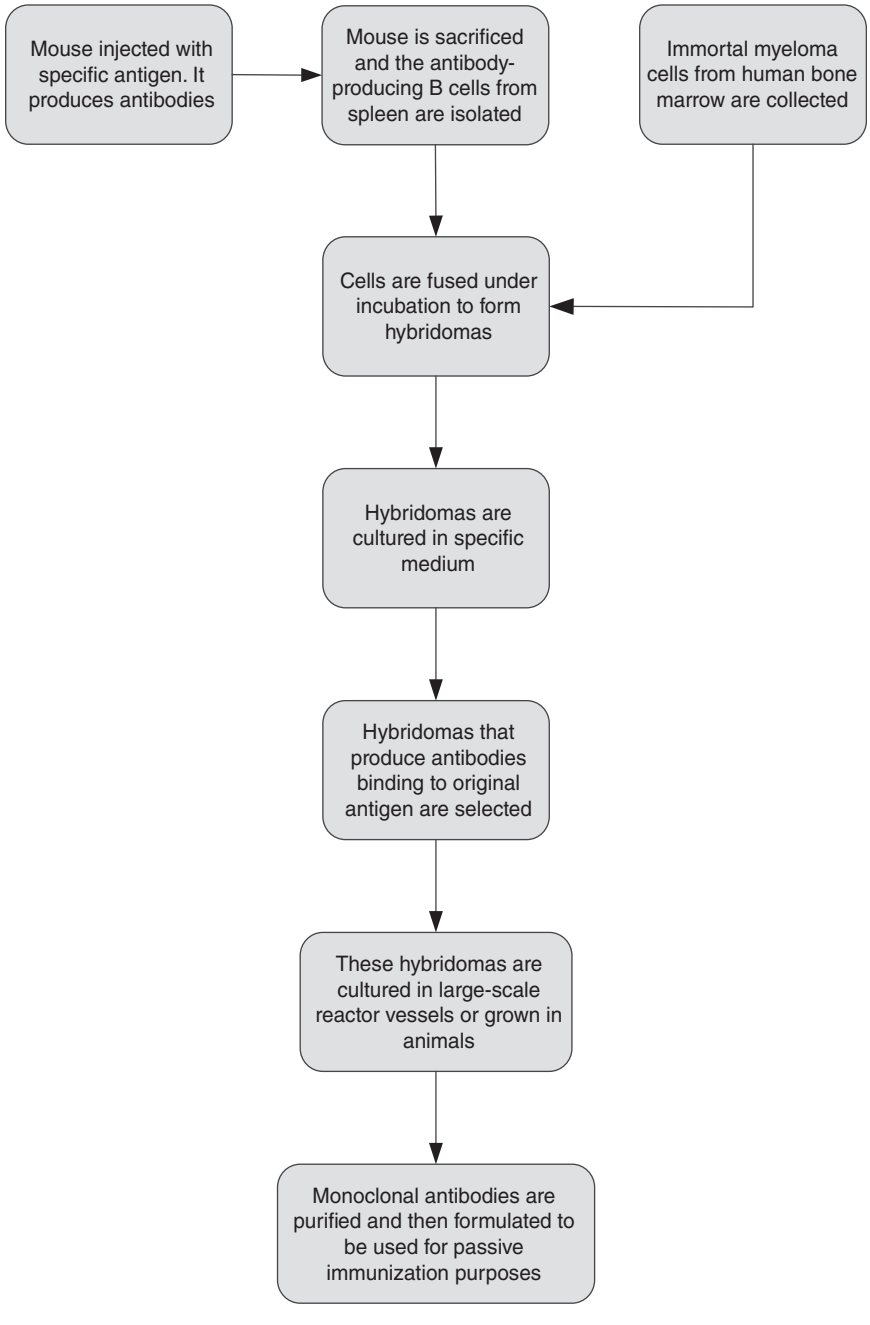



**Figure 4.5** Production of MAbs using the hybridoma technique.

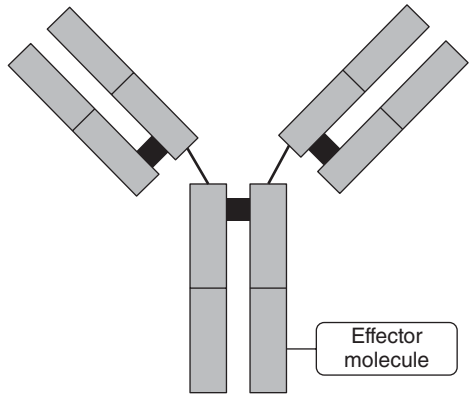



**Figure 4.6** Conjugate antibodies.

antibody-producing genes. In response to antigens, antibodies resembling the human antibodies are produced. Humira, the best-selling biopharmaceutical, is the first fully human antibody approved by FDA.

### 4.3.5 Conjugate Antibodies

Antibodies can also be prepared to carry "payloads." Materials such as toxins, enzymes, or even radioisotopes can be fused to the antibodies as effector molecules (Figure 4.6). The strategy here is to use antibodies as vehicles to deliver more effective treatment to specific target cells. Immunotoxins are fusion proteins consisting of a toxin connected to a MAb. Immunocytokines consist of a fusion of rDNA encoding the heavy chain of a MAb with the DNA encoding a cytokine. The aim is to obtain a high local concentration of cytokine to generate an antitumor response. Zevalin and Bexxar are two conjugate antibody drugs, which carry radioisotope yttrium ($^{90}$Y) and iodine ($^{131}$I) for the treatment of non-Hodgkin's lymphoma. Exhibit 4.8 provides more details concerning Zevalin and Bexxar.

Brentuximab vedotin (Adcetris) is a conjugate antibody directed at the protein CD30, which is expressed in Hodgkin's lymphoma and systemic anaplastic large cell lymphoma. The antibody carries a cytotoxic small molecule drug. After the antibody seeks out the CD30 disease target, the cancer-killing small molecule drug is released to perform its job. Adcetris was given an accelerated approval by FDA in 2011. Another recently approved antibody conjugate, in 2013, is trastuzumab emtansine (Kadcyla), which consists of the MAb trastuzumab (Herceptin) linked to the cytotoxic agentmertansine. A fuller description of this antibody conjugate is presented in Case Study #4.2.

Another variation to conjugate antibodies is to use bispecific antibodies. These are produced using chemical means and recombinant techniques to fuse separate hybridomas into a hybrid hybridoma (Figure 4.7). Bispecific antibodies use one arm of the Fv to target the antigen or tumor cell and the other arm carries the effector molecule of toxins, radioisotopes, or other drugs. Catumaxomab (Removab) is the first bispecific

**Exhibit 4.8 Zevalin and Bexxar**

Zevalin is a conjugate antibody consisting of a MAb linked to radioactive Indium-111 or Yttrium-90. It is a murine $IgG_1$ MAb known as ibritumomab, which binds to the CD20 antigen that is found on surface of normal and malignant B lymphocytes and on >90% of B-cell non-Hodgkin's lymphomas. Once bound, the radioactive emission of Indium-111 or Yttrium-90 causes cellular damage to the cancer cells.

Bexxar is a conjugate antibody consisting of radioactive Iodine-131. It is a murine IgG2a MAb known as tositumomab, which also binds to the CD20 antigen, similar to that of Zevalin.

While Zevalin is achieving moderate success, Bexxar encountered resistance in its acceptance due to treatment practice issues, reimbursement and delayed approval. Sales of Bexxar were terminated in February 2014.

*Source*: Data from 1. Food and Drug Administration 2001, *Ibritumomab Tiuxetan*, viewed February 10, 2014, http://www.fda.gov/downloads/drugs/developmentapprovalprocess/howdrugsaredevelopedandapproved/approvalapplications/therapeuticbiologicapplications/ucm113490.pdf; 2. Food and Drug Administration 2003, *Tositumomab*, viewed February 10, 2014, http://www.accessdata.fda.gov/drugsatfda_docs/label/2003/tosicor062703LB.pdf

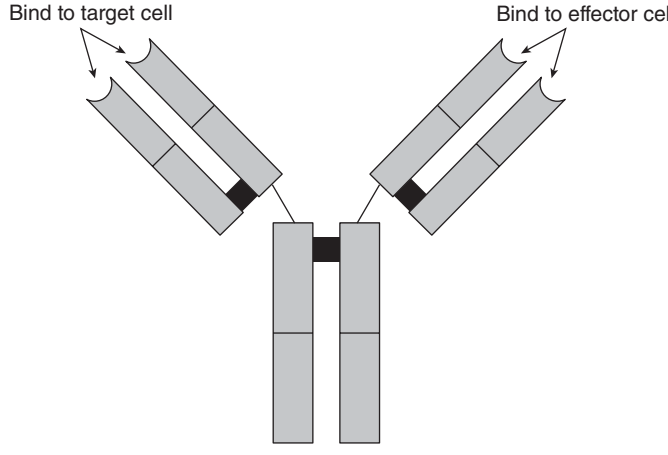



**Figure 4.7** Bispecific antibody.

antibody approved by EMA for intraperitoneal treatment of malignant ascites. It has a mouse IgG2a and rat IgG2b, one binds to T cells and the other binds to tumor cells. The Fc region also provides a third binding site.

Antibody is a very active area of research currently. Table 4.3 shows some examples of monoclonal antibody therapeutics approved recently by FDA, and many more are expected in the coming years.

**TABLE 4.3 Examples of Recent FDA-Approved Monoclonal Antibody Therapeutics**

| Antibody | Brand Name | Company | Approval Date | Type | Target | Indication (targeted disease) |
|---|---|---|---|---|---|---|
| Belimumab | Benlysta | GlaxoSmithKline | 2011 | Human | Inhibition of B-cell activating factor | Systemic lupus erythematosus |
| Bevacizumab | Avastin | Genentech/Roche | 2004 | Humanized | Vascular endothelial growth factor (VEGF) | Colorectal cancer, age-related macular degeneration (off-label) |
| Brentuximab vedotin | Adcetris | Seattle Genetics | 2011 | Chimeric | CD30 | Anaplastic large cell lymphoma (ALCL) and Hodgkin lymphoma |
| Canakinumab | Ilaris | Novartis | 2009 | Human | IL-1$\beta$ | Cryopyrin-associated periodic syndrome (CAPS) |
| Cetuximab | Erbitux | Bristol-Myers Squibb/Eli Lilly/Merck KGaA | 2004 | Chimeric | Epidermal growth factor receptor | Colorectal cancer, head and neck cancer |
| Certolizumab pegol | Cimzia | UCB (company) | 2008 | Humanized | Inhibition of TNF-$\alpha$ signaling | Crohn's disease |

(*continued*)

**TABLE 4.3 (*Continued*)**

| Antibody | Brand Name | Company | Approval Date | Type | Target | Indication (targeted disease) |
|---|---|---|---|---|---|---|
| Denosumab | Prolia, Xgeva | Amgen | 2010 | Human | RANK ligand inhibitor | Postmenopausal osteoporosis, solid tumor‘s bony metastases |
| Eculizumab | Soliris | Alexion Pharmaceuticals | 2007 | Humanized | Complement system protein C5 | Paroxysmal nocturnal hemoglobinuria |
| Golimumab | Simponi | Johnson & Johnson/Merck & Co, Inc. | 2009 | Human | TNF-alpha inhibitor | Rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis |
| Ipilimumab | Yervoy | Bristol-Myers Squibb | 2011 | Human | Blocks CTLA-4 | Melanoma |
| Natalizumab | Tysabri | Biogen Idec/Élan | 2006 | Humanized | Alpha-4 ($\alpha$4) integrin | Multiple sclerosis and Crohn’s disease |
| Omalizumab | Arzerra | Genmab | 2009 | Human | CD20 | Chronic lymphocytic leukemia |
| Omalizumab | Xolair | Genentech/Novartis | 2004 | Humanized | Immunoglobulin E (IgE) | Mainly allergy-related asthma |

| | | | | | | |
|---|---|---|---|---|---|---|
| Panitumumab | Vectibix | Amgen | 2006 | Human | Epidermal growth factor receptor | Colorectal cancer |
| Pertuzumab | Perjeta | Genentech | 2012 | Humanized | Binds to human epidermal growth factor receptor 2 protein (HER-2) | HER-2 positive metastatic breast cancer |
| Ranibizumab | Lucentis | Genentech/Novartis | 2006 | Humanized | Vascular endothelial growth factor A (VEGF-A) | Macular degeneration |
| Raxibacumab | | Human Genome Sciences | 2012 | Human | Neutralizes toxins produced by *B. Anthracis* | Inhalational anthrax |
| Tocilizumab or Atlizumab | Actemra and RoActemra | Genentech | 2010 | Humanized | Anti-IL-6R | Rheumatoid arthritis |
| Ustekinumab | Stelara | Centocor | 2012 | Human | Binds to p40 protein subunit of IL-12 and IL-13 | Plaque psoriasis |

## 4.4 CYTOKINES

Cytokines are produced mainly by the leukocytes (white blood cells). They are potent polypeptide molecules that regulate the immune and inflammation functions, as well as hemopoiesis (production of blood cells) and wound healing. There are two major classes of cytokines: (i) lymphokines and monokines, and (ii) growth factors.

### 4.4.1 Lymphokines and Monokines

Cytokines produced by lymphocytes are called lymphokines, and those produced by monocytes are termed monokines. Lymphocytes and monocytes are different types of white blood cells. The major lymphokines are interferons (IFNs) and some interleukins (ILs). Monokines include other interleukins and tumor necrosis factor (TNF).

***Interferons:*** There are two types of interferons: Type I, which includes IFN-$\alpha$, and IFN-$\beta$ and Type II consisting of IFN-$\gamma$. IFN-$\alpha$ and $\beta$ have about 30% homology in amino acid sequence. There are two more recently discovered Type I interferons; they are called IFN-$\omega$ and IFN-$\tau$. IFN-$\alpha$ and IFN-$\beta$ each has 166 amino acids, and IFN-$\gamma$ has 143. Both IFN-$\alpha$ and IFN-$\beta$ are of single chain structure and bind to the same type of cell surface receptors, whereas IFN-$\gamma$ is a dimer of two identical chains and interacts with another type of receptors. All our cells can produce Type I interferons when infected by viruses, bacteria, and fungi. However, only T cells and natural killer cells can produce Type II interferon. Type I interferon binds to receptor, which in turn activates tyrosine kinase phosphorylation and the subsequent transcription pathway that induces viral resistance. Similarly, Type II interferon binds to another receptor and activates the immune response. There is also a Type III interferon, but to date it is not universally recognized.

Because of its antiviral and anticancer effects, IFN-$\alpha$ is used in the treatment of hepatitis and various forms of cancer, such as Kaposi's sarcoma, non-Hodgkin's lymphoma, and hairy cell leukemia. Exhibit 4.9 describes the treatment of hepatitis C with IFN-$\alpha$, such as a pegylated interferon called PegIntron. IFN-$\beta$ (Betaseron) is used for treating multiple sclerosis, a chronic disease of the nervous system. The medical application of IFN-$\gamma$ is for cancer, AIDS, leprosy as well as chronic granulomatous disease (CGD – a defect in the ability of white blood cells to destroy bacteria and fungi) and osteopetrosis (hardening of the bones). Actimmune, an IFN-$\gamma$, is a single-chain polypeptide of 140 amino acids of 16 kDa produced by genetically modified *E. coli* in a fermentation process (refer to Section 10.5). It is approved for the treatment of CGD and osteopetrosis.

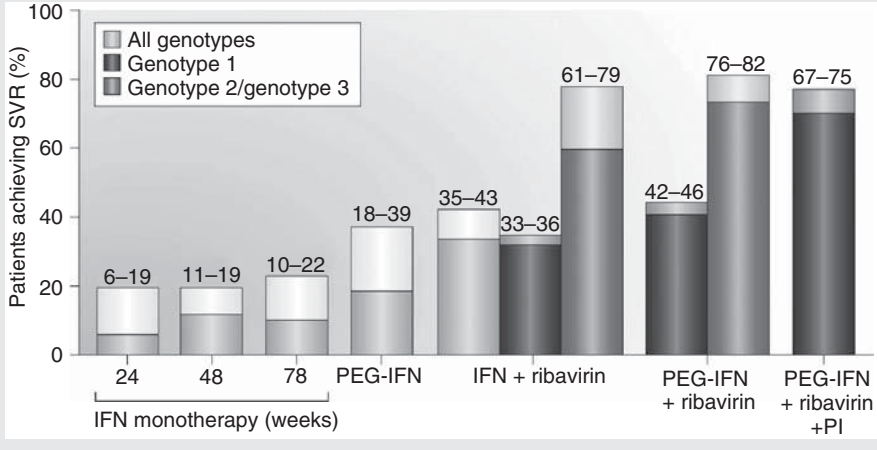
**Exhibit 4.9 Hepatitis C and Interferon/Pegylated Intron**

Hepatitis C is caused by a virus contracted through contaminated blood. Most infected patients show no sign of hepatitis for a long time. Of those infected, about 15% will clear the virus, and 85% develop chronic hepatitis. Up to 30% of patients

with chronic hepatitis C will develop cirrhosis within 20 years, and 5% will develop liver cancer. The WHO estimates that more than 170 million people worldwide are infected with hepatitis C.

Interferon is the approved treatment for hepatitis C. In general, there are four different treatments: (i) IFN-$\alpha$, (ii) combination therapy of IFN-$\alpha$ and another drug called ribavirin, (iii) pegylated IFN-$\alpha$ and (iv) pegylated IFN-$\alpha$ with ribavirin. Pegylated interferon contains polyethylene glycol, which increases the half-life (See Section 5.3.5) from 6 to 45 hours and slows down the body's absorption of interferon. In this way, a more controlled release of interferon is achieved to prolong absorption. Instead of a subcutaneous injection of three times weekly, the frequency can be reduced to once weekly.

The diagram below shows the evolution of treatment of hepatitis C. With IFN-$\alpha$ monotherapy, the treatment effectiveness is up to 22%. Using pegylated IFN-$\alpha$, the effectiveness reaches 39%, and combination of IFN-$\alpha$ and ribavirin even higher to 79% for genotype 2/genotype 3. Further improvement is shown by combination of pegylated IFN-$\alpha$ and ribavirin and also pegylated IFN-$\alpha$, ribavirin, and protease inhibitor at 82% (genotype 2/genotype 3) and 75% (genotype 1), respectively.




*Note*: SVR, sustained virological response – patient considered as achieving cure, IFN, interferon; PEG-IFN, pegylated interferon; PI, protease inhibitor; Genotype, specific genetic structure of hepatitis C; there are six major genotypes (1–6).

Another breakthrough development in hepatitis C is a new drug called Sovaldi, which has a treatment effectiveness of more than 90% for genotype 1 (refer to Exhibit 8.1).

Interferons were extracted and purified from human blood supplies up until the 1980s. The amount produced was very low. Since then, interferons have been produced using recombinant technology from a variety of cells: *E. coli*, fungus, yeast, and mammalian.

Patients receiving IFN experience side effects similar to influenza symptoms: headache, nausea, and tiredness. IFN also decreases red blood cells, white blood cells, and platelet counts. A measure of the effectiveness of IFN treatment is the marker called alanine aminotransferase in blood. The normal range is 10–70 U/L.

Source: Manns, MP and von Hahn, T 2013, 'Novel therapies for hepatitis C – one pill fits all?', *Nature Reviews Drug Discovery*, 12, pp. 595–610. Reproduced with permission of Macmillan Publishers Ltd

***Interleukins:*** Interleukins are proteins produced mainly by leukocytes. There are many interleukins within this family (Table 4.4). Interleukins have a number of functions, but are principally involved in mediating and directing immune cells to proliferate and differentiate. Each interleukin binds to a specific receptor and produces its response.

IL-2 is possibly the most studied interleukin. It is also called T cell growth factor. IL-2 is a 15 kDa glycoprotein produced by CD4+ T helper cells. It has 133 amino acids. There are four helical regions and a short $\beta$-sheet section (Figure 4.8).

IL-2 promotes the growth of B cells for antibody production and induces the release of IFN-$\gamma$ and TNF (see below). It has been approved by FDA for the treatment of different types of cancer, including metastatic melanoma and metastatic renal carcinoma. Examples of IL-2 for the treatment of malignant melanoma and a protein that targets IL-2 receptor in T-cell lymphoma are given in Exhibit 4.10.

Although IL-2 has not been approved to treat HIV/AIDS, many clinical trials using IL-2 are being conducted. The strategy is to complement the anti-HIV therapy by boosting the immune system with IL-2. The replacement therapy of IL-2 administered to AIDS patients increases production of CD4+ T cells and the activities of natural killer cells to combat HIV. Therapeutic IL-2 is manufactured using recombinant technology.

**TABLE 4.4 Selected Interleukins**

| Cytokine | Produced By | Major Functions |
|---|---|---|
| IL-2 | T cell | T cell growth factor |
| IL-3 | T cell, NK cell, mast cell | Growth factor for hematopoietic cells |
| IL-4 | T cell, mast cell | Growth factor for B cells, promotes IgE and IgG synthesis |
| IL-5 | T cell, mast cell | Stimulates growth and differentiation of eosinophils |
| IL-7 | Bone marrow, stroma cell | Growth factor for pre-T and pre-B cells |
| IL-10 | Macrophage, T cell | Inhibits macrophage function, control of immune response |
| IL-15 | T cell, epithelial cell | T cell growth factor |

*Source*: Coico, R, Sunshine, G and Benjamini, E 2003, *Immunology*, 5th edn., Wiley-Liss, New Jersey. Reproduced with permission of John Wiley and Sons Inc.

**Figure 4.8** Interleukin 2 (IL-2) molecule. (*Source*: Arkin, MM, Randal, M, Delano, WL et al. 2003, 'Binding of Small Molecules to an Adaptive Protein-Protein Interface', *Proceedings of the National Academy of Sciences USA*, 100, pp. 1603. Reproduced with permission of National Academy of Sciences, U.S.A.)

**Exhibit 4.10 Proleukin and Ontak**

Proleukin is a recombinant form of IL-2. It is approved for the treatment of malignant melanoma and renal cell cancer.

*Source*: Data from Food and Drug Administration 2009, *List of Orphan Products*, viewed June 15, 2014, www.fda.gov/ … /howtoapplyfororphanproductdesignation/ucm162066.xls

Ontak (denileukin diftitox) is a fusion protein for the treatment of persistent or recurrent T-cell lymphoma. Activated T-cells expressed IL-2 receptors. Ontak has a fragment that binds to the IL-2 receptor while the other part presents a diphtheria toxin to kill the activated T-cell.

*Source*: Data from Food and Drug Administration 2008, *Ontak*, viewed June 15, 2014, http://www.accessdata.fda.gov/drugsatfda_docs/label/2008/103767s5094lbl.pdf

***Tumor Necrosis Factor:*** There are two types of tumor necrosis factor: TNF-$\alpha$ and TNF-$\beta$. Of the two, TNF-$\alpha$ has been studied in more detail. TNF-$\alpha$ is a 157 amino acid polypeptide. It is a mediator of immune regulation, including the activation of macrophages and induction of the proliferation of T cells. Another TNF-$\alpha$ function is its cytotoxic effects on a number of tumor cells. Recent research, however, concentrates on its property in the stimulation of inflammation, in particular, in the case of rheumatoid arthritis. Clinical trials are being conducted with drugs to block TNF-$\alpha$ with anti-TNF-$\alpha$ monoclonal antibodies. These antibodies (Remicade, Enbrel) target the excessive levels of TNF-$\alpha$ in synovial fluids of joints and provide relief to sufferers of rheumatoid arthritis (Exhibit 4.11).

**Exhibit 4.11 Rheumatoid Arthritis and TNF-$\alpha$**

Rheumatoid arthritis is an autoimmune disease of the synovial lining of joints. Typically, the joints affected are those in the extremities: fingers, wrist, toes, and ankles. It is a debilitating disease in which ligaments may be damaged and joints deformed.

In the late 1980s, scientists found that TNF-$\alpha$ is involved in causing arthritis. Standard drug treatment for rheumatoid arthritis used to be methotrexate, steroids, and nonsteroidal anti-inflammatory drugs (NSAIDs). These drugs are nonspecific, and their effectiveness is variable. The new set of drugs in the late 1990s was designed to specifically target TNF-$\alpha$. Two drugs are especially effective; they are infliximab (Remicade, J&J) (a chimeric antibody that targets the TNF-$\alpha$) and etanercept (Enbrel, Pfizer) (a soluble protein receptor for TNF-$\alpha$ that neutralizes its effect).

The success of these two drugs provides impetus for the development of other anti-inflammatory drugs aiming at specific inflammatory agents.

### 4.4.2 Growth Factors

As the name implies, growth factors stimulate cell growth and maintenance. We will discuss the following growth factors:

- Erythropoietin
- Colony stimulating growth factors
- Vascular endothelial growth factors.

***Erythropoietin:*** Erythropoietin (EPO) (Figure 4.9) is a glycoprotein produced by specialized cells in the kidneys. It has 166 amino acids and a molecular weight of approximately 36 kDa. EPO stimulates the stem cells of bone marrow to produce red blood cells. It is used to treat anemia and chronic infections such as HIV and cancer treatment with chemotherapy where anemia is induced. Patients feel tired and breathless owing to the low level of red blood cells. EPO can be prescribed instead of blood transfusion.

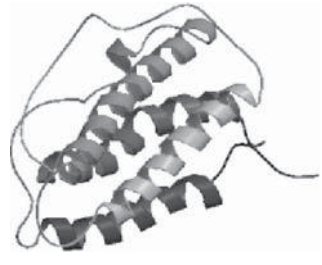


**Figure 4.9** Erythropoietin. (*Source*: Cheetham, JC, Smith, DM, Aoki, KH et al. 1998, 'NMR structure of human erythropoietin and a comparison with its receptor bound conformation', Nature Structural Biology, 5, pp. 861–866. Reproduced with permission of Macmillan Publishers Ltd.)

**Exhibit 4.12 Performance-enhancing Drugs**

To help them excel in sports, some athletes use drugs to boost their performance. There are several areas where drugs are used by athletes:

- To increase oxygen delivery
- To build muscle and bone
- To mask pain
- To mask use of other drugs
- As stimulants.

EPO is used in blood doping to generate more red blood cells for carrying oxygen. It is particularly favored by endurance athletes to enhance their performance. Human growth hormone (hGH, see description in Section 4.5.2) is used to build up muscle and bone strength. Both EPO and hGH are banned in sport.

The recombinant EPO and hGH produced are almost replicas of those that occur naturally in our body. Hence, it is very difficult to detect these banned substances if taken by athletes. Another difficulty is the need to develop reliable and sensitive test methods that take into account differences of these substances in athletes of different racial groups.

*Source:* Data from Zorpette, G 2000, 'All doped up—and going for gold', *Scientific American*, May, pp. 20–22.

Biopharmaceutical quantities of EPO are produced with recombinant cells. This is achieved through the isolation of the human gene that codes for EPO, and transfection of the gene into cell lines such as Chinese hamster ovary cells (refer to Section 10.5). The product is called rhEPO – recombinant human EPO (e.g., Epogen). EPO is normally administered subcutaneously and is generally well tolerated by patients.

EPO is considered a banned performance-enhancing drug in the sports arena, where athletes use EPO to boost their red blood cells with the expectation of boosting performance (refer to Exhibit 4.12 for a brief review of performance enhancing drugs and Section 11.10 on banned drugs in sports).

***Colony Stimulating Growth Factors:*** Growth factors such as granulocyte macrophage colony stimulating factor (GM-CSF) and macrophage colony stimulating factor (M-CSF) are involved in the regulation of the immune and inflammatory responses. GM-CSF is a glycoprotein with 127 amino acids and a molecular weight of about 22 kDa. It is produced by macrophages and T cells.

Clinically, GM-CSF is used to stimulate production of blood cells, in particular, patients who have received chemotherapy. M-CSF is a glycoprotein that can exist in different forms. The number of amino acids ranges from just over 200 to about 500, and

molecular weight varies between 45 and 90 kDa. M-CSF is being evaluated clinically for its antitumor activity.

***Vascular Endothelial Growth Factor:*** Vascular endothelial growth factor (VEGF) is a homodimeric glycoprotein with a molecular weight of 45 kDa. It is a major regulator of tumor angiogenesis (growth of blood vessels).

For cells to grow, a supply of oxygen is required. Our blood delivers oxygen to the cells, which are within a tenth of a millimeter from blood capillaries. For tumor cells, if they grow larger than a millimeter, they will be denied of oxygen if there are no new vessels formed. VEGF is used by tumor cells to form new blood vessels. It binds to receptors on the surface of endothelial cells and signals them to form new vessels. This will promote further tumor growth and metastasis, leading to the spread of tumor to other parts of the body.

Two MAbs (Avastin and Lucentis, both are angiogenesis inhibitors) have been approved by FDA to treat prostate cancer and macular degeneration through the use of the antibodies to target VEGF. They bind to the VEGF and stop angiogenesis – the growth of blood vessels (Exhibit 4.13).

## 4.5 HORMONES

Hormones are intercellular messengers. They are typically (i) steroids (e.g., estrogens, androgens, and mineral corticoids, which control the level of water and salts excreted by the kidney), (ii) polypeptides (e.g., insulin and endorphins), and (iii) amino acid derivatives (e.g., epinephrine, or adrenaline, and norepinephrine, or noradrenaline). Hormones maintain homeostasis – the balance of biological activities in the body; for example, insulin controls blood glucose level, epinephrine and norepinephrine mediate response to external environment, and growth hormone promotes normal healthy growth and development.

### 4.5.1 Insulin

Insulin is produced in the pancreas by $\beta$ cells in the region called the islets of Langerhans. It is a polypeptide hormone consisting of two chains: an A chain with 21 amino acids with an internal disulfide bond and a B chain with 30 amino acids. There are two disulfide bonds joining these two chains together (Figure 4.10). The molecular weight is around 6.8 kDa. Insulin regulates the blood glucose level to within a narrow range of 3.5–8.0 mmol/L of blood.

Insulin was originally (since the 1930s) obtained from porcine and bovine extracts. Bovine insulin differs from human insulin by three amino acids, and it can elicit an antibody response that reduces its effectiveness. Porcine insulin, however, differs in only one amino acid. An enzymatic process can yield insulin identical to the human form. Currently, insulin is produced via the rDNA process; it was the first recombinant

**Exhibit 4.13 Avastin and Lucentis**

*Avastin*: Bevacizumab (Avastin) is a monoclonal $IgG_1$ antibody. It binds to vascular endothelial growth factor (VEGF) and prevents VEGF from interacting with its receptors (Flt-1 and KDR) on the surface of endothelial cells. Thus it inhibits endothelial cell proliferation and new blood vessel formation, leading to the reduction of microvascular growth and inhibition of metastatic disease progression.

Bevacizumab is produced using CHO expression in a nutrient medium with gentamicin antibiotic. It has a molecular weight of 149 kDa. The antibody is humanized, with human framework and murine complementarity-determining regions.

*Source*: Data from Food and Drug Administration 2011, *Avastin*, viewed February 10, 2014, http://www.fda.gov/drugs/drugsafety/postmarketdrugsafetyinformationforpatientsandproviders/ucm193900.htm

*Lucentis*: Ranibizumab (Lucentis) is a humanized monoclonal $IgG_1$ antibody Fab fragment. It is designed for intraocular use for the treatment of age-related macular degeneration (AMD) – thinning of the retina, which affects the central vision of the elderly. The antibody binds to the vascular endothelial growth factor A (VEGF-A) and inhibits the biological activity.

Ranibizumab is produced in *E. coli* expression system in a nutrient medium containing the tetracycline antibiotic. It has a molecular weight of 48 kDa.

*Source*: Data from Food and Drug Administration 2012, *Lucentis*, viewed February 10, 2014, http://www.accessdata.fda.gov/drugsatfda_docs/label/2012/125156s0069s0076lbl.pdf

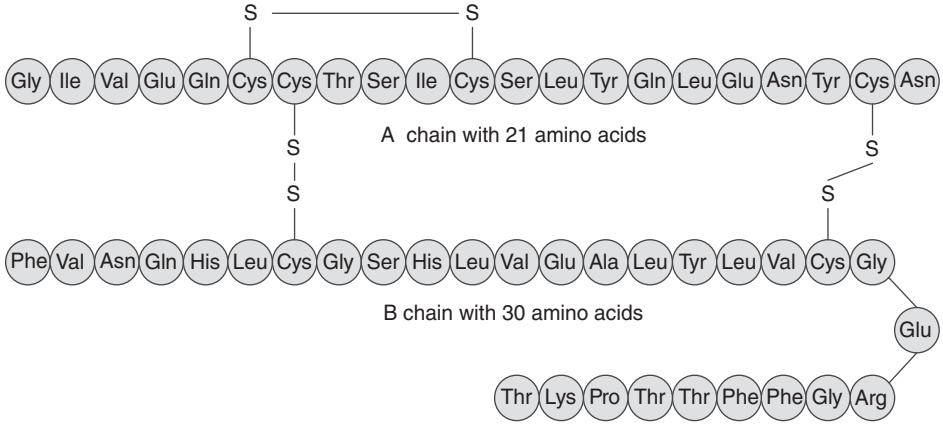



**Figure 4.10** Human insulin molecule. Refer to Table A2.1 for the names of amino acids.

**Exhibit 4.14 Diabetes Mellitus and Insulin**

Diabetes mellitus occurs when the human body does not produce enough insulin. This form of diabetes is called insulin-dependent diabetes mellitus (IDDM, or juvenile diabetes, or Type I diabetes). IDDM is an autoimmune disease (see Exhibit 4.7) in which the $\beta$ cells are targeted by the body's own immune system and progressively destroyed. Once destroyed, they are unable to produce insulin.

Production of insulin is triggered when there is a rise in blood sugar, for example, after a meal. Most of our body cells have insulin receptors, which bind to the insulin secreted. When the insulin binds to the receptor, other receptors on the cell are activated to absorb sugar (glucose) from the bloodstream into the cell.

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

Another form of diabetes is non-insulin-dependent diabetes mellitus (NIDDM, or adult diabetes, or Type II diabetes). In this case, insulin is produced, and a normal insulin level is detected in blood. But for various reasons its effect is reduced. This may be caused by a reduced number of insulin receptors on cells or reduced effectiveness in binding to these receptors. The cause is complex and may involve genetic make-up, changes in lifestyle, nutritional habits, and environmental factors.

biopharmaceutical approved by FDA in 1982. The recombinant insulin removes the reliance on animal sources of insulin and ensures that reliable and consistent insulin is manufactured under controlled manufacturing processes. A description of diabetes mellitus and insulin is presented in Exhibit 4.14.

In January 2006, FDA approved the inhalable insulin, Exubera for Types I and II diabetes. Details are presented in Exhibit 4.15.

### 4.5.2 Human Growth Hormone

Human growth hormone (hGH) is a polypeptide with 191 amino acids. It is secreted by the pituitary gland. This hormone stimulates the production of insulin-like growth factor-1 (IGF-1) from the liver. Most of the positive effects of hGH are mediated by the IGF-1 system, which also includes specific binding proteins.

A major function of hGH is the promotion of anabolic activity, that is, bone and tissue growth because of increase in metabolic processes. Other biological effects of hGH are stimulation of protein synthesis, elevation of blood glucose level, and improvement of liver function.

Overproduction of hGH during puberty leads to gigantism, and deficiency during this period results in dwarfism. The current main therapeutic use of hGH is for the

**Exhibit 4.15 Inhalable Insulin**

Exubera is an inhaled insulin. It represents a major step forward since the first insulin injection was approved in 1920s. The insulin particles are formulated to a certain micron size for deep lung delivery. An inhaler is used to achieve the delivery. The large surface area of the thin alveolar walls in the lungs allows for fast absorption of the insulin into the bloodstream.

A number of side effects, however, have been reported, such as coughing, shortness of breath, sore throat, and dry mouth. There is also concern over the prolonged delivery of insulin into the lungs.

It was expected to be a $2 billion drug, but in October 2007, Exubera was withdrawn from sales because of low demand by patients. The problem stems from a higher cost of the medication, cumbersome inhaler, confusing dosage calculations, and possible effect on pulmonary function.

In June 2014, FDA approved another fast-acting inhalable insulin product, Afrezza from MannKind Corp., to improve blood sugar level control in Type I and Type II diabetes. It consists of insulin powder packaged in a small hand-held device. The safety and effectiveness of the drug were evaluated in a total of 3,017 participants.

*Source*: Data from 1. Walsh, J 2014, *Insulin – Diabetes Mall*, viewed February 10, 2014, http://www.diabetesnet.com/about-diabetes/insulin/insulin-delivery/inhaled-insulin; 2. Food and Drug Administration, *FDA approves Afrezza to treat diabetes*, viewed July 2, 2014, http://www.fda.gov/newsevents/newsroom/pressannouncements/ucm403122.htm

treatment of short stature. As discussed in Exhibit 4.12, hGH is used by athletes illegally to enhance their performance. This hormone is also sold without prescription with claims of improvement to body composition (lean body mass, fat mass, fluid volume), bone strength, immune function, youthful vigor, and general well-being.

## 4.6 GENE THERAPY

In essence, gene therapy can be described as "good genes for bad genes." The technology involves the transfer of normal, functional genes to replace genetically faulty ones so that proper control of protein expression and biochemical processes can take place. Although this seems straightforward, the major question is "How do we get the normal genes to the intended location?"

This question revolves around the delivery tools for the genes. The transport system or vehicles used are called vectors (gene carriers). There are two basic gene therapy techniques: *in vitro* and *in situ* methods.

For the *in vitro* method, some of the patient's tissues, which have the genetic fault, are removed. Cells are selected from these tissues and normal genes are loaded into the cells with vectors. The modified cells are then returned to the patient to correct the

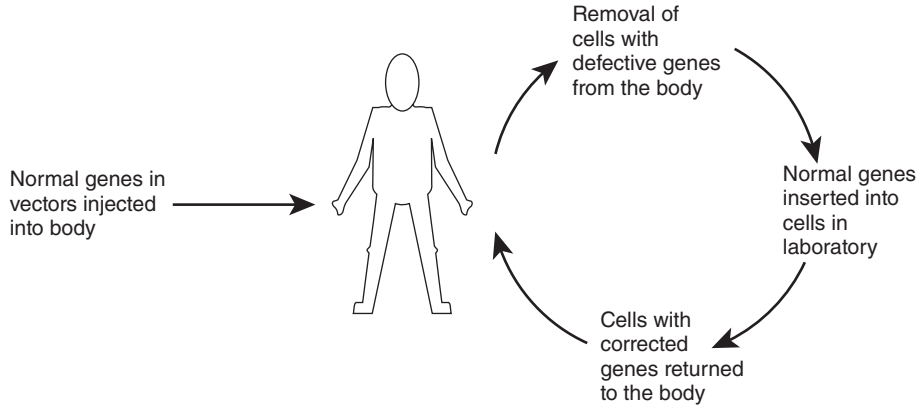



**Figure 4.11** Basis of gene therapy.

**TABLE 4.5 Vectors for Gene Therapy**

| Retrovirus | Adenovirus | Adeno-Associated Virus | Liposomes | Naked DNA |
|---|---|---|---|---|
| *Advantages* | | | | |
| Integrates genes to host chromosomes, chance of long-term stability | Large capacity for carrying foreign genes | Integrates genes to host chromosomes | Do not have viral genes, so do not cause disease | Do not have viral genes, so do not cause disease |
| *Disadvantages* | | | | |
| Integration is random, mostly on dividing cells | Transient function of genes | Small capacity for foreign genes | Less efficient than viruses | Inefficient gene transfer |

*Source*: Adapted from Friedman, T 1997, 'Overcoming the obstacles to gene therapy', *Scientific American*, June, pp. 96–101.

genetic fault. With the *in situ* method, genes encapsulated by the vectors are injected directly into the tissues to be treated. Figure 4.11 shows the basis for gene therapy.

Whether using the *in vitro* or *in situ* method, genes are first loaded onto the vectors. A number of vectors are used (Table 4.5).

The most common vectors used today are viruses, with retroviruses being the preferred candidates, as they are efficient vectors for entering humans and replicating their genes within human cells. Scientists take advantage of this biological function. Disease-causing genes from the viruses are removed, and the therapeutic genes are inserted. Retroviruses carrying the desired therapeutic genes are placed in the patient's body. When the viruses invade the cells, they "infect" these host cells, and the therapeutic genes are added to the host DNA. In this way, the new genes function and take over from the original faulty genes.

**Exhibit 4.16 Gene Therapy Trials**

The first gene therapy trial was conducted in September 1990. A 4-year-old girl with SCID (an inherited immune disorder disease, otherwise known as the "Bubble Boy" syndrome) was treated in Cleveland, USA. She is doing well even some 20 years after the treatment. A second girl with the same disorder underwent gene therapy and she too continues to do well.

These are the successes; there are many failures as well. Quite a number of gene therapy clinical trials have been conducted, mainly on cancer, but not many cases worked. In 1999, an 18-year old boy in Pennsylvania, USA, unexpectedly died from a reaction to gene therapy when he was treated for a metabolic disease. This trial raised many issues, and many trials with discrepancies and unreported adverse events were suspended by the FDA. The FDA has since introduced tighter controls for gene therapy trials.

Refer to Exhibit 6.17 for more information on gene therapy trials.

*Source*: Data from 1. Mayo Clinic, *Gene Therapy 2013*, viewed February 10, 2014, http://www.mayoclinic.org/tests-procedures/gene-therapy/basics/definition/PRC-20014778?p=1; 2. Richards, S 2012, 'Gene Therapy Arrives in Europe', *The Scientist*, viewed February 10, 2014, http://www.the-scientist.com/?articles.view/articleNo/33166/title/Gene-Therapy-Arrives-in-Europe/

Theoretically, this appears to be a fitting solution to gene problems. However, there are problems, such as immune and inflammation responses, toxicity, and means to target the intended cells. Nonviral vectors may overcome the problems with viral delivery agents. Lipids, in the form of liposomes and other lipid complexes, are being studied. Injection of DNA directly into a patient's muscle cells is another avenue being researched.

Another hurdle surrounding gene therapy is the identification of genes causing the disease. Effective cures can be possible only when there is a good understanding of the roles of particular genes in diseases. Some of the diseases to which gene therapy may be applicable are cancer, hemophilia, sickle cell anemia, cystic fibrosis, insulin-dependent diabetes mellitus (refer to Exhibit 4.14), emphysema, Alzheimer's disease, Huntington's disease, and severe combined immune deficiency (SCID).

Alipogene tiparvovec (Glybera) is the first gene therapy approved by EMA in 2012; it is indicated for the treatment for lipoprotein lipase deficiency where large amount of fat builds up in the blood. This is because of the lack of the lipase enzyme to break down fat from digested food. There are numerous gene therapy clinical trials for a variety of treatments in progress as shown in Exhibit 4.16. Another question on gene therapy is the ethical considerations. This issue is discussed in Section 11.6.

## 4.7 STEM CELLS AND CELL THERAPY

Stem cells are divided into three different categories: totipotent, pluripotent, and multipotent. A description of the genesis of stem cells is shown in Figure 4.12.

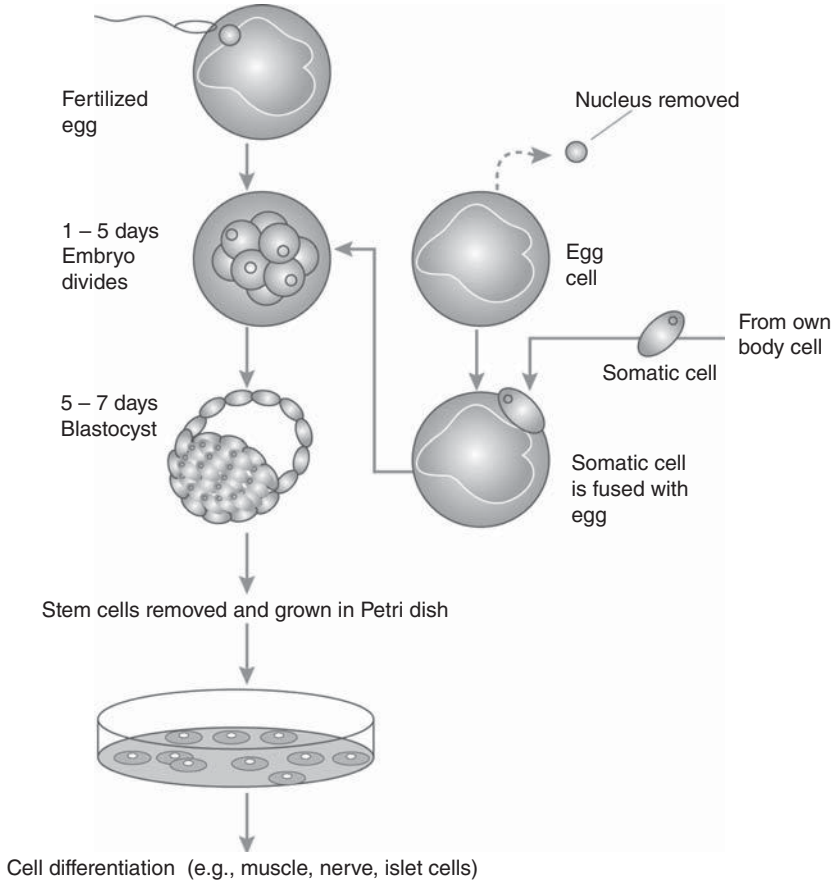



**Figure 4.12** Genesis of different stem cells.

Totipotent stem cells are obtained from embryos that are less than 5 days old. These cells have the full potential to develop into another individual and every cell type.

After about 5 days and several cycles of cell division, the totipotent cells form a hollow sphere of cells called a blastocyst. The blastocyst has an outer layer of cells surrounding clusters of cells. Those cells on the outside continue to divide and grow into the placenta and supporting tissues. The clusters of cells on the inside divide and form virtually all the cell types, except the placenta and supporting tissues, which form as a human being. These are the pluripotent stem cells, and they give rise to many different types of cells, but not a new individual.

Pluripotent cells continue to develop, differentiate, and specialize into different cells. They become the specialized stem cells, such as blood, skin, ansd nerve stem cells. These differentiated stem cells are multipotent, that is, they have the potential to produce specialized cells. For example, blood stem cells in bone marrow produce red blood cells, white blood cells, and platelets, but not other types of cells.

There are two general avenues for stem cell research: pluripotent and multipotent stem cells. Pluripotent stem cells are obtained by two methods. One method is to harvest the clusters of cells from the blastocysts of human embryos. Another method is the isolation of pluripotent cells from fetuses in terminated pregnancies. Multipotent stem cells are derived from umbilical cords or adult stem cells. However, because of the specialization of these cells, their potential to develop into a myriad of different cells is limited.

A burning issue is the ethics of obtaining pluripotent stem cells from embryos and fetuses. The US government has acted on this issue and declared that federal funds for stem cell research have to meet certain criteria. It stipulates that funding will be provided to research with only those stem cells that are obtained before August 9, 2001, as a cut-off date to limit research to preexisting stem cells. Other countries too have moratoria on the research of embryonic stem cells. Refer to Section 11.7 for an ethical debate on stem cells.

The potential contribution of stem cells to medical treatment lies in their capability to differentiate and grow into normal, healthy cells. Using pluripotent stem cells, scientists are devising means to culture them in the laboratories and coax them to grow into various specialized cells. Rather than gene therapy, with stem cells we have the potential of cell therapy to repair our diseased tissues and organs. This will circumvent the lack of donor organs. Stem cells also provide the possibility for healthy cells to cure disabilities such as strokes, Parkinson's disease, spinal injuries, and diabetes.

A drawback for stem cell therapy is the problem of cell rejection because of the host's immune system recognizing the cells as foreign. This rejection issue has to be overcome to ensure stem cell therapy is a viable treatment. A mix of antirejection drugs is often used to suppress rejection of the new stem cells.

Although research into stem cells is new, the use of stem cells for therapy has been with us for some time. Most of us are familiar with bone marrow transplant for patients with leukemia. This procedure involves finding a matching donor to harvest bone marrow stem cells and transfuse them to the patient with leukemia (refer Exhibit 4.17 for details).

Another cell therapy method includes the excision of cell from the body. These cells are then modified and returned to the host body. Provenge is an autologous (derived from the same individual) cellular immunotherapy for the treatment of metastatic prostate cancer. The technique for this therapy is presented in Exhibit 4.18.

## 4.8 CASE STUDY #4.1

### 4.8.1 Pneumococcal Vaccine and Meningococcal Vaccine

For children healthcare there are two important vaccines: pneumococcal vaccine and meningococcal vaccine. These vaccines help to prevent infections of pneumonia and meningitis to children.

#### *Pneumococcal Vaccine (Prevnar 13):*

Infection by the bacterium *Streptococcus pneumoniae* causes pneumococcal pneumonia. There are over 90 different strains of *S. pneumoniae*. These bacteria can infect the

**Exhibit 4.17 Bone Marrow Transplant**

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

Patients with leukemia have a condition in which the stem cells in the bone marrow malfunction and produce an excessive number of immature white blood cells, which interfere with normal blood cell production.

The aim of a bone marrow transplant is to replace the abnormal bone marrow stem cells with healthy stem cells from a donor. Healthy stem cells are normally harvested using a syringe to withdraw bone marrow from the rear hip bone of the donor. They are then infused into the patient via a catheter in the chest area. Before the infusion, the patient receives chemotherapy or radiotherapy to destroy the diseased bone marrow stem cells so that the infused stem cells have a chance to grow free of complications from diseased cells.

There are a number of terms used in the transplant procedure:

*Allogeneic transplant*: The person giving the bone marrow or stem cells is a genetically matched family member (usually a brother or sister).

*Unrelated allogeneic transplant*: The person donating marrow is unrelated to the patient.

*Syngeneic transplant*: The person donating the bone marrow or stem cell is an identical twin.

*Autologous transplant*: The patient donates his or her own bone marrow or stem cells before treatment, for reinfusion later. This happens when a patient is receiving radiotherapy or chemotherapy in such a high dose that the bone marrow is destroyed. The bone marrow stem cells collected previously are reinjected into the patient to reinforce the immune system.

*Source*: Data from National Institutes of Health 2014, *Bone Marrow Transplant*, viewed February 10, 2014, http://www.nlm.nih.gov/medlineplus/ency/article/003009.htm

sinuses and inner ear, as well as lungs, blood, and brain. Sometimes the infections can be fatal.

The vaccine, Prevnar 13, is a pneumococcal conjugate vaccine that contains 13 different strains of pneumococcal bacteria: serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. The polysaccharide sugars of the bacterial capsule are linked (conjugated) to a protein known as $CRM_{197}$, which is a recombinant variant of diphtheria toxin (*Corynebacterium diphtheriae*).

**Exhibit 4.18 Cell Therapy – Provenge**

Provenge is a cancer vaccine using cell therapy technique. Dendritic cells are removed from patients. These cells are treated with the prostate-specific antigen prostatic acid phosphatase (PAP), which is present in 95% of prostate cancer cases. The activated dendritic cells are returned to the patients and they stimulate the T cells to destroy cancer cells expressing the PAP, thus treating the tumor.

Provenge is the first immunotherapy approved by FDA in 2010 for the treatment of advanced prostate cancer.

*Source*: Data from 1. Jones, D 2007, 'Cancer vaccines on the horizon', *Nature Reviews Drug Discovery*, 6, pp. 333–334; 2. Dandreon 2013, Provenge, viewed February 10, 2014, http://www.dendreon.com/products/provenge/

FDA has approved Prevnar 13 as a pneumococcal vaccine for infants and children from 6 weeks through 17 years, and adults 50 and older. It is also approved for prevention of otitis media and invasive pneumococcal disease, including bacterial meningitis and bacteremia. Prevnar 13 is available in single-dose, prefilled syringes and is given to infants in a four-dose schedule at 2, 4, 6, and 12–15 months of age.

### *Meningococcal Vaccines (Nimenrix and Bexsero):*

Meningococcal diseases – meningitis and meningococcemia – are caused by the bacterium *Neisseria meningitides*. Meningitis infection occurs when the bacterium invades the cerebrospinal fluid; it causes inflammation and irritation to the menninges (the membranes surrounding the brain and spinal cord). The bacterium also causes blood infection (meningococcemia) and can result in whole-body inflammation. Both meningitis and meningococcemia can develop into serious and even fatal diseases.

Antibiotics are used to treat meningococcal diseases, but the most important method is prevention by immunization with a vaccine against *N. meningitides*. There are five strains (serotypes): A, B, C, Y, and W135 that are responsible for almost all the meningococcal diseases in humans.

Nimenrix is a tetravalent conjugate vaccine approved by EMA in 2012 against *N. meningitides* serotypes A, C, Y, and W135. It contains small amounts of polysaccharides from the four serotypes and conjugated to a carrier protein of attenuated tetanus toxoid. It is indicated for active immunization of individuals from the age of 12 months and above.

Vaccines against meningococcal serotype B proved elusive. Polysaccharide and conjugate vaccines are not effective against serotype B infection. The problem was overcome by the development of Bexsero, which adopted an approach called reverse

vaccinology. Reverse vaccinology uses the genome of the pathogen to identify all potential candidate antigens. It then uses a process of elimination to find those that would be successful in a vaccine. Bexsero was approved in 2013 by EMA; it is a vaccine for use in all age groups from 2 months of age and older.

The approval of Bexsero in the United States was still pending in mid-2014. In late 2013, there was an outbreak of meningitis serotype B at a Princeton University campus. To address the urgent public health need, the Centers for Disease Control and Prevention (CDC) worked with FDA to allow the importation of as yet unapproved Bexsero into the United States under the Expanded Access Program.

In June 2014, Pfizer submitted a Biologics License Application to FDA for a bivalent recombinant LP2086 (rLP2086) vaccine against *N. meningitides* serotype B.

*Source*: 1. Food and Drug Administration 2014, *Prevnar 13*, viewed Feb 8, 2014, http://www.fda.gov/biologicsbloodvaccines/vaccines/approvedproducts/ucm201667.htm; 2. European Medicines Agency 2014, *Nimenrix*: *EPAR – Summary for the public*, viewed Feb 8, 2014, http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/002226/human_med_001548.jsp&mid=WC0b01ac058001d124; 3. European Medicines Agency 2012, *Bexsero – Meningococcal group B Vaccine*, viewed Feb 8, 2014, http://www.ema.europa.eu/docs/en_GB/document_library/Summary_of_opinion_-_Initial_authorisation/human/002333/WC500134836.pdf; 4. Novartis 2014, *Bexsero, Meningococcal Group B Vaccine*, viewed Feb 8, 2014, http://bexsero.co.uk/healthcare-professional/index.htm; 5. Food and Drug Administration 2014, FDAVoice, *FDA is Working Closely with Manufacturers of Meningitis B vaccines*, viewed June 19, 2014, http://blogs.fda.gov/fdavoice/index.php/page/4/; 6. Pharmaceutical Processing 2014, *Pfizer Submits BLA for Investigational Meningococcal Vaccine*, viewed June 19, 2014, http://www.pharmpro.com/news/2014/06/pfizer-submits-bla-investigational-meningococcal-vaccine?et_cid=3999881&et_rid=151674301&location=top.

## 4.9 CASE STUDY #4.2

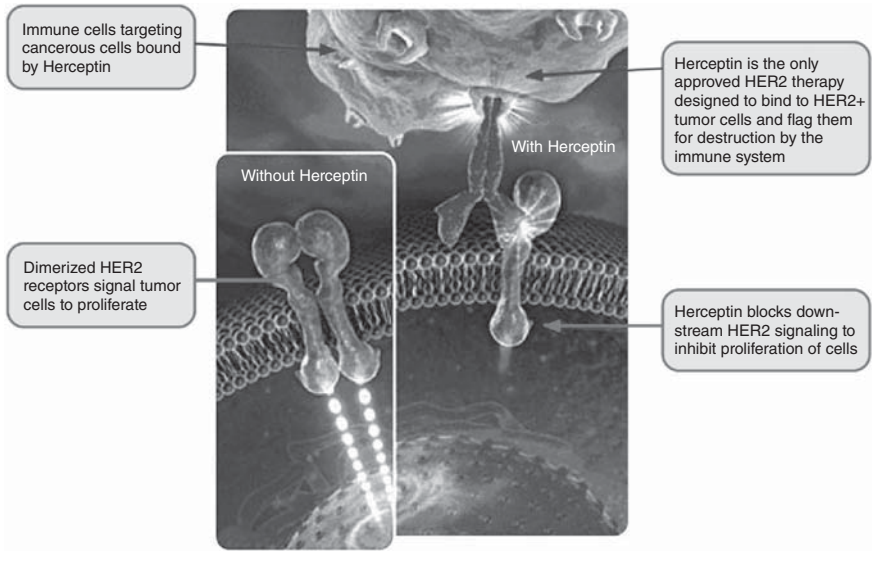
### 4.9.1 Herceptin and Tykerb

Studies found that in about 25–30% of early stage cancer, the cancer cells overexpress the HER2/neu receptors due to HER2/neu gene amplification. The name HER2/neu is derived from its structural similarity to human epidermal growth factor, HER1, and neu is a derivative of the oncogene from a neuoglioblastoma cell line.

#### *Trastuzumab (Herceptin):*

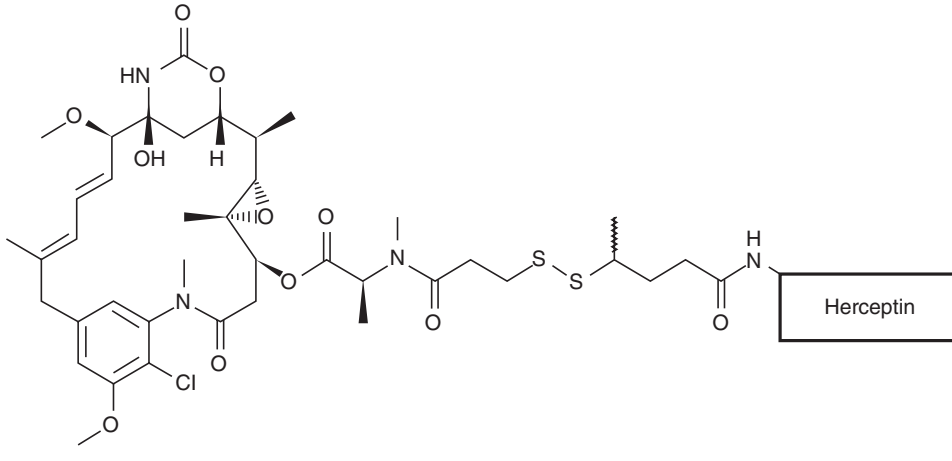
Herceptin is a humanized MAb that targets the HER2/neu receptor. It is an $IgG_1$ kappa antibody with a human framework and murine complementarity-determining regions (4D5) that bind to HER2/neu growth factor receptor. Women are selected for Herceptin treatment on the basis of immunohistochemistry (IHC) and fluorescence *in situ* hybridization (FISH) diagnostic tests that check for HER2 overexpression and gene amplification, respectively. Herceptin is supplied in sterile lyophilized form with 440 mg Trastuzumab, 400 mg $\alpha$, $\alpha$-trehalose dehydrate, 9.9 mg L-histidine HCl, 6.4 mg L-histidine, and 1.8 mg polysorbate 20.

Herceptin attaches to the HER2/neu receptor and activates the complement system (a series of serum and cell-associated proteins involved in immune response) to destroy those cells expressing such receptors. Through this action Herceptin disrupts the signaling pathway for breast cancer cell proliferation; refer to diagram below:




*Source*: *Genentech, Herceptin (trastuzumab)*, viewed February 8, 2014, http://www.gene.com/gene/products/information/oncology/herceptin/factsheet.html.

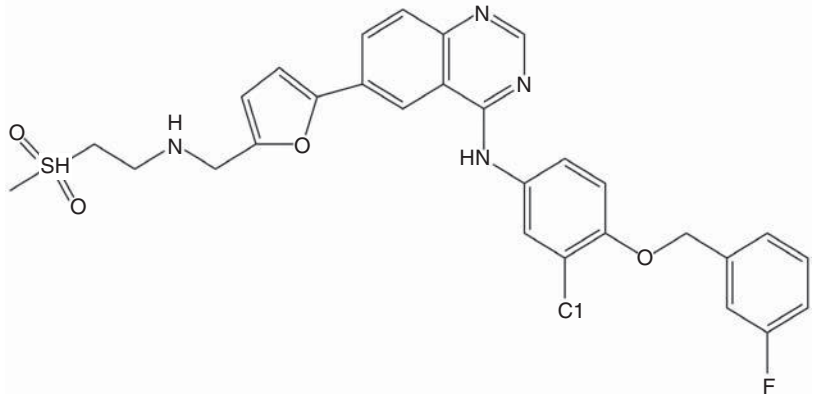
To improve the effectiveness of Herceptin, trastuzumab emtansine (Kadcyla) was developed. Kadcyla is a conjugate antibody consisting of trastuzumab (Herceptin) linked to a cytotoxic agent called mertansine as shown below. While Herceptin binds to the HER/2/neu receptor, mertansine enters the cancer cells and destroys them by binding to the tubulin. In this way only the cancer cells are targeted and destroyed.




### *Lapatinib (Tykerb):*

Tykerb is a small molecule drug that acts as a kinase inhibitor. It has been approved in 2007 by FDA as a combination therapy with capectabine (Xeloda) for the treatment of

advanced HER-2 positive breast cancer. The chemical name of the active ingredient is N-(3-chloro-4-{[(3-fluorophenyl)methyl]oxy}phenyl)-6-[5-({[2-(methylsulfonyl)ethyl] amino}methyl)-2-furanyl]-4-quinazolinamine-bis(4-methylbezenesulfonate)monohydr ate, $C_{29}H_{26}ClFN_4O_4S(C_7H_8O_3S)_2H_2O$, with a molecular weight of 943.5 and a chemical structure as shown below:




It is a yellow solid with a solubility in water of 0.007 mg/mL. The Tykerb tablet contains 405 mg of the active ingredient, and the inactives are magnesium stearate, microcrystalline cellulose, povidone, and sodium starch glycolate.

Tykerb attaches to the HER-1 and HER-2 receptors and blocks the tyrosine kinase reactions, hence turning off the growth of breast cancer cells. By widening the targets to beyond HER-2, which Herceptin attaches, Tykerb may help in those cases where Herceptin has failed. It is postulated that multikinase inhibitors such as Tykerb may be able to interfere with more biochemical signaling pathways to block the functions of the HER family type of receptors. Furthermore, Tykerb may also help in cases where the metastases had spread from the breast to the brain, and Herceptin may not be able to cross the blood–brain barrier (refer to Exhibit 5.4) with its large molecular size.

*Source*: 1. Jarvis, LM 2007, Battling Breast Cancer, *Chemical & Engineering News*, 84, pp. 21–27; 2. Food and Drug Administration 2010, *Tykerb (lapatinib) tablets*, viewed Feb 8, 2014, http://www.fda.gov/safety/medwatch/safetyinformation/ucm200945.htm; 3. Food and Drug Administration 2013, *Kadcyla*, viewed Feb 8, 2014, http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/125427lbl.pdf; 4. Moy, B. et al. 2007, 'Lapatinib', *Nature Reviews Drug Discovery*, 6, pp. 431 – 432.

## 4.10 SUMMARY OF IMPORTANT POINTS

1. Biopharmaceuticals are mainly protein-based molecules, which are copies of natural biological compounds. The aim is to provide these biopharmaceuticals to modify or alter the undesirable biological responses in our body in the disease states. Biopharmaceuticals have molecular weights of tens of thousands of Daltons, unlike the small molecule drugs of mostly less than 500 Da.

2. Vaccines are derived from the entire or fragments of pathogens, either through inactivation or attenuation of the pathogen or by generating the requisite molecules using recombinant technology. Adjuvants are added to enhance the efficacy of the vaccines.
3. Antibodies are proteins that mimic the natural antibodies or especially designed to interact with endogenous or exogenous protein molecules. They are "Y"-shaped molecules with the tips of two arms binding to antigens while the stem part is used to elicit the immune response to destroy the antigens. The variability of the tips means that they can bind to many different antigens. Parts of the tip can be replaced by toxins or radioactive elements to help destroy the antigens.
4. Cytokines such as interferons are for the treatment of hepatitis, cancer, and lymphoma, interleukins for enhancing immune response, and growth factors for the treatment of anemia and regulation of tumor angiogenesis.
5. Hormones in the forms of insulin are to treat diabetes and that of growth hormone for the promotion of bone and tissue growth.
6. Gene therapy is a technique to deliver genes into the body to replace faulty genes or insert new genes if they are missing in the body. To date, FDA has not approved any gene therapy product.
7. Stem cells and cell therapy is the use of pluripotent and multipotent cells to generate healthy cells and tissues to replace the faulty ones in disease conditions. The main ethical questions are the source of the cells and the possibility of working, leading to the cloning of humans.

## 4.11 REVIEW QUESTIONS

1. Describe the different types of vaccines: give examples of those produced by traditional methods and current techniques. Explain how the use of adjuvants can help to improve the efficacy of vaccines.
2. Discuss the structure and naming convention for the influenza virus. Provide reasons for the variations in the yearly compositions of the influenza vaccines.
3. What are the characteristics that make the influenza virus, for example, the avian influenza, a potential pandemic agent?
4. Compare and contrast the different types of antibody immunoglobulins. Provide a detailed description of the structure of the IgG antibody with particular reference to how it binds to antigens.
5. Explain why the humanization of antibodies is important, and, through the use of examples, demonstrate the progress made to the modification of antibodies as technology advances.
6. Provide examples for conjugating the antibodies with toxins and radioactive elements.
7. Describe how the human immune system works, focusing on the B and T cells.
8. What is the mechanism of action for drugs that target growth factors? Describe the mechanism of action for some of the latest drugs, such as Avastin.

9. Distinguish the technologies based on gene therapy and cell therapy. Describe the use of vectors for gene delivery. Describe how cell rejection can be overcome in cell therapy.
10. Demonstrate by citing examples the current treatment of breast cancer using small and large molecule drugs.

## 4.12 BRIEF ANSWERS AND EXPLANATIONS

1. Refer to Sections 4.2.1 and 4.2.2 for traditional and new vaccines. The mechanisms of vaccine efficacy enhancements are explained in Section 4.2.3.
2. Explain the roles of hemagglutinin and neuraminidase (Exhibits 3.7 and Exhibit 4.2), followed by the nomenclature for classifying influenza virus and the procedure that FDA and WHO recommend for the preparation of multivalent vaccines.
3. Avian influenza is extremely deadly, with 60% fatalities of infected human cases to date. The virus may become even more deadly through the process of reassortment and gradual adaptive mutation.
4. Refer to Section 4.3 and Figure 4.1 to compare and contrast the structures of IgG, IgD, IgE, IgA, and IgM. Explain the heavy and light chain structure of IgG and the complementarity-determining regions that bind to antigens.
5. The problem is the neutralizing or allergic reactions caused by the production of human antimouse antibodies as the body treats the MAbs as foreign (refer to Section 4.3.4). The humanization of antibodies, through chimeric to humanized and full human types, helps to address this problem.
6. Refer to Section 4.3.5.
7. Refer to Exhibit 4.7.
8. Section 4.4.2 shows the actions for EPO, CSFs, and VEGFs. The antiangiogenesis mechanism of Avastin is explained in Exhibit 4.13.
9. Refer to Sections 4.6 and 4.7. The vectors for gene therapy are tabulated in Table 4.5. In cell therapy antirejection drugs are used to suppress the rejection of transplanted cells.
10. Herceptin and Tykerb are examples of large and small molecule drugs prescribed for the treatment of breast cancer. Refer to Section 4.9 for details.

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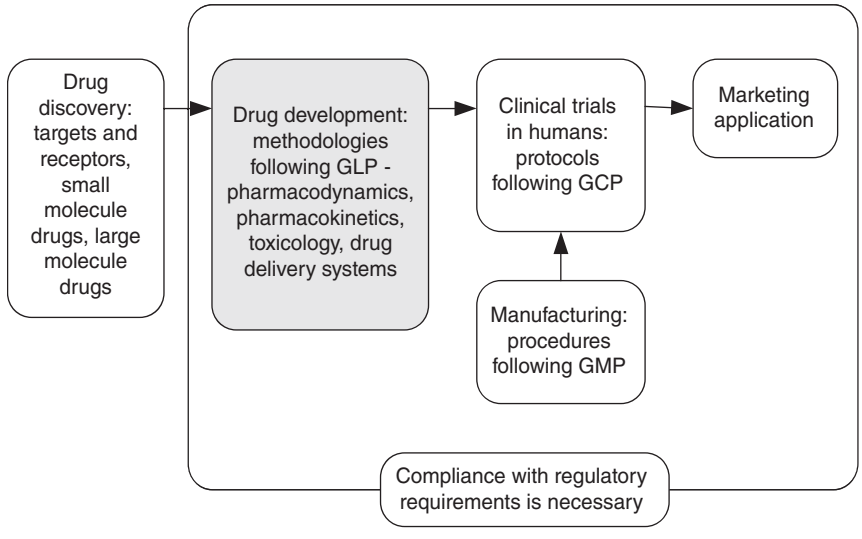
# CHAPTER 5

# DRUG DEVELOPMENT AND PRECLINICAL STUDIES




## 5.1 INTRODUCTION

In earlier chapters, we discussed the discovery of new drugs. After a lead compound has been identified, it is subjected to a development process to optimize its properties. The development process includes pharmacological studies of the lead compound and

*Drugs: From Discovery to Approval*, Third Edition. Rick Ng.

its effects on toxicity, carcinogenicity, mutagenicity, and reproductive development. These data are important for determining the safety and effectiveness of the lead compound as a potential drug.

An ideal drug is potent, efficacious, and specific, that is, it must have strong effects on a specific targeted biochemical pathway and minimal effects on all other pathways, to reduce side effects. In reality, no drugs are perfectly effective and absolutely safe. The aim of pharmacological studies is to obtain data on the safety and effectiveness of the lead compound. Many iterations of optimization of the lead compound may be necessary to yield a potential drug candidate for clinical trial.

The potency, efficacy, and safety of a drug depend on the chemical and structural specificity of drug–target interaction. In pharmacology, we are concerned with pharmacodynamics (PD), pharmacokinetics (PK), and toxicity. In simplified terms, PD deals with the actions of the drug on the target, whereas PK is about the actions of the body on the drug. Toxicity information in preclinical (also called nonclinical) studies provides us with confidence in the safety aspect of the potential drug. These data for PD, PK, and toxicity enable the dose and dosing regimen to be set for the clinical trials.

Although pharmaceutical firms are increasingly using *in vitro* methods to evaluate pharmacological responses, some aspects of pharmacological developments have no alternatives but to use *in vivo* tests in animals to study the effects of a potential drug in living systems. However, any study in support of preclinical safety and toxicity for a regulatory submission, including pharmacological and toxicity studies using animals, is regulated under Good Laboratory Practice (GLP) with strict guidelines, requiring scientists to follow established protocols. Readers are referred to FDA 21 CFR Part 58 *Good Laboratory Practice for Nonclinical Laboratory Studies* (*2013*). This regulation details the requirements for the conduct of nonclinical laboratory studies intended to support applications for clinical trials and marketing approvals (Investigational New Drug [IND], New Drug Application [NDA], and Biologics Licensing Application [BLA]; refer to Chapter 8) in the United States. It is important to point out that GLP is not designed to dictate the science to be used for the preclinical studies, but to ensure and to protect the integrity of the study so that the results are reliable. The contents list for this guideline is presented in Exhibit 5.1.

Examples of some of these requirements are:

- Personnel must have the education, training, and experience to conduct the nonclinical studies
- A quality assurance unit should be set up
- Materials for the studies must be appropriately tested for identity, strength, purity, stability, and uniformity
- Appropriate personnel, resources, facilities, equipment, materials, and methodologies must be available
- The studies must be conducted under specifically designed protocols with an appropriate quality system established to handle data, deviations, and reporting
- Animals must be isolated, their health status checked, and they must be given the appropriate welfare

- Nonclinical laboratory studies must be conducted in accordance with the protocols.

**Exhibit 5.1 FDA 21 CFR Part 58 *Good Laboratory Practice for Nonclinical Laboratory Studies*: Table of Contents**

Retention of records
Disqualification of Testing Facilities
Purpose
Grounds for disqualification
Notice of and opportunity for hearing on proposed disqualification
Final order on disqualification
Actions on disqualification
Public disclosure of information regarding disqualification
Alternative or additional actions to disqualification
Suspension or termination of a testing facility by a sponsor
Reinstatement of a disqualified testing facility

*Source:* Food and Drug Administration 2013, *CFR – Code of Federal Regulations Title 21 Part 58 Good Laboratory Practice for Nonclinical Laboratory Studies*, viewed February 25, 2014, http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?cfrpart=58&showfr=1

Drug development also extends to formulation and delivery. Most drugs that are administered to patients contain more than just the active pharmaceutical ingredients (API, the drug molecules that interact with the receptors or enzymes). Other chemical components are often added to improve manufacturing processing or to enhance stability and bioavailability of drugs, such as the use of adjuvants for vaccines. Effective delivery of drugs to target sites is an important factor to optimize efficacy and reduce side effects. The development process also includes the design and development of new manufacturing and testing methodologies for cost-effective production of drugs in compliance with regulatory requirements. Drugs are manufactured under Good Manufacturing Practice, which is discussed in Chapters 9 and 10.

## 5.2 PHARMACODYNAMICS

The chemical and structural aspects of pharmacodynamics PD are discussed in Chapters 2, 3, and 4, where we considered drug–target interactions. When a drug binds to a target, it may regulate the receptor as an agonist or antagonist, or act as an inducer or inhibitor in the case of an enzyme. The lock and key chemical and structural interaction is a priori to achieving a potent and safe drug. In this chapter, we focus on the quantitative mathematical relationships of drug–target interactions, in addition to the chemical and structural aspects covered in Chapters 2, 3 and 4.

PD is the study to determine dose–response effects. We are interested in finding out the effects of a drug on some particular response, such as heart rate, enzyme levels, antibodies production, or muscle relaxation or contraction. When a drug binds to a receptor, the ensuing response is complex. The following example is an idealized case,

which illustrates the drug–receptor interaction:


where D is the drug, R is the receptor, and D*R is the drug–receptor complex.

The response may be local or via a signal transduction process. The rate for the forward reaction of drug binding to receptor is proportional to the concentrations of both the drug and the receptor. Conversely, the rate for the reverse reaction, that is, dissociation of the drug–receptor complex, is proportional to the concentration of the drug–receptor complex. At equilibrium, both forward and reverse reactions are equal. Mathematically, we have:

$$k_1[\mathrm{D}][\mathrm{R}] = k_{-1}[\mathrm{D} * \mathrm{R}] \tag{5.1}$$

where $k_1$ is the forward reaction rate constant, $k_{-1}$ is the reverse reaction rate constant, and [D], [R], and [D*R] are the concentrations of the drug, receptor, and drug–receptor complex, respectively.

Rearranging Equation (5.1), we obtain:

$$\frac{[\mathrm{D}][\mathrm{R}]}{[\mathrm{D} * \mathrm{R}]} = \frac{k_{-1}}{k_1} = K_\mathrm{D} \tag{5.2}$$

where $K_\mathrm{D}$ is the equilibrium dissociation constant.

When half the receptors are bound, we have [R] = [D*R]. Substituting into Equation (5.2), $K_\mathrm{D}$ is equal to [D]. This means that $K_\mathrm{D}$ is the concentration of the drug that, at equilibrium, will bind to half the number of receptors.

If we consider all the available receptors as 100% and [D*R] are the occupied receptors with drug at the binding sites, then [R] – which is the percentage of free, unoccupied receptors – can be substituted with 100 – [D*R]. Equation (5.2) can be rewritten as:

$$[\mathrm{D}] = \frac{K_\mathrm{D}[\mathrm{D} * \mathrm{R}]}{100 - [\mathrm{D} * \mathrm{R}]} \tag{5.3}$$

Equation (5.3) is a hyperbolic function showing the relationship between dose of drug, [D], and its effects resulting from drug–receptor interaction, [D*R]. A graphical representation of this dose–effect, or dose–response curve, is shown in Figure 5.1. The graph shows that, at low doses, the effects are approximately linear in proportion to the doses. However, as the dose increases, there is gradually a diminishing return in effects. A maximum is reached and at this point all available receptors are bound with drug molecules. Further increase in dose does not generate any increase in effects. The point $E_{max}$ is the maximum effect, and $EC_{50}$ is the concentration of the drug that produces 50% of the maximum effect.

Very often, the dose–effect curve is redrawn using a logarithmic scale for the dose. This gives rise to a sigmoid curve, as shown in Figure 5.2. It is a mathematical transformation, which shows an approximate linear portion for the 20–80% maximal effect scale, which is usually the dose level for a therapeutic drug. Doses above 80% provide very little increase in therapeutic effects though with a concomitant rise in the risk of adverse reactions.

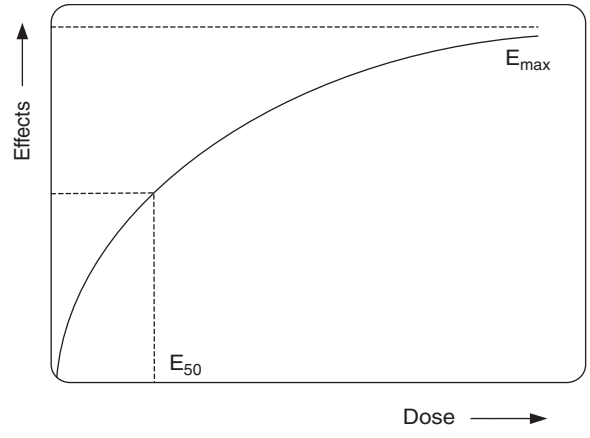



**Figure 5.1** Dose–effect curve.

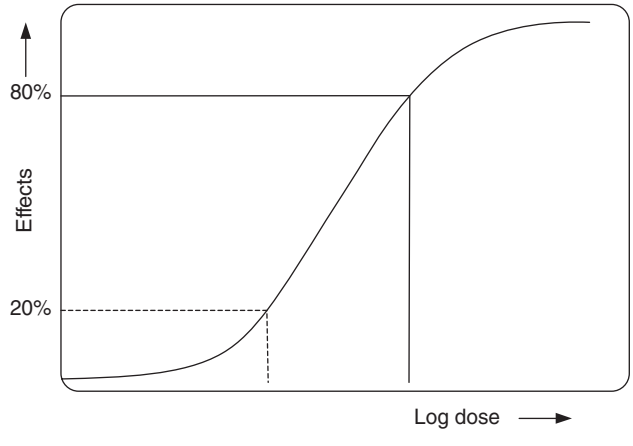



**Figure 5.2** Dose–effect curve with logarithmic scale for dose.

Scientists also study the potency, effectiveness, safety margin, and therapeutic index of a drug. These terms are described below with reference to Figures 5.3 and 5.4.

*Potency*: This is the dose required to generate an effect. A potent drug elicits an effect at a low dose.

*Effectiveness*: This is the intensity of the effect or response. It is a measure of the affinity of the drug for the receptor. An effective drug is one that can achieve effects in the vicinity of $E_{max}$.

*Therapeutic Index*: The index is given by the ratio:

$$\frac{LD_{50}}{ED_{50}} \tag{5.4}$$

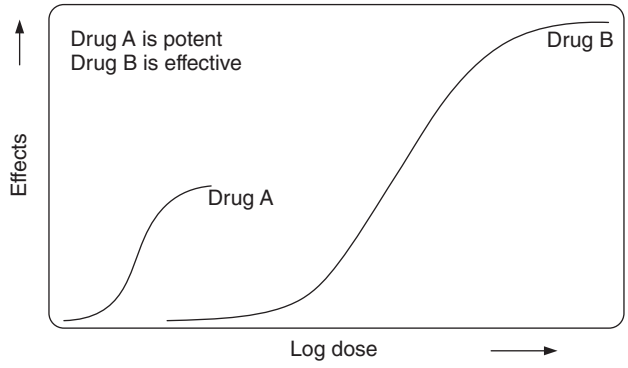



**Figure 5.3** Potency and effectiveness.

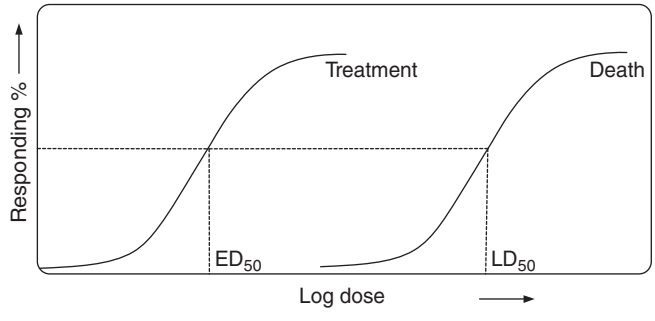



**Figure 5.4** Therapeutic index.

where $LD_{50}$ is the lethal dose for 50% of the population, and $ED_{50}$ is the effective dose for 50% of the population.

The lower the $ED_{50}$ compared to the $LD_{50}$ the higher the therapeutic index of the drug.

*Safety Margin*: This is the separation of two doses: one that produces therapeutic effects and one that elicits adverse reaction. The standard safety margin (SSM) is given by:

$$\text{SSM} = \frac{\text{LD}_1 - \text{ED}_{99}}{\text{ED}_{99}} \times 100 \tag{5.5}$$

where $LD_1$ is the lethal dose for 1% of the population, and $ED_{99}$ is the effective dose for 99% of the population.

A large safety margin is achieved when there is a significant difference between the $ED_{99}$ and $LD_1$ doses.

## 5.3 PHARMACOKINETICS

For a drug to interact with a target, it has to be present in sufficient concentration in the fluid medium surrounding the cells with receptors. Pharmacokinetics (PK) is the study of the kinetics of absorption, distribution, metabolism, and excretion (ADME) of drugs. It analyzes the way the human body deals with a drug after it has been administered and the transportation of the drug to the specific site for drug–receptor interaction. For example, a person has a headache and takes an aspirin to abate the pain. How does the aspirin travel from our mouth to reach the site in the brain where the headache is and act to reduce the pain?

There are several ways to administer a drug. They are as follows:

- Intravenous
- Oral
- Buccal
- Sublingual
- Rectal
- Subcutaneous
- Intramuscular
- Transdermal
- Topical
- Inhalational.

With the exception of intravenous administration, where a drug is injected directly into the bloodstream, all the routes of administration require the drug to be absorbed before it can enter the bloodstream for distribution to target sites. Metabolism may precede distribution to the site of action, for example, in the case of oral administration. The human body also has a clearance process to eliminate drugs through excretion. We consider absorption, distribution, metabolism, and excretion in the sections below, with reference to Figure 5.5.

### 5.3.1 Transport Mechanism

Except for intravenous injection, drug molecules have to cross cell membranes to reach target sites. There are four basic transport mechanisms:

- Passive diffusion
- Facilitated diffusion
- Active transport
- Pinocytosis.

***Passive Diffusion:*** Diffusion is the random movement of molecules in fluid. If a fluid is separated by a semipermeable membrane, more dissolved molecules will diffuse across the membrane from the higher concentration side to the lower concentration side

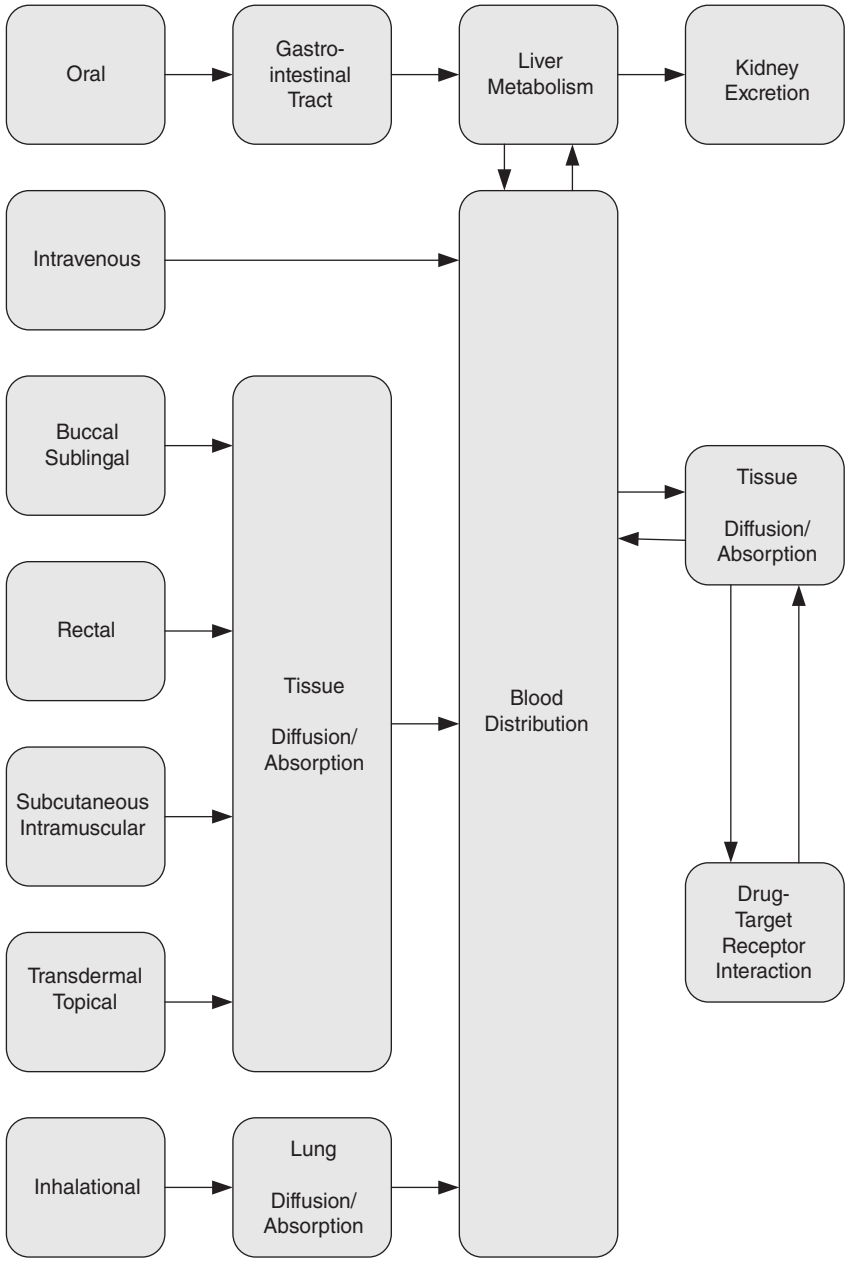



**Figure 5.5** Schematic representation of drug absorption, distribution, metabolism, and excretion.

than in the reverse direction. This process will continue until equilibrium is achieved, whereby both sides have the same concentration. When equilibrium is reached, there are equal numbers of molecules crossing the membrane in both directions.

Drug molecules are transported across cell membranes. Because of the lipid bilayer construction of the membrane (Appendix 2), nonpolar (lipid-soluble) molecules are able to diffuse and penetrate the cell membrane. Polar molecules, however, cannot penetrate the cell membrane readily via passive diffusion and rely on other transport mechanisms.

Lipid solubility determines the readiness of drug molecules to cross the gastrointestinal tract, blood–brain barrier, and other tissues. Molecular size is another factor that determines the diffusion of drugs across the membrane, with the smaller molecules able to diffuse more readily. Exhibit 5.2 describes the kinetics for the diffusion of drug molecules across the cell membrane.

***Facilitated Diffusion:*** Polar drug molecules have been observed to cross cell membranes. The transport mechanism is via carrier systems. Transmembrane carriers, such as proteins, are similar to receptors and bind to polar and nonpolar drug molecules. They facilitate the diffusion of drugs across the cell membrane. The facilitated diffusion rate is faster than passive diffusion and may be controlled by enzymes or hormones. Facilitated diffusion is from a region of high concentration to low concentration. However, these carriers, or transporters, may become saturated at high-drug concentration. In this case, the transportation rate plateaus until the carriers are cleared of the drugs in preparation for another cycle of transportation.

***Active Transport:*** The active transport mechanism requires energy to drive the transportation of drugs against the concentration gradient, from low to high. The transportation rate is dependent on the availability of carriers and energy supply via a number of biological pathways.

***Pinocytosis:*** Pinocytosis involves the engulfing of fluids by a cell. The process commences with the infolding of cell membrane around fluids containing the drug. The membrane then fuses and forms a vesicle with fluid core. In this way, the drug is taken into the cell interior within the vesicle.

### 5.3.2 Absorption

***Oral Administration:*** The oral route is the most common way of administering a drug. For a drug to be absorbed into the bloodstream, it has to be soluble in the fluids of our gastrointestinal tract. Drugs are often formulated with excipients (components other than the active drug) to improve manufacturing and dissolution processes (refer to Section 5.6).

Our gastrointestinal tract is lined with epithelial cells, and drugs have to cross the cell membrane (refer to Exhibit 5.2). In the stomach, where pH is low, drugs that are weak acids are absorbed faster. In the intestine, where pH is high, weak basic drugs are absorbed preferentially. Figure 5.6 shows the absorption of drugs under different pH environment.

**Exhibit 5.2 Diffusion of Drugs**

Most drugs are weak acids or bases. Under different pH conditions, they become ionized and cannot diffuse through the cell membrane. This ionization process is illustrated below:

Weak Acid: $AH \leftrightarrow A^- + H^+$

$$pK_a = pH + \log_{10}\frac{[AH]}{[A^-]} \tag{5.6}$$

Weak Base: $BH^+ \leftrightarrow B + H^+$

$$pK_a = pH + \log_{10}\frac{[BH^+]}{[B]} \tag{5.7}$$

AH and B are the unionized acid and base, respectively, and $A^-$ and $BH^+$ are the ionized forms. The lipid solubility of AH and B are dependent on the chemical structure of the drugs. In most instances, they are of sufficient solubility to diffuse across the cell membranes. However, as the equations show, the pH environment affects the ionization of a drug. We illustrate this with aspirin (a weak acid drug, $pK_a = 3.5$) as an example and apply Equation (5.6):

| Blood: high pH (7.4) environment | Stomach: low pH (3.0) environment |
|---|---|
| $3.5 = 7.4 + \log_{10}\frac{[AH]}{[A^-]}$ | $3.5 = 3.0 + \log_{10}\frac{[AH]}{[A^-]}$ |
| $\log_{10}\frac{[AH]}{[A^-]} = -3.9$ | $\log_{10}\frac{[AH]}{[A^-]} = 0.5$ |
| $\frac{[AH]}{[A^-]} = 0.00126$ | $\frac{[AH]}{[A^-]} = 3.16$ |
| The ionized form is dominant. Therefore, less lipid-soluble. | There is more of the unionized form. Therefore, more lipid-soluble. |

A similar method is used to calculate the unionized to ionized forms for basic drugs using Equation (5.7).

*Source*: Data from Rang, HP, Dale, MM, Ritter, JM and Gardner, R 1995, *Pharmacology*, 3rd edn., Churchill Livingstone, New York, p. 70.

In reality, there is more than just passive diffusion at work for drugs to traverse the cell membrane. Most drugs are absorbed in the intestine. Often, if an oral drug is taken and a fast response is desired, the drug is taken on an empty stomach to ensure a quick passage through the stomach for absorption in the intestine to take place.

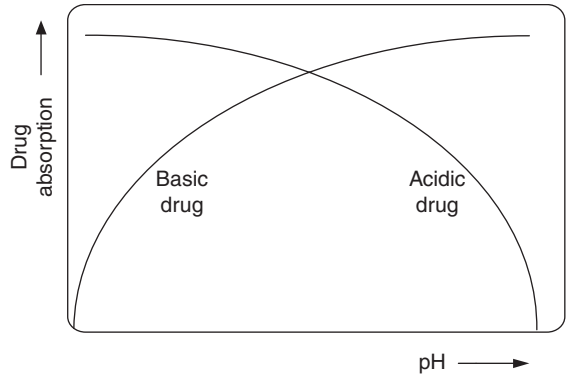



**Figure 5.6** Absorption of drugs in different pH environments.

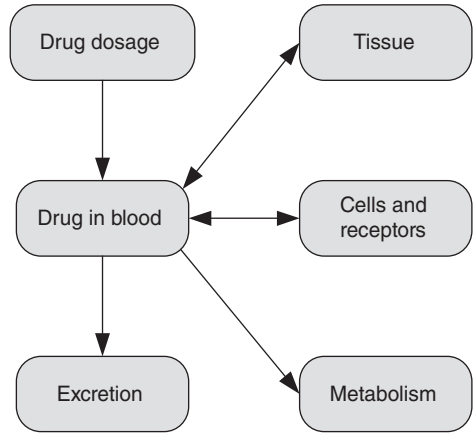



**Figure 5.7** Process of drug in the body.

Drugs absorbed through the gastrointestinal tract pass into the hepatic portal vein, which drains into the liver. The liver metabolizes the drug, which leads to reduction in the availability of the drug for interaction with receptors. This is called first pass metabolism. A schematic representation of the process of drug in the body is given in Figure 5.7.

A plot of the drug concentration in the bloodstream over time for a single dose is shown in Figure 5.8. At a certain time after administration, the rate of drug absorption equals the rate of clearance. This is an equilibrium condition called "steady state."

The area under the curve (AUC) represents the total amount of drug in the blood. It is a measurement of the bioavailability of the drug. Comparison of drug concentrations in the bloodstream administered via intravenous injection and oral route provides information for the bioavailability of the oral drug. This is because the oral drug is metabolized in the liver before reaching the general blood circulation (refer to Section 5.3.4), whereas in intravenous injection the total amount of drug is injected

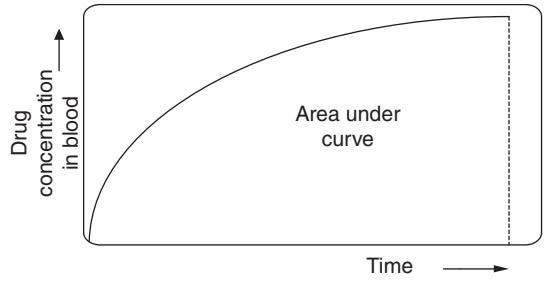



**Figure 5.8** Drug concentration in bloodstream versus time for a single dose.

directly into the bloodstream. In general, oral doses are higher than intravenous doses to take into account the effects of first pass metabolism.

***Buccal and Sublingual Administration:*** Drugs can be absorbed through the oral cavity. Buccal (between the gums and cheek) and sublingual (underneath the tongue) can be effective means of drug administration. In both cases, drugs can enter the blood circulation without first pass metabolism in the liver.

***Rectal Administration:*** Rectal administration of drugs may be applied when the patient is unable to take drug orally and some other routes are impractical. The drug administered via the rectum is absorbed and partially bypasses the liver. However, the absorption of drugs may be unreliable in certain cases.

***Subcutaneous and Intramuscular Administration:*** Subcutaneous and intramuscular administration can be used to deliver protein-based drugs. The absorption of drug is faster than with the oral route. The rate of absorption is determined by the blood flow pattern and diffusion of drug molecules in tissues.

***Transdermal and Topical Administration:*** Transdermal administration is to apply the drug on the skin surface. The drug is absorbed and transported by blood to receptors, which may be remote from the part of the skin where the transdermal patch is. The first pass metabolism is circumvented. Topical administration is to apply the drug for local effects. The typical areas for topical applications are the skin, eyes, throat, nose, and vagina.

***Inhalation Administration:*** Aerosol particles of drug can be inhaled into the lungs. Because of the large surface area of the alveoli, absorption is rapid and effective. As the lungs are richly supplied with capillaries, distribution of inhalational drugs is very quick (refer to Exhibit 4.15 on inhalable insulin).

***Intravenous Administration:*** When a drug is injected, the entire dose can be considered as being available in the bloodstream to be distributed to the target site. Hence, the dosage can be controlled, unlike with other routes of administration where

the bioavailability of the drug may be unpredictable because of diffusion and liver metabolic processes. Intravenous injection is the normal route for administration of protein-based drugs, as they are likely to be destroyed if taken orally because of the pH conditions in the gastrointestinal tract.

The onset of drug action with intravenous injection is quick, and this method is especially useful for emergency cases. However, intravenous injection is potentially the most dangerous. Once a drug is injected, there is no means to stop it from circulating throughout the body. The complete circulation of blood in the body takes about a minute, and hence an adverse reaction can occur almost instantaneously.

### 5.3.3 Distribution

When a drug is in the bloodstream, it is distributed to various tissues. The distribution pattern depends on a number of factors:

- Vascularity nature of the tissue
- Binding of the drug to protein molecules in blood plasma
- Diffusion of the drug.

When a tissue is perfused with blood supply, drug molecules in the blood are transported to the tissue rapidly until equilibrium is reached. On the contrary, the drug may bind to proteins in the blood such as albumin, rendering less of it available for distribution to tissues. In general, acid drugs bind to albumins and basic drugs to glycoproteins. The third factor for drug distribution is passive diffusion. Lipid-soluble drugs can cross the cell membrane more readily than polar drugs and move into the tissues to interact with receptors.

The volume of distribution (Vd) is an important parameter. It is represented by the following equation:

$$\mathrm{Vd} = \frac{\mathrm{Dose}}{\mathrm{Cb}} \tag{5.8}$$

where Vd is a hypothetical volume, and Cb is the concentration of drug in blood.

When Cb is low, Vd may turn out to be a large value, many times more than the volume of a person of around 60–70 L. Highly lipid-soluble drugs have a very high volume of distribution. Lipid insoluble drugs, which remain in the blood, have a low Vd.

For example, obesity affects Vd because lipid-soluble drugs diffuse into the adipose tissues of the obese person. Vd is a useful parameter for determining the loading dose for a drug to attain equilibrium after the drug is administered.

Distribution of drugs is restricted in two areas: the brain and the placenta. Refer to Exhibit 5.3 for a brief description on how drugs cross these barriers. Exhibit 5.4 presents a potential new method to deliver drugs across the blood–brain barrier.

### 5.3.4 Metabolism

Many drugs are metabolized in the body; their chemical structures are altered and pharmacological activity reduced. The liver is the major organ for metabolizing drugs; a secondary role is played by the kidneys. Some drugs are metabolized in tissue systems.

**Exhibit 5.3 Barriers to Drug Distribution**

**Blood–brain barrier (BBB)**

Distribution of drugs to the brain tissue is restricted in some types of drugs. The reason is that the brain has a sheath of connective tissue cells, the astrocytes, surrounding it, forming a barrier to passive diffusion for polar drugs. In addition, the endothelial cells of the brain capillaries are joined more tightly together, curtailing further the diffusion of polar drugs to the brain. Lipid-soluble drugs, however, can diffuse into the brain more readily and bring forth their effects.

For neuropharmaceuticals that target the brain, as in the case of neurodegenerative disorders (Alzheimer's, multiple sclerosis), psychiatric or psychotherapy, stroke, and infectious diseases, drug candidates are tested using *in vivo* and *in vitro* models to assess the transfer of the drug compound across the BBB.

**Placental barrier**

The placental barrier consists of several layers of cells between the maternal and fetal circulatory systems. Diffusion of polar drugs is limited. However, lipid-soluble drugs can pass through the barrier. Fetuses are rich in lipids and may form a reservoir for sequestering lipid-soluble drugs.

**Exhibit 5.4 Drug Delivery Across Blood–Brain Barrier**

Using knowledge that rabies virus can spread into the brain neurons, scientists mimic its delivery system. A short, 29 amino acids peptide chain is derived from the rabies virus glycoprotein (RVG). The RVG binds to the acetylcholine receptor on the neurons and the endothelium cells of the blood–brain barrier. Through this interaction, transvascular delivery is enabled.

The drug, in this case, a siRNA, was coupled to the RVG peptide and successfully delivered to the neurons in mice. This work opens up the possibility of using the RVG as delivery tools for drugs designed for interaction with brain neurons.

*Source*: Data from Kumar, P et al. 2007, 'Transvascular delivery of small interfering RNA to the central nervous system', *Nature*, 448, pp. 39–43.

Two types of biochemical metabolism reactions take place in the liver: Phase I and Phase II reactions. Phase I reactions are oxidation, reduction, and hydrolysis, which transform the drugs into metabolites. A family of enzymes called cytochrome P-450 (CYP) is responsible for these reactions. More than 50 CYP enzymes have been characterized, but only six are responsible for most of the drug metabolism. These are CYPIA2, CYP2C9, CYP2D6, CYP2A6, CYP2E1, and CYP3A4; they are found mainly

**Exhibit 5.5 Metabolism Studies**

The aim of metabolism studies is to (i) identify metabolic pathways and (ii) investigate the possibility of drug–drug interactions.

Pharmacogenetics influences the therapeutic effects of drugs. A drug that is normally metabolized by the P-450 2D6 enzyme will not be metabolized in about 7% of the Caucasian population. Coadministration of drugs may have different effects, which are either (i) additive, synergistic, or (ii) antagonistic.

Some drugs are administered in a prodrug form. They are metabolized, and the metabolites elicit the interactions with receptors.

P-450 enzymes have been cloned and *in vitro* studies can be performed using these enzyme systems. Metabolic pathways can be studied by incubating the drug with the P-450 enzymes. Similarly, drug–drug interactions can be studied.

*Source*: Data from Food and Drug Administration 2012, *Guidance for Industry: Drug Interaction Studies – Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations 2012*, viewed February 25, 2014, http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM292362.pdf

in the liver and convert lipid-soluble drugs to more water-soluble metabolites. Phase II reactions involve the addition or conjugation of subgroups, such as –OH, –NH, and –SH to the drug molecules. Enzymes other than P-450 are responsible for these reactions. These reactions give rise to more polar molecules, which are less lipid-soluble and are excreted from the body. Exhibit 5.5 describes some of the drug metabolism/drug interaction studies recommended by FDA.

### 5.3.5 Excretion

Drugs are excreted from the body by the following routes:

- Kidneys
- Lungs
- Intestine and colon
- Skin.

The kidneys are the most important organs for clearing drugs from the body. Water-soluble drugs are cleared more quickly than lipid-soluble drugs. Volatile and gaseous by-products of drugs are exhaled by the lungs. Some drugs are reabsorbed into the intestine and colon and later passed out as solid wastes. Another mechanism of clearance is for drugs to be excreted through the skin as perspiration. In terms of chemical reactions, drug elimination involves a number of processes such as conjugation, hydrolysis, oxidation, reduction, and proteolysis.

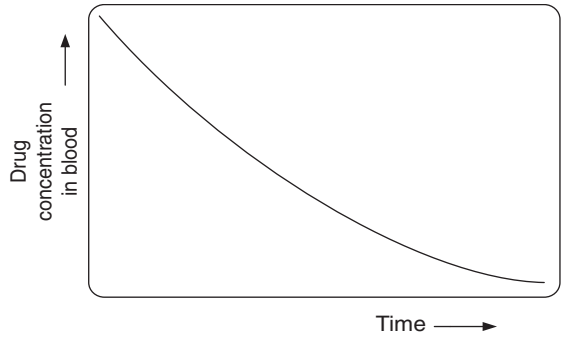



**Figure 5.9** Clearance of drug from the bloodstream.

The clearance of a drug is given by the following expression:

$$\text{CL} = \frac{\text{Rate of drug elimination}}{\text{Drug concentration in blood}} \tag{5.9}$$

A typical drug clearance curve is shown in Figure 5.9. The curve in Figure 5.9 is a first-order curve, that is, the elimination rate is proportional to the amount of drug in the bloodstream. As the amount of drug in blood reduces, the elimination rate also reduces. Another term often used is "half-life." This is the time taken to clear half (50%) of the remaining drug in the body. Mathematically, it is given by:

$$t_{1/2} = \frac{0.693 \times \text{Vd}}{\text{CL}} = \frac{0.693}{k} \tag{5.10}$$

where $k$ is the rate of drug elimination.

The half-life concept is further illustrated in Table 5.1.

In a first-order simple approximation the concentration of drug in the body at time $t$ is given by:

$$\text{Cd}(t) = \text{Cd}(0)\text{e}^{-kt} \tag{5.11}$$

**TABLE 5.1 Half-Life Calculations**

| | Amount of Drug in the Body | |
|---|---|---|
| Number of Half Lives | Percentage Eliminated | Percentage Remaining |
| 0 | 0.0 | 100.0 |
| 1 | 50.0 | 50.0 |
| 2 | 75.0 | 25.0 |
| 3 | 87.5 | 12.5 |
| 4 | 93.8 | 6.2 |
| 5 | 96.9 | 3.1 |
| 6 | 98.4 | 1.6 |

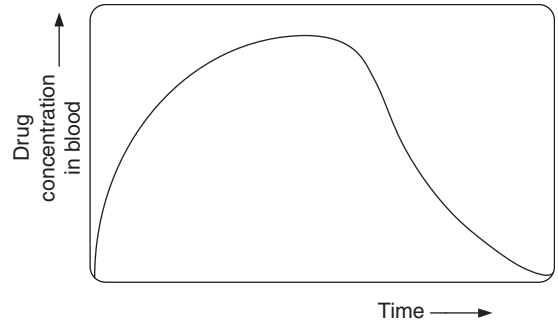



**Figure 5.10** Plasma concentration of drug after a single dose.

where Cd($t$) is the concentration of drug in the body at time $t$, Cd(0) is the concentration of drug in the body at time 0, and $k$ is rate of drug elimination.

The equation can be rearranged to give:

$$k = \frac{\ln(\text{Cd1}) - \ln(\text{Cd2})}{t2 - t1} \tag{5.12}$$

where ln(Cd1) is the natural logarithm of Cd at time 1 and ln(Cd2) for that at time 2, and $t1$ and $t2$ refer to time 1 and time 2, respectively.

### 5.3.6 Application of Pharmacokinetics Results

By combining Figures 5.8 and 5.9, we obtain a situation depicted in Figure 5.10. After a drug is absorbed, it enters the bloodstream, and the concentration builds up until a steady state is reached. As time passes, the elimination process takes over, and the concentration of the drug decreases.

Figure 5.10 would be the situation if a single dose of drug were given, but this is rarely the case. More than one dose is often administered to maintain the therapeutic level of the drug – the level that has been determined from pharmacodynamics studies of dose–response versus drug concentration. Before the drug is cleared by the excretion process, another dose is given to keep the drug concentration in a steady state and achieve maximal effects. This is illustrated in Figure 5.11.

Sometimes a larger dose is administered first; this is called a loading dose (refer to Section 5.3.3). It quickly builds up to the steady state level. After that, smaller doses are given to maintain the steady state.

## 5.4 TOXICOLOGY

In addition to the preclinical research of pharmacodynamics and pharmacokinetics, the study of the toxicology of a potential drug is critical to demonstrate that it is safe before given to humans in clinical trials. Toxicological studies show the functional and morphological effects of the drug. They are performed by determining the mode, site, and degree of action, dose relationship, sex differences, latency, and progression and reversibility of these effects.

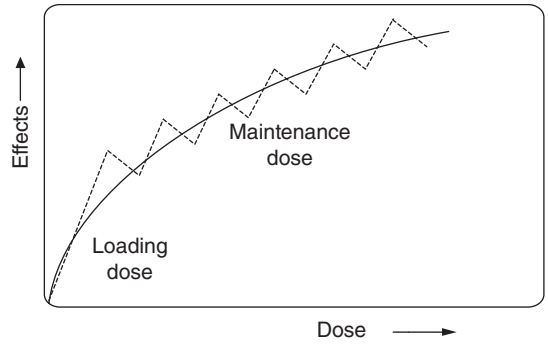



**Figure 5.11** Multiple doses to maintain maximal effect.

We summarize in Exhibit 5.6 the International Conference on Harmonization (ICH) guidelines for toxicological and pharmacological studies (refer to Section 7.11 for more information about ICH). Appendix 7 shows the type of information on pharmacodynamics, pharmacokinetics, and toxicology that regulatory reviewers examine when a potential drug is filed for Investigational New Drug (human clinical trial) and New Drug Application (marketing authorization of drug) approval. The information is extracted from the FDA *Guidance for Reviewers*: *Pharmacology/Toxicity Review Format* (2001).

### 5.4.1 Toxicity

Determining the toxicity of a drug is a must. The maximum tolerable dose and area under curve are established in rodents and nonrodents. There are two types of toxicity studies: single dose and repeated dose. Single dose acute toxicity testing is conducted for several purposes, including the determination of repeated doses, identification of organs subjected to toxicity, and provision of data for starting doses in human clinical trials.

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

Repeated dose chronic toxicity studies are performed on two species of animals, a rodent and nonrodent. The aim is to evaluate the longer-term effects of the drug in animals. Plasma drug concentrations are measured and pharmacokinetics analyzes are performed. Vital functions studied are cardiovascular, respiratory, and nervous systems. Animals are retained at the end of the study to check toxicity recovery. Table 5.2 shows the duration of the animal studies required in support of clinical trial and marketing authorization applications. Appendix 7 summarizes the information to be submitted to regulatory authorities.

In general, there is a 50-fold approach for toxicity studies. This means the high dose in the toxicity studies in animals should be selected to produce a 50-fold exposure margin over the anticipated clinical exposure at the highest dose proposed for Phase 2 and 3

**Exhibit 5.6 ICH Guidelines on Safety Studies**

| | |
|---|---|
| *S1*: | *Carcinogenicity Studies* |
| S1 | Rodent Carcinogenicity Studies for Human Pharmaceuticals |
| S1A | Need for Carcinogenicity Studies of Pharmaceuticals |
| S1B | Testing for Carcinogenicity in Pharmaceuticals |
| S1C(R2) | Dose Selection for Carcinogenicity Studies of Pharmaceuticals |
| *S2*: | *Genotoxicity Studies* |
| S2(R1) | Guidance on Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use |
| *S3*: | *Toxicokinetics and Pharmacokinetics* |
| S3A | Note for Guidance on Toxicokinetics: The Assessment of Systemic Exposure in Toxicity Studies |
| S3B | Pharmacokinetics: Guidance for Repeated Dose Tissue Distribution Studies |
| *S4*: | *Toxicity Testing* |
| S4 | Duration of Chronic Toxicity Testing in Animals (Rodent and Nonrodent Toxicity Testing) |
| *S5*: | *Reproductive Toxicology* |
| S5(R2) | Detection of Toxicity to Reproduction for Medicinal Products and Toxicity to Male Fertility |
| *S6*: | *Biotechnological Products* |
| S6 | Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals |
| *S7*: | *Pharmacology Studies* |
| S7A | Safety Pharmacology Studies for Human Pharmaceuticals |
| S7B | The Nonclinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals |
| *S8*: | *Immunotoxicology Studies* |
| S8 | Immunotoxicity Studies for Human Pharmaceuticals |
| *S9* | *Nonclinical Evaluation for Anticancer Pharmaceuticals* |
| S9 | Nonclinical Evaluation for Anticancer Pharmaceuticals |
| *S10* | *Photosafety Evaluation* |
| S10 | Photosafety Evaluation of Pharmaceuticals |

*Source*: International Conference on Harmonization 2014, *Safety Guidelines*, viewed February 23, 2014, http://www.ich.org/products/guidelines/safety/article/safety-guidelines.html. Reproduced with permission of ICH.

studies in humans. It applies to small molecule drugs, but there are exceptions based on routes of administration, for example, topical, inhalational. High-dose selection for nonclinical studies of large molecule drugs are described in ICH *Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals*, *S6*(*R1*).

### 5.4.2 Carcinogenicity

Carcinogenicity studies are carried out to identify the tumor-causing potential of a drug. Drugs are administered to animals continuously for at least 6 months. Rats are normally

**TABLE 5.2 Duration of Repeated Dose Toxicity Studies to Support Clinical Trials and Marketing Authorizations**

| Maximum Duration of Clinical Trials | Minimum Duration of Repeated Dose Toxicity Studies to Support *Clinical Trials* | |
|---|---|---|
| | Rodents | Non-rodents |
| Up to 2 weeks | 2 weeks* | 2 weeks |
| Between 2 weeks and 6 months | Same duration as clinical trials 6 months | Same duration as clinical trials 9 months† |
| >6 months | | |

| Maximum Duration of Clinical Trials | Minimum Duration of Repeated Dose Toxicity Studies to Support *Marketing Authorizations* | |
|---|---|---|
| | Rodents | Non-rodents |
| Up to 2 weeks | 1 month | 1 month |
| >2 weeks to 1 month | 3 months | 3 months |
| >1 month to 3 months | 6 months | 6 months |
| >3 months | 6 months | 9 months† |

Readers are referred to Food and Drug Administration 2008, *Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals*, viewed Feb 23, 2014, http://www.fda.gov/downloads/regulatoryinformation/guidances/ucm129524.pdf

*In Japan, women of child bearing potential can be included in clinical trials if repeated dose toxicity studies of at least 2 weeks in at least one species (generally rodents) have been completed with careful histopathological evaluation of the ovary.

†Specific conditions for EU and pediatric applications.

used, but another rodent study may be required. The studies are performed using the drug administration route intended for humans. Data for hormone levels, growth factors, and tissue enzymatic activities are gathered. At the end of the experiments, the animals are sacrificed and the tissues examined. Appendix 7 summarizes the information to be submitted to regulatory authorities.

### 5.4.3 Genotoxicity

These studies are to determine if the drug compound can induce mutations to genes. A standard battery of tests is:

- Assessment of genotoxicity in a bacterial reverse mutation test (Ames Test, refer to Exhibit 5.7)
- Detection of chromosomal damage using *in vitro* method (Mouse Lymphoma tk Test, a test to evaluate the potential of drug in causing mutations to thymidine kinase [tk])
- Detection of chromosomal damage using rodent hematopoietic cells.

**Exhibit 5.7 Ames Test**

The Ames test is based on the reversion of mutations in the bacterium *Salmonella typhimurium*. Mutant strains of *S. typhimurium*, those with mutations in the *his* operon, are unable to grow without the addition of the amino acid histidine. The drug to be tested is mixed with *S. typhimurium* and a small amount of histidine in a nutrient medium. After the histidine is consumed, the growth will stop if the drug is not a mutagen. However, if the drug is a mutagen, it will induce a reversion in the *his* operon, and the bacterium will continue to grow.

### 5.4.4 Reproductive Toxicology

The aim of these studies is to assess the effect of the potential drug on mammalian reproduction. All the stages, from premating through conception, pregnancy, and birth to the growth of the offspring, are studied. Rats are the predominant species used, and rabbit is the preferred nonrodent model. The route of administration is similar to the intended route for humans. At least three dosage levels and control groups are used (control groups are dosed with drug excipients or vehicles to provide comparable basis for analysis). For the females, effects such as hormonal cycles, pregnancy, and embryo development are studied. For the males, effects on the reproductive organs are analyzed. Other parameters studied are detailed in Appendix 7.

## 5.5 ANIMAL TESTS, *IN VITRO* ASSAYS, AND *IN SILICO* METHODS

The use of animals for pharmacological and toxicological studies has yielded invaluable information for drug development. However, many drug candidates failed in Phase I and II clinical trials because the animal models were insufficient to represent human systems and functions for some drugs. Efficacy and acceptable toxicities derived from animal models were not replicated in humans (Exhibit 5.8). In recent years, the direction in the development of drugs has shifted toward the use of *ex vivo*, *in vitro* assays and even *in silico* methods. Nevertheless, some tests must still be confirmed in animals.

Where animals are used, mice and rats are the preferred models. Other species used are hamsters, guinea pigs, and rabbits. These animals are bred in a specially controlled environment, under specific pathogen-free (SPF) conditions, to ensure that they do not carry infections or pathogens before being used in various tests. Different breeds or strains of animals are used, for example, BALB/c mice are used for immunity studies, pound mice for metabolic syndrome, and prediabetes assessments and Fischer – 344 mice for carcinoma evaluations. Nude mice (mice without body hair) have a genetic mutation that results in the absence of thymus, which leads to a suppressed immune system because of a reduction in T-cells. They are used as models to study the immune system and leukemia. More recent additions are transgenic animals with knockout genes. For example, mice with knockout p53 genes have high incidence of tumor growth. Some of these animal models are labeled with bioluminescence (enzymes that emit light) such that the progress of the disease can be tracked through imaging technology.

**Exhibit 5.8 Clinical Trial Failures**

Only 1 in 10 Investigational New Drugs (IND) will become approved as drugs. Half the IND failures are because of unacceptable efficacy. Another third fail because of safety issues.

Toxicity failures occur because:

- Toxicity in animals is not fully understood and potential toxicity in humans cannot be estimated
- Toxicity in animals is understood and potential toxicity in humans is not acceptable
- Acceptable therapeutic margins (efficacy vs. toxicity) cannot be established
- Toxicities in animals do not predict toxicity in human trials.

See also Exhibit 6.18

*Source*: Data from Johnson, DE 2000, 'Predicting human safety: Screening and computational approaches', *Drug Discovery Today*, 5, pp. 445–454.

Experimental use of animals is controlled under Good Laboratory Practice (GLP), and study protocols are submitted to the Animal Research Ethics Committee for approval. Studies using animals can proceed only with the approval of the Ethics Committee, which consists of technical personnel, including a veterinarian, as well as lay people who evaluate the study from different perspectives.

*In vitro* assays are increasingly being used. Reasons are costs, availability of more rapid results, and avoidance of negative publicity. Assays such as cytochrome P-450 enzymes, Ames test, and the mouse lymphoma tk test are *in vitro* methods. For absorption studies, Caco-2 (Exhibit 5.9) and Maudin-Darby canine kidney cell assays are now routinely used. Hepatocyte cell lines with metabolism capacity are being developed to test drug metabolism and toxicity. Exhibit 5.10 shows the *in vitro* and *in vivo* tests for the assessment of the effects of drugs on ventricular repolarization and irregular heartbeat. All these examples show that, where possible, pharmaceutical firms are gradually dispensing with animal studies.

**Exhibit 5.9 Caco-2 Cell Assays**

The Caco-2 cells are derived from human colorectal carcinoma. When these cells are cultured on semipermeable membranes, they grow into epithelial cells that are very similar to intestinal epithelial cells. The permeability of drugs across these Caco-2 cells provides model tools for the study of drug absorption.

**Exhibit 5.10 Ventricular Repolarization and Rapid Irregular Heartbeat (ICHS7B)**

The human heart pumps blood throughout the body. Electrical signals are required to contract the heart muscles for the pumping motions. These electrical signals are due to the movements of chemical ions in and out of the heart muscle cells through the voltage-gated ion channels on the cell membrane surface.

When $Na^+$ and $Ca^{2+}$ ions move into the muscle cells, the membrane potential becomes positively charged, a process called depolarization. A result is that the heart muscle cells contract and blood is pumped out of the heart. After the contraction, $K^+$ ions move into and $Na^+$, and $Ca^{2+}$ ions leave the cells. The cell membrane potential becomes negative and this is the repolarization process, a resting stage for the heart before the next "contraction–rest" cycle repeats itself.

Some drugs have the effects of delaying the time interval between depolarization and repolarization. When the time interval is prolonged, there is an increased risk of ventricular tachyarrhythmia, which can cause irregular heartbeats and sudden death.

The ICH Guideline, S7B – The Nonclinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals – sets out the requirements to use both *in vivo* and *in vitro* tests on the potential drugs. Data from these tests are part of the information to be submitted to regulatory authorities for marketing approval.

*Source*: Data from International Conference on Harmonization 2005, *The Nonclinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals S7B*, viewed March 6, 2014, http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Safety/S7B/Step4/S7B_Guideline.pdf

With better understanding of drug functions and information from huge databases, predictive *in silico* ADME algorithms have been designed. These algorithms encompass information derived from *in vivo* and *in vitro* studies; they consider molecular interactions, biological data, pharmacological results, and toxicological endpoints. A description of *in silico* toxicology is given in Exhibit 5.11.

The aim of all the laboratory and animal studies is to understand the effects of the potential drug in living systems. These studies cannot guarantee the safety and efficacy of the drug in humans, but they can enhance the reliability and predictive value. Results from these studies provide a basis for starting dose for clinical trials in humans. The *Guidance for Industry*: *Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers* from FDA (July 2005) outlines the derivation of the maximum recommended starting dose (MRSD) for a drug to be used in humans for the first time. This dose is based on the following derivation algorithm:

- Determine the no observed adverse effect level (NOAEL) in animals – the highest dose level that does not produce a significant increase in adverse effects.

**Exhibit 5.11 *In Silico* Toxicology**

*In silico* toxicology is the use of computational technologies to analyze data and models and predict the toxicology of pharmaceutical substances.

Some of the *in silico* toxicology methodologies are as follows:

- Data mining from toxicity databases. Such databases are available, for example, from FDA/CDER
- Prediction of toxicity on the basis of mathematical calculations of Quantitative Structure Activity Relationships (QSARs) using chemical compounds of known toxicity
- Human knowledge-based models with expert rules for features or functional groups/structures that could lead to toxicity effects
- ADME-Tox software for the prediction of metabolic, pharmacokinetic, and toxicological issues related to drug metabolism, disposition, and effects.

*Source*: Data from Valerio Jr, LG 2009, 'In silico toxicology for the pharmaceutical sciences', *Toxicology and Applied Pharmacology*, 241, pp. 356–370.

- Convert the NOAEL to human equivalent dose (HED) using the data from Table 5.3 (calculations are based on body surface areas).

## 5.6 FORMULATIONS AND DELIVERY SYSTEMS

Development of manufacturing processes for the production of a drug (active pharmaceutical ingredients – APIs), initially to supply enough materials for laboratory testing, then for human clinical trials, and ultimately as production batches of drug products when approved by regulatory authorities, proceeds as soon as the clinical candidate is identified. There are two distinct manufacturing processes: synthetic chemistry for pharmaceuticals and recombinant DNA technology for biopharmaceuticals. Manufacturing processes are discussed in Chapter 10.

Apart from pharmacological and toxicological studies, the drug development process encompasses meticulous and methodical work in the following areas:

- Formulation of the drug product, which includes active pharmaceutical ingredients and excipients, into final form suitable to be administered to patients
- Study of drug delivery systems to improve effective presentation of the drug to patients for enhancing certain characteristics and improving patient compliance.

The last two items are discussed below.

**TABLE 5.3 Conversion of Animal Doses to Human Equivalent Doses (HED) on the Basis of Body Surface Area**

| Species | To convert animal dose in mg/kg to dose in mg/m$^2$, multiply by kg/m$^2$ below: | To convert animal dose in mg/kg to HED* in mg/kg, either: | |
|---|---|---|---|
| | | Divide animal dose by: | Multiply animal dose by: |
| **Human** | 37 | | |
| **Child (20 kg)**† | 25 | | |
| Mouse | 3 | 12.3 | 0.08 |
| Hamster | 5 | 7.4 | 0.13 |
| Rat | 6 | 6.2 | 0.16 |
| Ferret | 7 | 5.3 | 0.19 |
| Guinea pig | 8 | 4.6 | 0.22 |
| Rabbit | 12 | 3.1 | 0.32 |
| Dog | 20 | 1.8 | 0.54 |
| **Primates** | | | |
| Monkeys‡ | 12 | 3.1 | 0.32 |
| Marmoset | 6 | 6.2 | 0.16 |
| Squirrel monkey | 7 | 5.3 | 0.19 |
| Baboon | 20 | 1.8 | 0.54 |
| Micro-pig | 27 | 1.4 | 0.73 |
| Mini-pig | 35 | 1.1 | 0.95 |

*Source*: Food and Drug Administration 2005, Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers.

*Assumes 60 kg human. For species not listed or for weights outside the standard ranges, human equivalent dose can be calculated from the formula:
$\text{HED} = \text{animal dose in mg/kg} \times (\text{animal weight in kilograms/human weight in kilograms})^{0.33}$.

†This is provided for reference only, as healthy children will rarely be volunteers for Phase I trials.

‡For example, cynomolgus, rhesus, stumptail.

*Note*: Column 2 is for information only. For HED calculations, either column 3 or column 4 is used.

### 5.6.1 Formulations

Most prescribed drugs are formulated with the active pharmaceutical ingredients and excipients. The formulations of selected drugs are presented in Exhibit 5.12. According to the *US Pharmacopeia and National Formulary* definition, excipients are "any component, other than the active substance(s), intentionally added to the formulation of a dosage form". The reasons for the addition of excipients are to:

- Control the release of drug substance in the body
- Improve the half-life of the drug substance (refer to Exhibit 4.9)
- Improve the assimilation process and bioavailability
- Enhance drug dissolution as disintegration promoters
- Extend the stability and shelf-life of the drug as antioxidants or preservatives

**Exhibit 5.12 Selected Small Molecule Drug Formulations**

**Prilosec**

Antiulcerant in 10, 20, and 40 mg doses.
Active ingredient: omeprazole.
Excipients: cellulose, disodium hydrogen phosphate, hydroxypropyl cellulose, hydroxypropyl methylcellulose, lactose, mannitol, sodium lauryl sulfate, etc.

**Prozac**

Antidepressant in 10, 20, and 40 mg doses.
Active ingredient: fluoxethine hydrochloride.
Excipients: starch, gelatin, silicone, titanium dioxide, iron oxide, etc.

**Lipitor**

Cholesterol reducer in 10, 20, 40, and 80 mg doses.
Active ingredient: atorvastatin calcium.
Excipients: calcium carbonate, candelilla wax, croscarmellose sodium, hydroxypropyl cellulose, lactose monohydrate, magnesium stearate, microcrystalline cellulose, polysorbate 80, simethicone emulsion.

**Celebrex**

Anti-inflammatory in 100 and 200 mg doses.
Active ingredient: celecoxib.
Excipients: croscarmellose sodium, edible inks, gelatin, lactose monohydrate, magnesium stearate, povidone, sodium lauryl sulfate, and titanium dioxide.

*Source*: Data from Food and Drug Administration, Center for Drugs Evaluation and Research website, http://www.fda.gov/cder/

- Aid in the manufacturing processes in the form of fillers, lubricants, wetting agents, and solubilizers
- Mask an unpleasant taste of the active pharmaceutical ingredient
- Use as an aid for the identification of the product.

According to the International Pharmaceutical Excipients Council, the most commonly used excipients in the United States are:

- Simethicone emulsion – antifoam
- Selenium – antioxidant
- Vitamins A, C, E – antioxidant
- Hydroxypropyl cellulose – binder

- Hydroxypropyl methylcellulose – binder
- Ethylcellulose – binder
- Lactose – binder
- Starch (corn) – binder
- Gelatin – capsule shell
- Silicon dioxide – colorant
- Titanium dioxide – colorant
- Microcrystalline cellulose – disintegrant
- Sodium starch glycolate – disintegrant
- Sodium carboxymethyl cellulose – disintegrant
- Polysorbate – emulsifier
- Calcium carbonate – filler
- Calcium phosphate – filler
- Talc – filler
- Calcium stearate – lubricant
- Magnesium stearate – lubricant
- Stearic acid – lubricant
- Sucrose – sweetener.

FDA maintains a database of approved excipients (*Drug Information*: *Electronic Orange Book*, http://www.fda.gov/cder/ob/default.htm). Standards and tests for regulatory acceptable excipients are included in the *US Pharmacopeia and National Formulary*. Two such tests, dissolution and stability, are included in Exhibit 5.13 for reference. For new excipients to be included in a drug formulation, they have to satisfy one of the following criteria:

- Determination by FDA that the substance is "generally recognized as safe" (GRAS) according to 21 CFR 182, 184, and 186
- Approval by FDA as a food additive under 21 CFR 171
- Excipients referenced in the New Drug Application, showing that they have been tested in laboratory and clinical trials.

The foregoing applies mainly for small molecule drugs. In the case of large molecule drugs, formulations are undertaken first and foremost to improve stability as proteins are prone to undergo physical and chemical changes and to obtain high concentrations of drug to enable convenient subcutaneous (versus intravenous) administration. These changes may involve aggregation due to dimerization, trimerization, and higher order associations, as well as crystallization and precipitation. Degradation processes such as deamidation, oxidation, hydrolysis, isomerization, proteolysis, and disulfide bond formation/dissociation may also occur. Large molecule drug formulations typically consist of buffers, surfactants, and stabilizers in liquid form or as lyophilized (freeze-dried) powder to be reconstituted with sterile water before administration via the parenteral route to the patient (Exhibits 5.14 and 5.15).

**Exhibit 5.13 Dissolution and Stability Tests**

**Food and Drug Administration 1997, *Guidance for Industry*: *Dissolution Testing of Immediate Release Solid Dosage Forms***

Dissolution tests using the basket method (50/100 rpm) or the paddle method (50/75 rpm) under mild test conditions are used to generate a dissolution profile at 15-min intervals. The pH range is 1.2–6.8; pH up to 8.0 may be tested with justification. The temperature is $37 \pm 0.5\,^{\circ}C$. Methods are described in the *US Pharmacopeia*. Test requirements vary depending on the solubility of drug products. *In vitro* test may need validation to confirm *in vivo* results.

**European Medicines Agency 2008, *Guideline on Stability Testing*: *Stability Testing of Existing Active Substances and Related Finished Products***

Active substances and finished products are evaluated for their thermal stability and, if applicable, sensitivity to moisture. Properties and characteristics of the actives and finished products are tested after temperature and humidity exposures to determine the storage conditions and consequential effects on shelf-life. Study conditions are as set out below:

For General Case

| Study | Storage condition | Minimum Time Period Covered by Data at Submission |
|---|---|---|
| Long term | $25 \pm 2\,^{\circ}C/60\%RH \pm 5\%RH$ or $30 \pm 2\,^{\circ}C/65\%RH \pm 5\%RH$ | 6 months |
| Intermediate | $30 \pm 2\,^{\circ}C/65\%RH \pm 5\%RH$ | 6 months |
| Accelerated | $40 \pm 2\,^{\circ}C/75\%RH \pm 5\%RH$ | 6 months |

Different storage conditions apply for active substances and finished products that require storage in refrigerator, freezer, or $-20\,^{\circ}C$. Finished products packaged in semipermeable containers are subject to a different set of storage conditions.

Photostability is tested by the exposure of drug products to visible and ultraviolet light sources.

For drugs to be administered by the parenteral route, some general requirements are as follows:

- Intravascular – clear solution, iso-osmotic (serum and cellular fluid osmotic pressure is around 285–290 mOsm), $pH \approx 7.4$
- Subcutaneous or intramuscular – suspensions allowable but avoid extreme pH, citrate as buffer should not be used as it causes pain.

**Exhibit 5.14 Selected Large Molecule Drug Formulations**

**Avastin**

Avastin is for the treatment of colorectal cancer.

It is supplied in 100 and 400 mg dosages.

The 100 mg formulation consists of 240 mg α,α trehalose dihydrate, 23.2 mg sodium phosphate (monobasic, monohydrate), 4.8 mg sodium phosphate (dibasic, anhydrous), 1.6 mg polysorbate 20, and water-for-injection.

*Source*: Food and Drug Administration 2011, *Avastin*, viewed Apr 18, 2014, http://www.accessdata.fda.gov/drugsatfda_docs/label/2011/125085s225lbl.pdf

**Enbrel**

Enbrel is for the treatment of rheumatoid arthritis.

It is supplied as a sterile, white, preservative-free, lyophilized powder for parenteral administration after reconstitution with 1 mL of the supplied Sterile Bacteriostatic Water for Injection, USP (containing 0.9% benzyl alcohol) prior to parenteral injection.

*Source*: Food and Drug Administration 1999, *Enbrel*, viewed Apr 18, 2014, http://www.fda.gov/downloads/drugs/developmentapprovalprocess/howdrugsaredevelopedandapproved/approvalapplications/therapeuticbiologicapplications/ucm088842.pdf

**PEG-Intron**

PEG-Intron is for the treatment of hepatitis C.

The product consists of a covalent conjugate of the recombinant interferon-α-2b with monomethoxy polyethylene glycol (PEG) supplied in vials with 74, 118.4, 177.6, or 222 μg of the active and 1.11 mg sodium phosphate (dibasic, anhydrous), 1.11 mg sodium phosphate (monobasic, dihydrate), 59.2 mg sucrose, and 0.074 mg polysorbate 80. The powder is reconstituted with sterile water-for-injection.

*Source*: Data from Food and Drug Administration 2010, *PEG-Intron*, viewed April 18, 2014, http://www.fda.gov/downloads/advisorycommittees/committeesmeetingmaterials/pediatricadvisorycommittee/ucm235414.pdf

To study and derive an effective formulation, a suite of analytical methods must be developed to evaluate the formulation. Some suggested assays for biopharmaceutical drug formulation evaluations are listed below:

- Bioassay – activity of formulation
- Immunoassay – purity assessment
- pH – chemical stability

**Exhibit 5.15 Excipients for lyophilized formulations**

Lyophilized excipients are as follows:

- Bulking agent – Mannitol
- Stabilizing agent – monosaccharides (glucose), disaccharides (sucrose, lactose, maltose, and trehalose)
- Surfactant – nonionic: polyethylene sorbitan monolaurate (Tween 20, Tween 80), pluronic, Triton, sodium dodecyl sulfate (SDS)
- Buffering agents – phosphate, citric, glutaric, succinic, carbonic acid
- Chelating agents – to bind trace metals, EDTA 0.01–0.05%
- Antioxidants – block specific chain reaction, 0.01–0.05%
- Preservatives – for multidose formulations, antimicrobial agents, phenol (0.3–0.5%), chlorobutanol (0.3–0.5%), benzyl alcohol (1.0–3.0%)
- Isotonic agents – Osmolality 285–290 mOsm; Mannitol, sucrose, glycine, glycerol, and sodium chloride.

- SDS-PAGE – protein characterization and purity
- HPLC – purity, identity, and stability
- IEF – modifications of protein
- N-terminal sequencing – identity of protein
- UV – concentration and aggregation
- DSC – differential scanning calorimetry – structural stability
- Circular dichroism – secondary and tertiary conformations.

The use of antioxidants, preservatives and container, and closure are other aspects of formulations that are considered with regard to how the drug is to be delivered and the target patient group for the drug.

### 5.6.2 Drug delivery Systems

Delivery systems have come a long way from pills, syrups, and injectables. As we have discussed earlier, the ADME process means that most drugs administered to us have tortuous paths to reach their targets, and in many instances the bioavailability is reduced. A traditional means to overcome the vagaries of ADME is to have larger doses or more frequent administrations. These types of treatments have complications: (i) potential for adverse events, and (ii) ensuring patient compliance to take the medication regularly. New delivery systems are devised to overcome these problems.

The oral route for drug administration is convenient and does not normally require physician's intervention. Most protein-based drugs are, however, not administered via the oral route because they are destroyed by the low pH medium in the stomach. One means to overcome this is the use of enteric coating for some drugs. Drugs are coated

**TABLE 5.4 Bioavailability of Oral Drugs**

| Bioavailability | Dosage Form |
|---|---|
| Fastest | Solutions |
| ↓ | Suspensions |
| | Capsules |
| | Tablets |
| | Coated tablets |
| Slowest | Controlled-release form |

with cellulose acetate phthalate, which can withstand the acid environment in the stomach and yet readily dissolve in the slightly alkaline environment of the intestine. In this way, the protein-based drugs can have a safe passage to the intestine for absorption to take place. Table 5.4 shows the bioavailability of oral drugs in various dosage forms.

Another method is to prolong the release of the drug in the bloodstream. This will reduce the frequency for taking the drug, for example, from several times a day to once per day or even once per week. To achieve this, drug molecules are encapsulated within polymer matrices. These are known as microspheres, polymer micelles, and hydrogels. The polymers are made with biodegradable materials and, through processes of hydrolysis, drug molecules are released at controlled rates as the polymer is degraded. The degradation process can be triggered by pH, temperature, electric field, or even ultrasound. Exhibit 5.16 provides further description on these polymeric delivery systems.

**Exhibit 5.16 Polymeric Drug Delivery Systems**

Two new developments are the dendrimers (highly branched, globular, synthetic macromolecules) and modified buckyballs. Together with hydrogels, they are tailored to provide targeted delivery.

The dendrimers form small micelles, which transport small molecules within their matrices or act as hubs for covalent bonding to drug molecules, extending like dendrites. In this way, they can shepherd high concentrations of drugs to targets.

Buckyballs are cage-like molecules of fullerenes. They are robust and can carry radioactive drugs to targets. Research is directed at using these buckyballs as delivery systems for the treatment of cancer.

Hydrogels are 3D cross-linked polymer networks. They can withstand acid conditions and release the entrapped drug molecules. Purdue University researchers have used a poly[methacrylic acid-g-poly(ethylene glycol)] hydrogel to encapsulate insulin, which could be released by pH trigger.

*Source*: Data from Vogelson, CT 2001, 'Advances in drug delivery systems', *Modern Drug Discovery*, 4, April, pp. 49–50, 52.

**Exhibit 5.17 Needleless Injection**

The sight of hypodermic syringe is enough to send shivers down the spines of most patients, besides the agony of enduring the pain.

Needleless injections are new devices to bypass this problem. Drugs in powder or liquid form can be injected into the subcutaneous layer in the following ways:

- Propelled by a jet stream of compressed air
- Fired as pellets similar to that of bullets from rifles
- Electroporation (a temporary application of direct current, which disturbs the skin surface and allows penetration of the drug molecules)
- Drug particles can be vaporized and 'vaped' into the lungs for therapy
- Combination of nanotechnology and ultrasound to deliver the drugs to the target sites.

Needleless injection is ideal for frequent injections, as in the cases of insulin and growth hormone, which are administered routinely.

Other delivery systems are transdermal patches, metered dose inhalers, nasal sprays, implantable devices, and needle-free injections. A description of needleless injection is given in Exhibit 5.17.

## 5.7 NANOTECHNOLOGY

Nanotechnology is the science of matters with sizes in the range of 1–100 nm ($1–100 \times 10^{-9}$ m). These are scales of large molecules; for example, the sizes of some familiar matters are: DNA – 1–2 nm; virus – 3–50 nm; red blood cell – ≈300 nm.

At these nano scales, matters behave quite differently from how they would in the macro level that we are accustomed to. Properties such as conductivity, magnetism, melting, and boiling points, and reactivity may be dissimilar at nano and larger magnitudes because of quantum mechanical behavior of small structures at molecular dimensions.

Nanotechnology provides a means to manufacture particles with very high surface area to mass ratio, and together with their unique properties, may provide opportunities for more surface interactions and biochemical reactions to ensue. One use of nanotechnology is for drug delivery devices. Nano cages with embedded drugs can be delivered to their targets with high specificity and enabled interactions to take place to alter the disease pathways. For example, nanoparticles of iron oxide are linked to chemotherapy drug, doxorubicin, loaded in liposome to target breast cancer cells. Once the nanoparticles have entered the cancer cells, the nanoparticles are set to vibrate using radiofrequency, thus rupturing the liposome and releasing doxorubicin to home in on the tumor cells.

Abraxane, a plant alkaloid chemotherapy drug, is formulated with the active ingredient (paclitaxel) bound to nanoparticles of albumin as delivery vehicle. It was approved by FDA for breast cancer treatment initially and later widened to include non-small-cell lung cancer in 2013.

## 5.8 CASE STUDY #5.1

### 5.8.1 Abilify and Enbrel

Presented below are the pharmacodynamics and pharmacokinetics for Abilify and Enbrel.

***Abilify:***

(a) Indication:
Abilify is approved for the treatment of schizophrenia and bipolar disorder.

(b) Pharmacodynamics:
Its mechanism of action is a combination of being a partial agonist at dopamine D2 and serotonin 5HT1a receptors, and as an antagonist at serotonin 5HT2a receptors.

(c) Pharmacokinetics:

Absorption – Peak plasma concentration occurs within 3–5 h after dosing. Oral bioavailability is 87% of tablet formulation. Each tablet contains 5 mg of aripiprazole, the active ingredient.

Distribution – Aripiprazole is widely distributed throughout the body with an apparent volume of distribution of 4.9 L/kg, indicating extensive extravascular distribution.

Metabolism – Aripiprazole is metabolized in the liver by dehydrogenation, hydroxylation, and N-dealkylation. At steady state, dehydro-aripiprazole, the active metabolite, represents about 40% of aripiprazole AUC in plasma.

Excretion – The mean excretion half-life is approximately 75 h in active metabolizers and 146 h in poor metabolizers. The clearance of aripiprazole is 0.7 mL/min/kg and is mainly through the liver.

*Source*: European Medicines Agency 2013, *Abilify*: *EPAR – Product Information*, viewed Feb 20, 2014, http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000471/human_med_000619.jsp&mid=WC0b01ac058001d124

***Enbrel:***

(a) Indication: Enbrel is approved for the treatment of rheumatoid arthritis, juvenile idiopathic arthritis, psoriatic arthritis, ankylosing spondylitis, plaque psoriasis, and pediatric plaque psoriasis.

(b) Pharmacodynamics: The mechanism of action is acting as a tumor necrosis factor alpha (TNF-α) inhibitor. The active ingredient, etanercept, acts as a receptor molecule for TNF-α, thereby inhibiting TNF-α from causing the inflammatory process in arthritis and psoriasis.

(c) Pharmacokinetics:

Absorption – Etanercept is slowly absorbed from the site of subcutaneous injection, reaching maximum concentration approximately 48 h after a single dose. The absolute bioavailability is 76%.

Distribution – The volume of distribution is 10.4 L at steady state.

Metabolism – There is no first pass liver metabolism as Enbrel is injected subcutaneously.

Excretion – The half-life is approximately 70 h. Clearance is approximately 0.066 L/h in patients with rheumatoid arthritis.

*Source*: European Medicines Agency 2014, *Enbrel*: *EPAR – Product Information*, viewed Feb 20, 2014, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000262/WC500027361.pdf

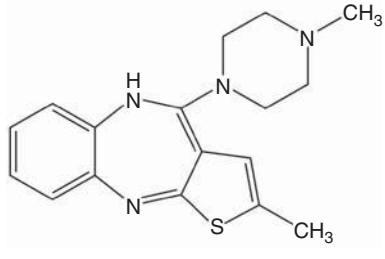
## 5.9 CASE STUDY #5.2

### 5.9.1 Zyprexa and Aranesp

Examples of PD, PK, Toxicology, and Formulation for two more drugs, Zyprexa (a small molecule drug) and Aranesp (a large molecule drug), are described below.

### 5.9.2 Zeprexa

***Description:*** Zeprexa (olanzapine) is an antipsychotic drug. The chemical formula is 2-methyl-4(4-methyl-1-piperazinyl)10H-thieno[-2-3-b][1,5]benzodiazepine. The molecular weight is 312.44 Da.




***PD:*** Zeprexa is a selective monoaminergic antagonist with high affinity for the following receptors: serotonin 5HT, dopamine, muscarinic, histamine, and adrenergic. Its action on schizophrenia is through the antagonism in serotonin and dopamine.

***PK:*** Following oral dose, plasma peak concentration is approximately 6 h. About 40% is eliminated through first pass metabolism. The half-life is from 21 to 54 h and plasma clearance is from 12 to 47 h. Daily administration will lead to steady state of plasma concentration in about a week with concentration twice that of the single dose. Metabolism of Zyprexa is by the cytochrome P450 oxidation.

***Toxicology:*** At 17 times the maximum human dose (on mg/kg basis), dogs developed reversible neutropenia and/or reversible hemolytic anemia between 1 and 10 months of treatment. Mice given doses twice the maximum human dose (on mg/kg basis) showed decrease in lymphocytes and neutrophils in studies of 3 months' duration.

***Formulation:*** The formulation consists of excipients such as carnauba wax, crospovidone, hydroxylpropyl cellulose, hydroxypropyl methylcellulose, lactose, magnesium stearate, microcrystalline cellulose, and other inactive ingredients.

### 5.9.3 Aranesp

***Description:*** Aranesp is an erythropoiesis protein (EPO) produced using rDNA technology in CHO cells. It has 165 amino acids, and the molecular weight is 30–37 kDa.

***PD:*** Patients with chronic renal failure (CRF) and those receiving chemotherapy developed anemia because of deficiency in erythropoietin. Aranesp stimulates the production of red blood cells (RBCs). It mimics the natural erythropoietin and interacts with the progenitor stem cells to produce RBCs. The increased level of hemoglobin is observed after 2–6 weeks on Aranesp treatment.

***PK:*** For therapeutic range of 0.45–4.5 μg/kg, maximum plasma concentration, half-life, and AUC are linear with respect to dose. Following subcutaneous injection, the absorption is slow and rate limiting. The half-life ranges from 27 to 89 h. Peak plasma concentration is 34 h after SC administration for CRF patients and 90 h for cancer patients.

***Toxicology:*** Animals treated with Aranesp showed no evidence of abnormal mitogenic and tumorigenic responses. In some studies, Aranesp appears to increase the beneficial effects of radiotherapy.

***Formulation:*** Aranesp is formulated as a sterile, colorless, preservative-free protein solution for intravenous (IV) or subcutaneous (SC) administration. There are two formulations: the polysorbate solution include excipients such as polysorbate 80, sodium phosphate monobasic monohydrate, sodium phosphate dibasic anhydrous, and sodium chloride in water-for-injection, and the albumin solution contains albumin, sodium phosphate monobasic monohydrate, sodium phosphate dibasic anhydrous, and sodium chloride in water-for-injection. The pH for both formulations is $6.2 \pm 0.2$.

*Source*: (i) Food and Drug Administration 2007, *Aranesp*, viewed Jun 21, 2014, http://www.accessdata.fda.gov/drugsatfda_docs/label/2007/103951s5164lbl.pdf; (ii) Food and Drug Administration 2003, *Zeprexa*, viewed Jun 21, 2014, http://www.accessdata.fda.gov/drugsatfda_docs/label/2004/20592se1-019_zyprexa_lbl.pdf; (iii) Bunn, HF 2007, 'EPO binding to receptor: New agents that stimulates erythropoiesis', *Reviews in Translational Hematology*, 109, pp. 868–873.

## 5.10 SUMMARY OF IMPORTANT POINTS

1. Pharmacodynamics (PD) is the study of interactions between the drugs and the body whereas pharmacokinetics (PK) describes the absorption, distribution, metabolism, and excretion (ADME) of the drugs by the body.
2. PD studies allow us to understand the potency, effectiveness, therapeutic index, and safety margins of drugs. PK information on ADME provides us with an understanding of how drugs are transported, diffused into bloodstream, and become available to the cells and act on the target sites.
3. Drugs are administered by various means: from oral to intravenous to topical. The oral route is a relatively slow process where the drugs must be absorbed across the gastrointestinal tract and then passed through the liver and metabolized before it becomes available to bind to receptors and perform its intended function. On the contrary, intravenous application is quick but has the potential of fast systemic reaction if adverse reactions occur. In the case of topical administration, the effects of the drug are localized.
4. Drug development has to evaluate the toxicity of drugs to the body. Animals, mainly rodents, are used to study toxicities. The evaluation should also consider the effect of drugs in causing cancers, tendency in inducing mutations, and the consequences on reproduction.
5. Over time, more and more laboratory and cell-based assays are used to study ADME and toxicities of drugs.
6. Preclinical studies on safety and toxicity of drugs are conducted according to regulatory guidelines such as FDA 21 CFR Part 58 *Good Laboratory Practice for Nonclinical Laboratory Studies*, or the ICH safety-guidelines (Exhibit 5.6).
7. The active drug molecule is formulated with excipients to aid in manufacturing processes and the delivery process of the active to the receptors or enzymes. Excipients also help in maintaining the stability of the active component, in shipping, storage, and administration into the body. Other functions for the excipients are to modulate the bioavailability of the drug and prolonging the half-life of the drug.
8. Nanotechnology is a new technique that may result in more targeted delivery of drug molecules to the active sites, thus improving bioavailability and reducing adverse events.
9. Most small molecule drugs are formulated for oral delivery while large molecule biopharmaceuticals are injected via parenteral means of intravenous, intramuscular, subcutaneous, and infusion.

## 5.11 REVIEW QUESTIONS

1. Distinguish between PD and PK. For PD, explain the term $K_D$ and show how drugs interact with receptors. For PK, explain the mechanisms of ADME.
2. What do the terms potency and effectiveness of a drug mean? Explain the definitions of therapeutic index and safety margin.
3. Using acid–base theories for drugs, explain how ionization of a drug accounts for its solubility.
4. Using graphs explain the absorption and clearance of a drug. What methods are used to prolong the availability of a drug in the body?
5. An IV dose of 500 mg is administered. The table below shows the drug concentration in blood, taken over a 10 h period. Determine the rate of elimination and the half-life of the drug.

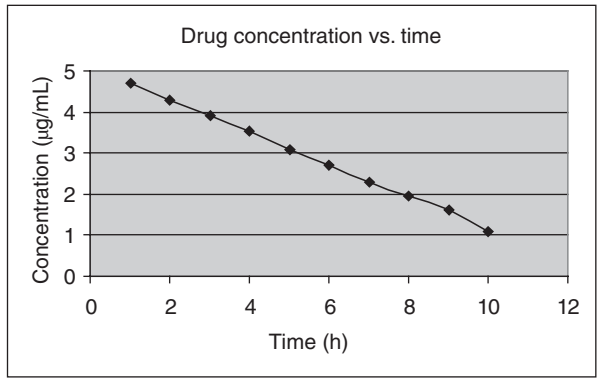
| *Time* (*h*) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| *Conc.* (μg/mL) | 110 | 74 | 50 | 34 | 21 | 14 | 9 | 6 | 3 | 2 |

6. Explain the use of animals in drug testing. Provide examples to show alternative methods for testing the drugs. Discuss the pros and cons of each type of testing.
7. List the common excipients used and what are the regulatory requirements for approving excipients?
8. Describe nanotechnology and clarify its role in drug application.

## 5.12 BRIEF ANSWERS AND EXPLANATIONS

1. Refer to Sections 5.2 and 5.3. Use Equations (5.1), (5.2), and (5.3) to describe the term $K_D$. Refer to Sections 5.3.2–5.3.5 to explain ADME.
2. Potency of a drug refers to the quantity of drug that generates a response while effectiveness is the intensity of the response. Equations (5.4) and (5.5) show the therapeutic index and safety margin. In general more specific drugs, such as those designed through the rational approach to bind to particular receptor/s, for example Relenza and Tamiflu (Exhibit 3.7), are expected to have higher therapeutic index and safety margin.
3. Refer to Exhibit 5.2.
4. Use Figures 5.8–5.10, and 5.11 to explain the increase in drug concentration following administration and subsequently being cleared through the excretion route. The concept of drug half-life shows the elimination rate of a drug. One practice to prolong and sustain availability of drugs in the body is to increase the initial loading dose and then follow by reduced maintenance dosages. Another means is to prepare formulations for controlled-release of drugs, for example the pegylated interferon for Hepatitis C treatment (Exhibit 4.9).

5. By plotting the graph using natural logarithm for the concentration, we obtain the following:




This is a linear graph and the slope is the rate of elimination. Alternatively we substitute the values in Equation (5.10):

$$k = \frac{\ln(Cd1) - \ln(Cd2)}{t2 - t1} = \frac{\ln(110) - \ln(2)}{10 - 1} = \frac{4.7 - 1.1}{9}$$

$$= 0.4/\text{hour}$$

$$t_{1/2} = \frac{0.693}{k} = 1.73 \text{ hour.}$$

6. Over the years the use of animal testing has yielded invaluable information on drug PD and PK before being administered to humans. For the study of many diseases, special breeds of animals are used as model systems to test the efficacy and safety of the drug candidates, refer to Section 5.5. Increasingly more and more *in vitro* assays are being used, partly to reduce pressure from animal rights groups and partly due to advances in assay development. It should also be noted that animal studies have to follow GLP, including the use of appropriate animal care facility and protocols.
7. Refer to Section 5.6.1.
8. Refer to Section 5.7.

## 5.13 FURTHER READING

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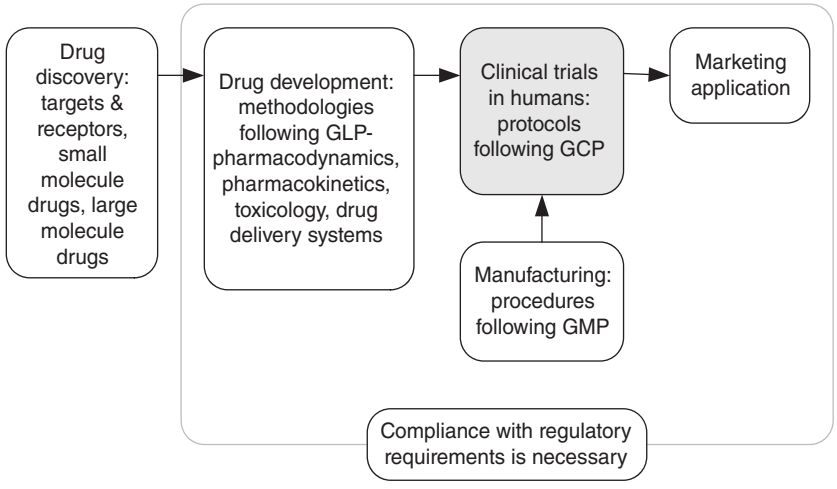
# CHAPTER 6

# CLINICAL TRIALS




## 6.1 DEFINITION OF CLINICAL TRIAL

After a lead compound has been optimized and tested in the laboratory, and pharmacological studies have been conducted to show that the lead compound has the potential to become a drug and that the safety studies are satisfactory, it is ready for clinical trial in humans.

*Drugs: From Discovery to Approval*, Third Edition. Rick Ng.

What is a clinical trial? According to the International Conference on Harmonization (ICH, refer to Section 7.11) a clinical trial or study is:

> Any investigation in human subjects intended to discover or verify the clinical, pharmacological and/or other pharmacodynamic effects of an investigational product, and/or to identify any adverse reactions to an investigational product, and/or to study absorption, distribution, metabolism, and excretion of an investigational product with the object of ascertaining its safety and/or efficacy.

## 6.2 ETHICAL CONSIDERATIONS

Before a drug is put forward for a clinical trial, there are ethical and regulatory constraints for the design and conduct of a clinical trial that have to be considered.

The United States National Institutes of Health (NIH) has stipulated seven ethical requirements to ensure that, before a trial begins, there is proper consideration of ethical issues and the trial subjects are protected. The essential tenet is that the potential exploitation of human subjects must be minimized and the risk–benefit ratio must be favorable. These seven ethical requirements are:

- Social value
- Scientific validity
- Fair subject selection
- Informed consent
- Favorable risk–benefit ratio
- Independent review
- Respect for human subjects.

### 6.2.1 Social Value

This requirement is to ensure that the clinical trial is justified on the basis of scientific research and will result in improvements in health or advancement of scientific knowledge. In this way, resources are not directed at nonmeaningful clinical research and human subjects are not being exploited.

### 6.2.2 Scientific Validity

The clinical trial should be conducted methodically with clear objectives and outcomes that are statistically verifiable. The preclinical and toxicological data should have been carefully analyzed and should confirm the scientific finding. The trial should not be biased and should be able to be executed without unreasonable caveats and conditions.

### 6.2.3 Fair Subject Selection

Selection of subjects is based on scientific objectives and not on whether the subject is privileged or vulnerable or because of convenience (Exhibit 6.1). Inclusion and

**Exhibit 6.1 An Example of an Early Clinical Trial**

In 1917, comparative studies were carried out in Georgia, USA, to evaluate the effects of diets on children with pellagra.

Children were selected from orphanages. This practice would not be allowed today, as institutionalized children who could not defend their rights were taken advantage of.

*Source*: Data from National Institutes of Health 2003, *What Makes Clinical Research Ethical?*, viewed March 31, 2014, http://www.bioethics.nih.gov/slides/10-29-03-Emmanuel.pdf

exclusion criteria are well thought out and designed solely to satisfy the scientific basis being put forward. There must be documented evidence to support the choice of selection criteria.

### 6.2.4 Informed Consent

Subjects are to be informed about the aims, methods, risks, and benefits of the trial. The availability of alternatives should be explained to the subjects. Subjects should not be pressured into enrolling in the trial, but rather should voluntarily join in, and that they are able to leave the trial at any time without duress or penalty. For young and incapacitated people who are not able to understand the requirements and implications of the trial, proxy decision from their representatives (parents or guardians) must be obtained. An example of the format for an Informed Consent Form recommended by the World Health Organization (WHO) is presented in Exhibit 6.2.

### 6.2.5 Favorable Risk–Benefit Ratio

The risk–benefit ratio should be analyzed and, wherever possible, clinical trial subjects should be exposed to minimal risk and maximal benefit. The risk–benefit ratio should be based on proven scientific data gathered at the preclinical stage. A clinical trial should not be conducted if there is doubt about the risk–benefit ratio.

### 6.2.6 Independent Review Board/Independent Ethics Committee (IRB/IEC)

An independent review is to ensure that a separate party assesses the clinical trial so the question of conflict of interest is addressed. The IRB/IEC acts as a third party to oversee the welfare of the trial subjects and ensure that the trial is conducted in accordance with the study being put forward.

The members of IRB/IEC may consist of clinicians, scientists, lawyers, religious leaders, and laypeople to represent different viewpoints and protect the rights of the subjects. The investigator is to inform the IRB/IEC if there are changes in the research activity. Such changes, if they present risks to the subjects, have to be approved before the trial continues. The IRB/IEC has the right to stop a trial or require that procedures and methods be changed.

**Exhibit 6.2 Format for an Informed Consent Form**

Institution Letterhead
Informed Consent form for _____
Name of Principal Investigator
Name of Organization
Name of Sponsor
Name of Proposal and Version

Part I: Information Sheet
- Introduction
- Purpose of the Research
- Types of Research Intervention
- Participant Selection
- Voluntary Participation
- Information of the Trial Drug
- Procedure and Protocol
- Duration
- Side Effects
- Risks
- Benefits
- Reimbursements
- Confidentiality
- Sharing of Results
- Right to Refuse or Withdraw
- Alternatives to participating
- Who to Contact

Part II: Certificate of Consent
- Consent Statement by Participant (including oral consent by illiterate participant)
- Signature (Thumb-print by illiterate participant) and Date by Participant
- Statement by the Researcher/Person taking Consent
- Signature and Date by Researcher/Person

*Source*: Data from World Health Organization 2014, *Informed Consent Form Templates*, viewed March 31, 2014, http://www.who.int/rpc/research_ethics/informed_consent/en/

### 6.2.7 Respect for Human Subjects

Subjects should be protected and their progress in the trial monitored closely, and appropriate treatments should be provided. New developments in the trial, either risks or benefits, must be relayed to the subjects without prejudice, and the subject's decisions should be honored.

Outcomes from the trial must be communicated to the subjects promptly and in an unbiased way. In addition to the ethical guidelines by the NIH, the World Medical Association has formalized a document called the *Declaration of Helsinki—Ethical Principles for Medical Research Involving Human Subjects* to describe the constraints on research involving human beings. Those countries that have signed this declaration are bound by the ethical principles. An extract of this document is given in Exhibit 6.3.

## 6.3 CLINICAL TRIALS

Clinical trials are divided into four phases. These are Phase I–IV (Figure 6.1). These trials are conducted with specific purposes to evaluate the safety and effectiveness of the drug in defined population groups. A recent proposal is to conduct "Phase 0" – the trialing of microdose on subjects. Exhibit 6.4 provides more details on this new topic.

### 6.3.1 Phase I

The Phase I clinical trial is the first experiment in which a drug is tested on the human body. The primary aim of the trial is to assess the safety and tolerability of the experimental drug. Other areas of study are pharmacokinetics (absorption, distribution, metabolism, and excretion), pharmacodynamics, and drug activity.

Normally, healthy volunteers are recruited for the Phase I trial. In many cases, volunteers are compensated financially in terms of travel expenses, lost work time, and other reimbursements for participation in the Phase I trial. However, in some situations, patients who are critically ill or with terminal disease are presented with the option to be included in the trial after due consideration of the risk–benefit ratio. Phase I trials are usually conducted with open label, that is, the subjects are aware of the drugs that they are being given.

The typical number of subjects recruited for Phase I trial is 20–80 people. The starting doses are based on the results of preclinical work as described in Chapter 5. Doses are increased as the trial progresses for subjects recruited at later stages, as the effect of the experimental drug becomes apparent. Subjects are monitored closely to check their tolerance of the drug and incidents of side effects. Depending on the study, samples of blood, urine or stool, and other physiological information may be obtained for analysis to evaluate absorption, distribution, metabolism, and elimination of the drug in the body. Other observations about how the subject feels (e.g., pain, headache, fever, malaise, and irritability), vital signs (blood pressure, heart rate), and behavioral matters are taken into account.

Depending on the complexity of the trial, the cost for Phase I is around US$10 million, and the trial may last from several months to a year.

**Exhibit 6.3 World Medical Association Declaration of Helsinki**

***Ethical Principles for Medical Research Involving Human Subjects***

The World Medical Association has developed the Declaration of Helsinki as a statement of ethical principles to provide guidance to physicians and other participants in medical research involving human subjects. Medical research involving human subjects includes research on identifiable human material or identifiable data.

It is the duty of the physician to promote and safeguard the health of the people. The physician's knowledge and conscience are dedicated to the fulfillment of this duty.

The Declaration of Geneva of the World Medical Association binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares, "Medical progress is based on research, which ultimately must rest in part on experimentation involving human subjects. In medical research on human subjects, considerations related to the well being of the human subject should take precedence over the interests of science and society."

The primary purpose of medical research involving human subjects is to improve prophylactic, diagnostic and therapeutic procedures, and the understanding of the etiology and pathogenesis of disease. Even the best proven prophylactic, diagnostic, and therapeutic methods must continuously be challenged through research for their effectiveness, efficiency, accessibility, and quality.

In current medical practice and in medical research, most prophylactic and diagnostic and therapeutic procedures involve risks and burdens.

Medical research is subject to ethical standards that promote respect for all human beings and protect their health and rights. Some research populations are vulnerable and need special protection. The particular needs of the economically and medically disadvantaged must be recognized. Special attention is also required for those who cannot give or refuse consent for themselves, for those who may be subject to giving consent under duress, for those who will not benefit personally from the research and for those for whom the research is combined with care.

Research investigators should be aware of the ethical, legal, and regulatory requirements for research on human subjects in their own countries as well as applicable international requirements. No national ethical, legal, or regulatory requirement should be allowed to reduce or eliminate any of the protections for human subjects set forth in this Declaration.

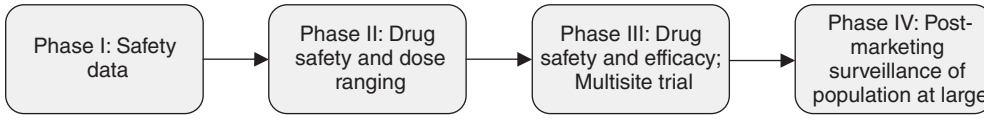



**Figure 6.1** The four phases of clinical trials.

**Exhibit 6.4 Phase 0, Microdosing**

The term microdosing, or Phase 0, refers to the *in vivo* testing of drug candidates in humans at very low dosages. Typically, the dosages are 100 times less than the intended therapeutic dose. At this dosage whole-body reaction is unlikely to happen, yet with sensitive analytical techniques, cellular responses can be studied. Through this, pharmacokinetics are evaluated at very low risk. Such study can be carried out before much time and expenses are spent on nonclinical animal studies and enable viable drugs to be identified earlier and with less costs.

For this method, the drug candidate is labeled with radioisotope, such as carbon-14. The ADME of the compound within the body can be monitored by analyzing samples using high sensitivity instrumentation, for example, accelerator mass spectroscopy.

*Source*: Data from Garner, RC and Lappin, G 2006, 'The phase 0 microdosing concept', *British Journal of Clinical Pharmacology*, 61, pp. 367–70.

### 6.3.2 Phase II

The aim of the Phase II clinical trial is to examine the safety and effectiveness of the drug in the targeted disease group. A series of doses of varying strengths may be used. Sometimes this is referred to as therapeutic exploratory trial.

It is now common to conduct Phase II trials with a control group in conjunction with the test group given the drug. The control group is given either the current standard treatment or placebo (an inert nondrug substance). Again, the risk–benefit profile has to be assessed as to whether the trial should use placebo or standard treatment to ensure the subjects' well-being is not compromised during the trial.

In situations where there is no effective treatment for the targeted disease, the use of placebo in the trial does not pose ethical concerns. However, when effective treatment is available for the targeted disease, there is ethical acceptability question to be addressed when placebo is used. It is required that the use of placebo should not cause serious harm or irreversible morbidity to the subjects.

Patients are randomized to either the control group (with standard treatment or placebo) or the drug group without bias. The randomization procedure is important, because the information will provide comparative data about the safety and effectiveness of the drug versus standard treatment or placebo. Phase II clinical trials can be divided into IIa and IIb. Phase IIa is concerned with proof of concept, efficacy, and mechanistic studies, and Phase IIb is a dose-range-finding study with efficacy as the primary endpoints.

Another practice is to blind the trial, which means that the subjects are not privy to whether they receive the placebo or drug. In some trials, even the investigator is unaware of whether the subject is in the control or active group. This is called a double-blind trial. The rationale is to eliminate the possibility of bias affecting the trial results.

The result of the Phase II trial is the information needed to determine the effective dose and the dosing regimen of frequency and duration. Specific clinical endpoints

or markers are used to assess interaction of drug and disease. There are two types of markers: definitive and surrogate. For example, in the case of cancer or hypertension, the definitive markers are mortality and stroke, respectively, and the surrogate markers may be tumor size, or cancer-associated proteins p53, TGF-$\alpha$ in the case of cancer, and blood pressure or cholesterol level in hypertension. Statistical analysis is carried out to evaluate the influence of the drug on different patient groups, to determine the optimum conditions.

For Phase II, the number of patients is normally in the vicinity of 50–500. The trial may take 1–2 years or more to complete, depending on the study numbers and availability of patients. The cost for such a trial can be more than US$20 million.

The success rate of Phase I and II studies is estimated as around 20–30%. An example of a Phase II trial is presented in Exhibit 6.5.

**Exhibit 6.5 Phase II Study: Effect of Insulin Sensitizer Metformin on AD Biomarkers**

Clinical and preclinical evidence proves that brain insulin resistance may play a role in the pathogenesis and/or progression of Alzheimer's disease (AD) and that ameliorating insulin action in the brain may benefit cognition symptomatically and modify disease pathology.

*Study Type:* Interventional

*Study Design:*

- Allocation: Randomized
- Endpoint Classification: Safety/Efficacy Study
- Intervention Model: Crossover Assignment
- Masking: Double Blind (Subject, Caregiver, Investigator, Outcomes Assessor)
- Primary Purpose: Treatment

*Official Title:* A Phase II Trial to Study the Effect of Metformin on AD Biomarkers: A Randomized Placebo-Controlled Crossover Pilot Study of Metformin Effects on Cognitive, Physiological, and Biochemical Biomarkers of MCI and Dementia due to AD127 or early dementia due to AD128

*Primary Outcome Measures:*

- Cognitive Biomarker Outcomes – AD Assessment Scale – Cognitive subscale

*Secondary Outcome Measures:*

- Neurophysiological Biomarker Outcome – Cogstate Computerized Psychometric battery Dementia Rating Scale

*Other Outcome Measures:*

- Biochemical Biomarker Outcome – Brain MRI, Lumbar Puncture for CSF

*Estimated Enrollment:* 30

*Study Start Date:* January 2013

*Estimated Study Completion Date:* December 2016
*Eligibility:*

- Ages Eligible for Study: 55–80 years
- Genders Eligible for Study: Both
- Accepts Healthy Volunteers: No

*Inclusion Criteria:*

- Ages: 55–80 years
- Sex distribution: male and female
- Diagnosis of MCI due to AD127 or early dementia due to AD128 with: (i) age 55–80, (ii) complaint of cognitive decline, (iii) abnormal performance on the Logical Memory subtest of the Wechsler Memory Scale, (iv) MMSE >21, (v) CDR 0.5–1, (vi) positive topographic (MRI, FDG-PET) or molecular (CSF, amyloid imaging) biomarker consistent with AD, and (vii) no history of diabetes or other exclusions
- Fluent in English or Spanish
- Education >5, literate, and/or good working history that precludes consideration of mental retardation
- Visual and auditory acuity sufficient for neuropsychological testing and auditory evoked potential EEG
- Geriatric Depression Scale <6
- Modified Hachinski Ischemic Score <4
- No major health issues or diseases expected to interfere with the study
- Willing to complete all baseline assessments and study procedures
- Stable on all permitted medications for 8 weeks
- Not pregnant, lactating, or of child-bearing potential (women must be >2 years postmenopausal or surgically sterile)
- No history of diabetes
- Fasting blood glucose <126 and/or HgbA1c <6.4
- Study partner with frequent contact with patient willing to accompany patient to visits and complete partner study forms
- No contraindication to metformin

*Exclusion Criteria:*

- Any CNS disease other than suspected incipient AD, such as clinical stroke, brain tumor, normal pressure hydrocephalus, brain tumor, multiple sclerosis, significant head trauma with persistent neurological of cognitive deficits or complaints, Parkinson's disease, frontotemporal dementia, or other neurodegenerative diseases
- Screening/baseline MRI scans with evidence of infarction or other focal lesions in critical memory structures that may be related to cognitive dysfunction

- Major active psychiatric illness (e.g., depression, bipolar disorder, obsessive compulsive disorder, schizophrenia) within the previous years
- History of alcohol or other substance abuse or dependence within the past 2 years
- Pacemakers, aneurysm clips, artificial heart valves, ear implants, metal fragments or foreign objects in the eyes, skin, or body or claustrophobia that would preclude MRI scanning
- History of past or current diabetes, pancreatic or liver disease, renal disease
- Any significant systemic illness or unstable medical condition that could affect compliance with study
- Laboratory abnormalities in B12, TFTs, RPR, Lyme, or other common lab parameters that might contribute to cognition or participation in study
- Coagulopathy or anticoagulant therapy (such as coumadin) increasing the risk for LP resulting in PT/PTT and INR within 1.5 standard deviations over the upper normal limit
- Compromised renal function at screening as determined by creatinine clearance <30 mL/min on the basis of Cockcroft-Gault calculation
- Liver dysfunction at screening as evidenced by alanine transaminase (ALT/SGPT) values >2× upper limit of normal or aspartate transaminase (AST/SGOT) values >3× or total bilirubin >2×
- Has received acetylcholinesterase inhibitor and/or memantine and/or any other medicine that affects the central nervous system for less than 4 months or has less than 2 months stable therapy on these treatments by baseline visit
- Current use of specified medications with psychoactive properties that deleteriously affect cognition (e.g., certain antidepressants, anticholinergics, antihistamines, antipsychotics, sedative hypnotics, anxiolytics)
- Use of investigational agents 1 month prior to entry and for the duration of the trial
- Exceptions to these guidelines may be considered on a case-by-case basis at the discretion of the protocol director.

*Source*: Data from National Institutes of Health 2013, *ClinicalTrials.gov, Effect of Insulin Sensitizer Metformin on AD Biomarkers*, viewed March 31, 2014, http://clinicaltrials.gov/ct2/show/NCT01965756?term=phase+ii+alzheimer&rank=37

### 6.3.3 Phase III

After the successful completion of the Phase II trial, the objective of Phase III is to confirm the efficacy (therapeutic confirmatory) of the drug in a large patient group. Phase III is an extension of Phase II, and the trial is normally conducted in several hospitals in different demographic locations, to determine the influence of ethnic

responses, together with incorporation of new criteria for fine-tuning the trial. This trial is also known as a multisite trial.

Because the results are crucial to the determination of the drug's effectiveness, the Phase III trial is referred to as the pivotal trial, as it can make or break the success of a drug. The methodology of the trial has to be carefully prepared so that meaningful results can be gathered at the conclusion of the trial. Extensive statistical analyses are performed to evaluate the data. If for any reason the drug does not show significant advantage over current treatment, the result may be refined and certain subgroups are analyzed to determine if the effects are greater in one group than another. The study results provide comprehensive data for understanding the critical parameters of safety and effectiveness of the drug.

These results enable the pharmaceutical company to set the dosage, treatment frequency, duration, and target patient groups for the drug. The information and analyses gathered, together with preclinical data on safety and the Chemistry, Manufacturing, and Control information (CMC – manufacturing information, refer to Chapter 8), are submitted to regulatory authorities to seek approval to market the drug. An example of a Phase III trial is presented in Exhibit 6.6.

**Exhibit 6.6 Phase III Study: Clinical Study of Efficacy and Safety of Vaccae™ to Prevent Tuberculosis**

Mycobacterium Vaccae for Injection (Trade Name "Vaccae") is a kind of bioproducts developed by Anhui Zhifei Longcom Biopharmaceutical Co. Ltd. and received "The New Drug Certificate" in 1999. Vaccae has been approved for adjuvant therapy of tuberculosis (TB) and is also the only recommended drug in TB immunotherapy by WHO. It was approved for production and sale by Anhui Zhifei Longcom Biopharmaceutical Co. Ltd. in 2001, and received favorable comment in the therapy of tuberculosis.

The purpose of this study is to add new indications for Vaccae, mainly to prevent Tuberculosis for high-risk groups of Tuberculosis Infection. In December 2012, China Food and Drug Administration approved of the planned Phase III Clinical Study of Efficacy and Safety of Mycobacterium Vaccae to Prevent Tuberculosis in high-risk groups of Tuberculosis Infection. In the test, 10,000 cases whose skin tests of Pure Protein Derivative (PPD) are strongly positive are enrolled. Using random, double-blind, and placebo-controlled methods, the study is carried out to evaluate the efficacy and safety of Vaccae in preventing Tuberculosis. Meanwhile, in this test, TB incidence and degree of pathological changes of experimental group are lower than that of control group, and no drug-related Serious Adverse Events (SAEs) are reported in treatment groups.

*Study Type:* Interventional

*Study Design:* Allocation: Randomized Endpoint Classification: Safety/Efficacy Study Intervention Model: Parallel Assignment Masking: Double Blind (Subject, Investigator) Primary Purpose: Prevention

*Official Title:* Phase III Clinical Study of Efficacy and Safety of Mycobacterium Vaccae to Prevent Tuberculosis in High-Risk Groups of Tuberculosis Infection

*Primary Outcome Measures:*

- The whole TB incidence after injection of Vaccae [Time Frame: Terminal Stage: 2 years after the last group of subjects enrolled] [Designated as safety issue: Yes]
- Medium-term: 1 year after the last group of subjects enrolled or after observation of 38 cases of the disease, Terminal Stage: 2 years after the last group of subjects enrolled or after observation of 76 cases of the disease.

*Secondary Outcome Measures:*

- Lesion degree (Bacteriology indicators, cavity) of patients [Time Frame: 2 years after the last group of subjects enrolled or after observation of 76 cases of the disease] [Designated as safety issue: Yes]
- Medium-term: 1 year after the last group of subjects enrolled or after observation of 38 cases of the disease, Terminal Stage: 2 years after the last group of subjects enrolled or after observation of 76 cases of the disease
- Systemic and local reactions and adverse events [Time Frame: within 30 days after last dosing] [Designated as safety issue: Yes] within 30 days after last dosing
- The relation between skin test results and paroxysm of TB-PPD [Time Frame: Terminal Stage: 2 years after the last group of subjects enrolled or after observation of 76 cases of the disease] [Designated as safety issue: Yes]
- Medium-term: 1 year after the last group of subjects enrolled or after observation of 38 cases of the disease, Terminal Stage: 2 years after the last group of subjects enrolled or after observation of 76 cases of the disease

Total Enrollment: 10,000

Study starting date: September 2013

Estimated Study Completion date: April 2016

*Eligibility:*

Ages Eligible for Study: 15–65 years old, all genders

*Inclusion Criteria:*

- Aged from 15 to 65 years old, all genders.
- Skin test of Tuberculin Pure Protein Derivative (TB-PPD) is strongly positive (the average diameter of PPD skin test induration is greater than or equal to 15 mm, and (or) local blisters, necrosis)
- Agreed to participate in the test and sign the informed consent
- Subjects agreed to participate in the experiments and voluntarily signed the informed consent (guardians of 15–17-year-old subjects should agree, meanwhile)

- The subjects should comply with the requirements of the clinical trial protocol and be followed
- Have not participated in any other clinical trial for nearly three months
- Women of childbearing age from 15 to 49 years should agree with urine pregnancy tests and take effective birth control measures in 2 years after the medication
- Axillary temperature is normal.

*Exclusion Criteria:*

**The first injection drug exclusion criteria:**

- Suffering from any other serious disease, for example, during cancer treatment, autoimmune disease, progressive atherosclerosis, diabetes accompanied with complications, chronic obstructive pulmonary disease (COPD) needing oxygen therapy, acute or progressive liver or kidney disease, congestive heart failure, etc.
- Known allergy to experiment drugs
- People with history of specific diagnosis of TB, extrapulmonary tuberculosis, or have been cured
- People have history of allergy, convulsions, epilepsy, cerebropathy, neurological symptoms and signs
- Patients who have impaired or abnormal immune function, for example, patients treated with immunosuppressor or immunopotentiator, received immunoglobulin preparation or blood products or plasma extraction outside the gastrointestinal tract in 3 months, human immunodeficiency virus, or related diseases
- Oral corticosteroids
- Patients who have been using oral corticosteroids for more than 1 week, or hormone medication *in vitro* for a long time
- Acute febrile illness and infection
- Pregnant or lactating women, or women who have birth plan in following 2 years
- Any other cases that may influence the test evaluation.

**The second–sixth injection drug exclusion criteria:**

- Subjects whose compliance is poor and cannot take medicine on time or according to the amount
- Patients who are using medicine and food that can influence the result
- Pregnant subjects during the test
- Patients who are reluctant to continue and require exit.

*Source*: Data from National Institutes of Health 2013, ClinicalTrials.gov, *Clinical Study of Efficacy and Safety of VaccaeTM to Prevent Tuberculosis*, viewed March 31, 2014, http://clinicaltrials.gov/ct2/show/NCT01979900?term=Phase+III&rank=32

Patient numbers for Phase III can vary from several hundreds to thousands. The larger number is normally for trials involving infectious diseases such as influenza or vaccines, as these may require recruitment of up to tens of thousands of people to provide a larger sample size for detecting "rare" but serious side effects. Statistical proof to show the efficacy of the drug at the targeted patient group has to be established. At least two Phase III trials need to be conducted. Because of the magnitude of the trial, the duration may be 3–5 years and the cost is around US$50–100 million. Refer to Section 6.10 Case Study #6.1 for more information on the Plavix and Gardasil trials.

### 6.3.4 Phase IV

Phase IV clinical trials are postmarketing approval trials to monitor the efficacy and side effects of the drug in an uncontrolled real-life situation where the drug is prescribed to the wider patient population. This is also known as a postmarket surveillance trial. Information about the effectiveness of the drug compared with established treatment, side effects, patient's quality of life, and cost-effectiveness is collated.

## 6.4 REGULATORY REQUIREMENTS FOR CLINICAL TRIALS

Clinical trials are performed under Good Clinical Practice (GCP). Up to now, there has been no reference to the regulatory requirement. The reality is that every trial has to be approved by the regulatory authority in the country or region where the clinical trial is to be conducted. Moreover, the trial must be carried out in compliance with their regulatory requirements to comply with GCP. Otherwise, the trials may be considered as noncompliant and become invalid.

A normal course of event in initiating a clinical trial is for the sponsor (see below) to prepare an Investigator's Brochure and select an investigator to conduct the trial. The sponsor and investigator then prepare the trial protocol, which is submitted to the Institutional Review Board or Independent Ethics Committee for approval. An application to the regulatory authority, such as the US Food and Drug Administration (FDA) or the Medicines and Healthcare Products Regulatory Agency (MHRA) of the United Kingdom, is then submitted (refer to Chapter 8).

Different countries have different requirements for clinical trials. However, the two main documents that most clinical trials are based on are the documents from FDA and ICH. The relevant documents are:

- FDA 21 CFR Parts 50, 56, 312
- ICH Harmonized Tripartite *Guideline for Good Clinical Practice, E6*. Table 6.1 shows the ICH documents related to clinical trials (refer to Section 7.11 for more information about ICH).

In the United States, an Investigational New Drug (IND) application has to be filed with FDA. For other countries, a notification has to be submitted to the respective regulatory authorities. For example, Clinical Trial Exemption (CTX) applications are

**TABLE 6.1 ICH Clinical Study Efficacy Guidelines**

| Document | Title |
|---|---|
| E1 | The extent of population exposure to assess clinical safety for drugs intended for long-term treatment of non-life-threatening conditions |
| E2A | Clinical safety data management: definitions and standards for expedited reporting |
| E2B (R3) | Clinical safety data management: data elements for transmission of individual case safety reports |
| E2c (R2) | Periodic benefit–risk evaluation report |
| E2D | Postapproval safety data management: definitions and standards for expedited reporting |
| E2E | Pharmacovigilance planning |
| E2F | Development safety update report |
| *Clinical study reports* | |
| E3 | Structure and content of clinical study reports |
| E4 | Dose-response information to support drug registration |
| *Ethnic Factors* | |
| E5 (R1) | Ethnic factors in the acceptability of foreign clinical data |
| *Good clinical practice* | |
| E6 (R1) | Good clinical practice |
| *Clinical trials* | |
| E7 | Studies in support of special populations: geriatrics |
| E8 | General consideration of clinical trials |
| E9 | Statistical principles for clinical trials |
| E10 | Choice of control group and related issues in clinical trials |
| E11 | Clinical investigation of medicinal products in the pediatric population |
| *Guidelines for clinical evaluation by therapeutic category* | |
| E12 | Principles for clinical evaluation of new antihypertensive drugs |
| *Clinical evaluation* | |
| E14 | The clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs |
| *Pharmacogenomics* | |
| E15 | Definition for genomic biomarkers, pharmacogenomics, pharmacogenetics, genomic data, and sample coding categories |
| E16 | Biomarkers related to drug or biotechnology product development: content, structure, and format of qualification submissions |

*Source*: International Conference on Harmonization 2014, *Efficacy Guidelines*, viewed March 27, 2014, http://www.ich.org/products/guidelines/efficacy/article/efficacy-guidelines.html.

required for the UK, Clinical Trial Notification (CTN) and CTX for Australia, and a Clinical Trial Certificate (CTC) for Singapore, and the European Medicines Agency (EMEA). A more extensive discussion concerning regulatory authorities and the processes and procedures of applications is presented in Chapters 7 and 8. The relevant authority will review the application. Clinical trial may commence 30 days after an IND application is filed with FDA. FDA reviews the IND for safety to ensure subjects are not exposed to unreasonable risks and may put a clinical hold on commencement of the trial until queries are satisfactorily resolved.

The scope of this book does not allow a discussion on all the requirements for GCP here. Readers are referred to Exhibit 6.7 for the headings in the relevant regulatory documents to gain further understanding of the requirements. Some important issues are, however, discussed below to clarify the important aspects and requirements for clinical trials in accordance to GCP. Some of these aspects are:

- Investigator
- Investigator's brochure
- Informed consent
- Protocol
- Inclusion and exclusion criteria
- Case report form
- Randomization, placebo-controlled and double-blinded
- Monitoring
- Adverse events
- Statistics
- Sponsor
- Clinical research organization
- Surrogate markers.

### 6.4.1 Investigator

The investigator is the person who conducts the trial. If there is a team in the investigation, then there is a Principal Investigator. This person is normally an expert in the field of the disease to be investigated. The investigator's responsibility is to ensure that GCP is being implemented in the course of the trial and the subjects' rights and welfare are respected. Another important point is that the investigator has to maintain impartiality. He or she is not an employee of the company (the sponsor where the drug is developed), to show that there is transparency and no conflict of interest, nor is there financial gain if the drug is successful.

### 6.4.2 Investigator's Brochure

The Investigator's Brochure is a collection of information prepared and updated by the sponsor for the investigator. The information consists of all the data relevant to the drug under investigation, including properties of the drug, the PK and PD, and toxicity results on animals (Exhibit 6.8).

**Exhibit 6.7 Examples of GCP Requirements**

***Main Heading from 21CFR Part 50 Protection of Human Subjects***

Subpart A – General Provisions
Subpart B – Informed Consent of Human Subjects
Subpart C – Protection Pertaining to Clinical Investigations Involving Prisoners as Subjects

***Main Heading from 21CFR Part 312 – Investigational New Drug Application***

Subpart A – General Provisions
Subpart B – IND
Subpart C – Administrative Actions
Subpart D – Responsibilities of Sponsors and Investigators
Subpart E – Drugs Intended to Treat Life-threatening and Severely debilitating Illnesses
Subpart F – Miscellaneous
Subpart G – Drugs for Investigational Use in Laboratory Research Animals or *In Vitro* Tests.

***Main Heading from 21CFR Part 56 – Institutional Review Board***

Subpart A – General Provisions
Subpart B – Organization and Personnel
Subpart C – IRB Functions and Operations
Subpart D – Records and Reports
Subpart E – Administrative Action for Noncompliance

***ICH Harmonized Tripartite Guideline for Good Clinical Practice***

*Section II*
Introduction
Glossary
The Principle of ICH GCP
Institutional Review Board/Independent Ethics Committee (IRB/IEC)
Investigator
Sponsor
Clinical Trial Protocol and Protocol Amendments
Investigator's Brochure
Essential Documents for the Conduct of a Clinical Trial

**Exhibit 6.8 Investigator's Brochure**

***Description of the drug***

Physical, chemical, and biological properties
Dosage form, storage conditions, stability

***Pharmacology***

Pharmacodynamics
Pharmacokinetics
Toxicology

*Source*: Data from International Conference on Harmonization 1996, *Guideline for Good Clinical Practice, E6 (R1)*, viewed March 31, 2014, http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E6_R1/Step4/E6_R1__Guideline.pdf

### 6.4.3 Informed Consent

This is described earlier in Section 6.2.4 and is a fundamental aspect that has to be included in a clinical trial.

### 6.4.4 Protocol

This document sets out how a trial is to be conducted. It contains the rationale for the clinical trial, methodology on how the trial is designed, the number of subjects to be recruited, the biomarkers (refer to Exhibit 6.9) or endpoints to show effectiveness of the drug, statistical methods to be used to analyze the data, how the subjects are protected in the trial, informed consent and confidentiality, as well as welfare and frequency of monitoring. Exhibit 6.10 summarizes the required information for a protocol.

### 6.4.5 Inclusion and Exclusion Criteria

These criteria set out the conditions under which a person may or may not be included in the trial. The criteria may mention the disease type, medical history, age group, sex, and so on. It is necessary to set out the parameters for the criteria to enable meaningful analysis to be made for assessment of the safety and effectiveness of the experimental drug. Subjects are screened before commencement to ensure that they meet the recruitment criteria before being admitted to the trial.

### 6.4.6 Case Report Form

All the information as set out in the protocol relating to a subject is recorded in the Case Report Form (CRF). The commencement of the trial will include gathering baseline

**Exhibit 6.9 Biomarkers**

According to the Biomarker Definitions Working Group, a biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. Biomarkers are used to measure a patient's pharmacological response to a drug to indicate the safety and effectiveness of the drug. They represent the end points of the patient's state of health.

In clinical trials, biomarkers are used to indicate a particular disease state and its progression. They may be used as surrogate markers in the evaluation of the effectiveness of a drug as representative of the natural endpoint such as survival rate or irreversible morbidity.

Biomarkers include, for example, cholesterol level, blood pressure, viral load, enzyme concentration, tumor size, and so on.

Appendix 8 tabulates some of the biomarkers regularly tested in the laboratory.

Source: Data from Biomarkers Definitions Working Group 2001, 'Biomarkers and Surrogate Endpoints: Preferred Definitions and Conceptual Framework', *Clinical Pharmacology and Therapeutics*, 69, pp. 89–95.

data from the subjects. Then at each defined stage of a trial, the designed markers or endpoints are analyzed and recorded. These may include dosing information, observations, vital signs, blood analysis, targeted enzyme levels, hormonal changes, and so on. There are also records for patient's comments, adverse events, and investigator's spontaneous comments. The CRFs are part of the regulatory document, and the data are statistically analyzed and submitted to regulatory authorities for marketing approval of the drug. All CRFs must have the study number, site/center number, and subject identification number. An example of a hypothetical CRF is presented in Exhibit 6.11.

### 6.4.7 Randomization, Placebo-Controlled, and Double-Blinded

Some trials are conducted with open labels, that is, the subjects are aware of the type of drugs that they have been provided. However, in most trials, the subjects are divided into treatment and control groups using statistical randomization process (Exhibit 6.12). The aim is to reduce bias in the studies.

In a double-blinded study, both the investigator and the subjects are unaware of whether they receive the drug or the placebo. The randomization code is held in confidence and is opened at the end of the trial for data analysis or in cases where adverse events or death occurred.

### 6.4.8 Monitoring

An important aspect of the trial is the meticulous monitoring required. This is a process to interact with the subjects: monitoring their well-being, the effects of drug and placebo, adverse events, and so on. Information is recorded in the CRFs. All the

**Exhibit 6.10 Clinical Trial Protocol**

***Information to be included (ICH GCP)***

Protocol title
Name and address of Sponsor and Monitor
Name of authorized person
Name of Sponsor's medical expert
Name of Investigator responsible for the trial
Name of physician responsible for trial-related medical decisions
Name of Clinical Laboratory and other Institutions involved in the trial
Name and description of the clinical trial protocol
Summary of results from nonclinical studies
Potential risks and benefits to human subjects
Description and justification for route of administration, dosage, and treatment plan
Compliance to GCP
Description of the population to be studied
Reference literature and related data
Standard Operating Procedures.

*Source*: Data from International Conference on Harmonization 1996, *Guideline for Good Clinical Practice, E6(R1)*, viewed March 31, 2014, http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E6_R1/Step4/E6_R1__Guideline.pdf

processes are recorded in accordance with Standard Operating Procedures, which describe how the trial is to be conducted, and GCP.

### 6.4.9 Adverse Events

These are the unintended reactions of the subjects as a consequence of taking the drug or placebo. Subjects are checked and, if the adverse events are serious, subjects may be temporarily removed from the trial. If there is a persistence of adverse event, the subject may be withdrawn from the trial. The randomization code may be broken (opened) to determine whether the subject has been given the drug or placebo. Refer to Exhibit 6.13 for a clinical trial, which resulted in unexpected events. There are regulatory guidelines for accessing the adverse events to enable informed decisions to be made. Some examples of these "toxicity gradings" are presented in Appendix 9.

### 6.4.10 Statistics

Statistics plays a major role in the design of the clinical trial. The groups or subgroups to be studied, frequencies, dosages, and markers used to monitor drug efficacy are all

**Exhibit 6.11 An Example of a Case Report Form**

CASE REPORT FORM

Personal Data
Patient's Last name: _____ First name: _____ Middle initial/s: _____
Age: _____ years Sex: M/F Race: _____ Ethnicity: _____
Address: _____
Telephone number: _____ Email: _____

Study Data
Study number: _____ Site/Center number: _____ IRB number: _____
Patient number: _____
Date of visit: _____ Details: _____
Clinical Data
Height: _____ Weight: _____
Symptoms, signs and adverse reactions: _____
Associated disease history: _____
Medication taken: _____

Laboratory Analysis
Hemoglobin: _____ Platelet count: _____
Bilirubin: _____ ALT: _____
Cholesterol/LDL: _____ Cholesterol/HDL: _____

Other Details:
Patient's comments: _____
Physician's name/address/telephone number: _____
Person completing this form: _____
Signature: _____ Date: _____

important factors to consider. Statistical analysis provides the means to demonstrate, at a certain confidence level, whether the drug is effective. This is normally reported in the form of a statistical power test, analyzing the Type I and Type II errors. A more detailed discussion on statistics in clinical trials is presented in Exhibit 6.14.

### 6.4.11 Sponsor

This is the organization or individual that initiates the clinical trial and finances the study. The organization may be a government department, pharmaceutical company, university, nonprofit organization or individual. Normally, however, the sponsor is a pharmaceutical company.

**Exhibit 6.12 Randomization Techniques**

*Randomized parallel group fixed dose*: Subjects are divided into several groups, such as Placebo, 10 mg, 20 mg, and 40 mg. Subjects continue with this regimen for the duration of the trial.

*Randomized parallel group forced titration*: Subjects are divided into placebo and active groups. Active groups all start with the same dose, for example, 10 mg. One group continues with 10 mg, another group later increases to 20 mg and stays at this dose. A third active group then increases from 10 mg to 20 mg and finally to 40 mg progressively.

*Randomized parallel group optional titration*: Subjects are divided into placebo and active groups. Active groups all start with the same dose, say, 10 mg. Depending on response and safety assessment, dose can be increased to 20 mg and then 40 mg for selected subjects.

*Randomized crossover design*: Subjects are divided into placebo and active groups. After some time these two groups crossover, the initial placebo group now becoming the active group and vice versa. There may be a washout period before the crossover to enable the effect of the placebo and active to washout. This method requires a smaller number of subjects and is useful in cases for studying rare or more stable illnesses.

*Randomized Latin square design*: This is a crossover design with dose ranging. For example, the regimens for six separate groups are: (i) Placebo, 10 mg, 20 mg, (ii) Placebo, 20 mg, 10 mg, (iii) 10 mg, 20 mg, Placebo, (iv) 10 mg, Placebo, 20 mg, (v) 20 mg, Placebo, 10 mg, and (vi) 20 mg, 10 mg, Placebo. This is a very powerful method to show the efficacy of the drug under trial.

*Randomized factorial design*: This is a design where two or more treatments are evaluated simultaneously. The simplest example is the $2 \times 2$ factorial design. For example, there are two treatments, A and B. The possible combinations are (i) Treatment A, (ii) Treatment B, (iii) Combination Treatment A and B, and (iv) No treatment.

*Source*: Data from Monkhouse, DC and Rhodes, CT (eds.) 1998, *Drug Products for Clinical Trials*, Marcel Dekker, Inc., New York.

### 6.4.12 Clinical Research Organization

This is the organization that is contracted by the sponsor to conduct and monitor the trial. It also provides a certain measure of independence to the trial and enhances the validity of the trial results to be unencumbered by conflict of interest.

### 6.4.13 Surrogate Markers

Sometimes it is not possible to measure the direct effect of the drug. Endpoints or surrogate biomarkers are used to monitor the pharmacodynamics and pharmacokinetics of the drug. These markers may be changes in blood pressure, cholesterol level,

**Exhibit 6.13 A Monoclonal Antibody Trial – TGN1412**

TGN1412 (CD28-SuperMAb) is a humanized monoclonal antibody that binds and acts as an agonist for the CD28 receptor of the immune system's T cell. It is intended to treat B cell chronic lymphocytic leukemia (B-CLL) and rheumatoid arthritis.

The first human clinical trial was conducted in March 2006 in the United Kingdom but unexpectedly caused systemic failures in the subjects, even though the dose administered was 500 times lower than the dose that is safe for animals, on a per kilogram basis. Of the eight subjects enrolled, two were on placebo. The six active subjects were hospitalized and four showed signs of multiple organ dysfunction, with one having signs of developing cancer. The trial was stopped.

Later investigation confirmed the subjects experienced a "cytokine storm," and their white blood cells vanished almost completely on administration of the MAb. Because earlier nonclinical work using primates found no visible side effects at significantly higher dose, the case with humans appeared to be totally unexpected. This led the regulatory authority MHRA in April 2006 to suggest that the problem was most likely to be caused by "unforeseen biological actions in humans."

*Source*: Data from Medicines and Healthcare Products Regulatory Agency 2006, *Clinical Final Report on TGN1412*, viewed March 31, 2014, http://webarchive.nationalarchives.gov.uk/20090714014254/http://www.nelm.nhs.uk/en/NeLM-Area/News/488530/488574/488579/

concentrations of certain enzymes, proteins, blood glucose levels, and similar factors (refer to Table 6.2 for serum tumor markers and Appendix 8 for general biomarkers).

**Exhibit 6.14 Statistics for Clinical Trial**

On the basis of the design of the trial protocol, statistics are used to calculate the number of people to be recruited for the trial, how the trial should be randomized (Exhibit 6.12), and finally analysis of the data. Statistics provide a nonbiased means to evaluate the trial results.

The objective of clinical trials is to demonstrate the safety and effectiveness of the drug compared to placebo or control. The statistical method normally used is known as hypothesis testing.

For example, we wish set up the null hypothesis ($H_0$) and claim that there is no difference ($\delta$) between the control or placebo ($\mu_C$) and the drug being trialed ($\mu_D$). This is set against the alternative hypothesis ($H_A$) that states that indeed there is a difference ($\delta$) between the control and drug under trial. Mathematically, the representation is given below:

$$H_0 : \mu_C - \mu_D = 0$$

$$H_A : \mu_C - \mu_D \neq 0$$

where $\mu_C$ and $\mu_D$ stand for the true mean of the population in the control group and the drug group, respectively.

In a trial the parameters for comparison may be the mean level of cholesterol (in the case of a cholesterol lowering drug), the amount of antibodies in the body (a vaccine trial), or the reduction in the size and severity of tumor (a cancer trial). A test statistic is normally used to compute and compare the means for the placebo and active groups. One method is the $z$ distribution for testing the hypothesis with respect to differences in two means:

$$Z = \frac{\overline{x}_D - \overline{x}_C}{\sigma\sqrt{\frac{1}{N_D} + \frac{1}{N_C}}}$$

where $\overline{x}_D$ and $\overline{x}_C$ are the mean of their respective samples, $N_D$ and $N_C$ are the respective sample size for the drug and control groups, and $\sigma$ is the standard error.

Another important aspect is to ensure that we limit the errors in drawing the wrong conclusion. These are described as Type I and Type II errors:

Type I error ($\alpha$, *false positive*) – the probability of wrongly concluding that a difference exists where in fact there is no real difference, thus putting a useless medicine into the market. Normally, a 5% level of significance is chosen, which means there is a 95% confidence in the decision, that is, $\alpha = 0.05$. The value of $\alpha$ may need to be even smaller for the testing efficacy of a potentially dangerous medication. This value for $\alpha$ is customarily a condition required by the regulatory agency and is typically around 0.05–0.1.

Type II error ($\beta$, *false negative*) – the probability of wrongly concluding that there is no difference when in fact there is a difference, which means keeping a good medicine away from patients and manufacturer misses opportunity to market the drug. Type II error is normally limited to 5–20%, that is, $\beta = 0.05$–$0.2$. The boundaries of Type II error are normally set by the company.

This leads to the term Power $(1 - \beta)$, which quantifies the ability of the study to find the true differences of various values of $\delta$. It is the probability of rejecting the null hypothesis when it is false or determining that the alternative hypothesis is true when indeed it is true.

Clinical trials are carried out to show that the null hypothesis is false. The $p$ value is the probability of having an effect by chance if the null hypothesis were actually true. The null hypothesis is rejected in favor of the alternative hypothesis when the $p$ value is less than $\alpha$.

Once the parameters for the hypothesis and Type I and Type II errors are set, the total number of subjects ($2N$) to be recruited to join the trial can be determined by the equation below:

$$2N = \frac{4(Z_\alpha + Z_\beta)^2\sigma^2}{\delta^2}$$

where $Z_\alpha$ and $Z_\beta$ are obtained from tables of the standardized normal distribution for given $\alpha$ and $\beta$; $\sigma$ and $\delta$ are as defined previously.

The number of subjects may need to be more than the calculated figure as the trial has to account for dropouts and subject noncompliances over the duration of the trial.

## 6.5 CLINICAL DATA MANAGEMENT

An important part of clinical trial is clinical data management. This includes data capture, coding, and data presentation.

Data capture is the collection of clinical data onto a database for analysis. An important criterion is that the investigator's record on the CRF must be faithfully transferred

**TABLE 6.2 Serum Tumor Markers**

| Cancer Antigen | Description | Indication |
|---|---|---|
| Cancer antigen (CA) 27.29 | MAb to a glycoprotein present on apical surface of normal epithelial cells; CA 27.29 elevated in 1/3 early stage breast cancer and 2/3 late-stage breast cancer | Breast cancer |
| Carcinoembryonic antigen (CEA) | Oncofetal glycoprotein expressed in normal mucosal cells and overexpressed in adenocarcinoma | Colorectal cancer |
| Cancer antigen (CA) 19.9 | Intracellular adhesion molecule | Primarily pancreatic and biliary tract cancers |
| Alpha-fetoprotein (AFP) | Major protein of fetal serum | Hepatocellular carcinoma and nonseminomatous germ cell tumors |
| Beta subunit human chorionic gonadotropin ($\beta$-hCG) | Glycoprotein hormone | Germ cell tumors |
| Cancer antigen (CA) 125 | Glycoprotein expressed in epithelium | Ovarian cancer |
| Prostate-specific antigen (PSA) | Glycoprotein produced by prostatic epithelium | Prostate cancer |

*Source*: Adapted from Perkins, GL, Slater, ED, Sanders, GK and Prichard, JG 2003, 'Serum Tumor Markers', *American Family Physician*, 68, pp. 1075–1082.

to the database. This includes single or double entry for verification of data to ensure accuracy. Data entry can occur in a central location or remotely from one or more trial sites. The linking of results from a central laboratory, computer systems, or monitoring devices is advantageous but would require control in terms of accuracy and security. Cost, accuracy, speed, security, and regulatory compliance are critical factors for data capture.

Coding is the entry of data from various sources into the database. Data may be in the forms of alphabetic, alphanumeric, and numeric terms. Observations and comments, for example, are coded into standard medical terminologies agreeable and have the same meanings to the healthcare professionals in different countries and acceptable to the regulatory authorities. Coding in standard terminologies thus enables a multitude of activities to be performed: information exchange, searches, analyses, retrievals, sorting, and data manipulations. The standard terminologies adopted are those of the ICH *Medical Dictionary for Regulatory Activities M1* (MedDRA). In addition, MedDRA is in multilingual format, which allows users to code the terminologies in their native languages. Exhibit 6.15 provides further information about the MedDRA structure and terms used (refer to Section 7.11 for more information on ICH).

Data are reviewed by many people, from investigators to biostatisticians, quality personnel, and regulatory authorities. As such the presentation of data should be accurate, clear, concise, and easily understood by the reader. Data can be divided into categorical (e.g., medications, adverse events) or continuous (e.g., cholesterol levels, heart rate), and these can be presented using appropriate tools such as graphs, charts, or tables. The use of patient synopsis forms that show key data for patients and summary presentations with statistics and graphs help reviewers understand the data clearly.

**Exhibit 6.15 MedDRA Terminologies**

To facilitate data entry and retrieval, the MedDRA terminologies are arranged in hierarchical structure. There are five levels of structures as below:

- Lowest Level Terms (LLTs) – more than 70,000 terms, reflect how an observation is reported in practice
- Preferred Terms (PTs) – more than 20,000 terms, single concepts for symptoms, signs, disease diagnoses, therapeutic indications, investigations, surgical or medical procedures, and medical, social, or family history characteristics
- High Level Terms (HLTs) – more than 1,700 terms, related PTs are grouped together into HLTs on the basis of anatomy, pathology, physiology, etiology, or function
- High Level Group Terms (HLGT) – more than 330 terms, HLTs are in turn linked to (HLGTs)
- System Organ Classes (SOC) – 26 terms, HLGTs are grouped into 26 SOCs, which are grouped by etiology (e.g., Infections and infestations), manifestation site (e.g., Gastrointestinal disorders), or purpose (e.g., Surgical

and medical procedures). There is also an SOC accounting for social circumstances.

The 26 SOC are:

- Blood and lymphatic system disorders
- Cardiac disorders
- Congenital, familial, and genetic disorders
- Ear and labyrinth disorders
- Endocrine disorders
- Eye disorders
- Gastrointestinal disorders
- General disorders and administration site conditions
- Hepatobiliary disorders
- Immune system disorders
- Infections and Infestations
- Injury, poisoning, and procedural complications
- Investigations
- Metabolism and nutrition disorders
- Musculoskeletal and connective tissue disorders
- Neoplasms benign, malignant, and unspecified (viz. cysts and polyps)
- Nervous system disorders
- Pregnancy, puerperium, and perinatal conditions
- Psychiatric disorders
- Renal and urinary disorders
- Reproductive system and breast disorders
- Respiratory, thoracic, and mediastinal disorders
- Skin and subcutaneous tissue disorders
- Social circumstances
- Surgical and medical procedures
- Vascular disorders

Examples of coding are:

**Example 1.**

- LTs – Arrhythmia NOS, Arrhythmia, Dysrhythmia, Other specified cardiac arrythmias
- PT – Arrhythmia
- HLT – Rate and rhythm disorders NEC

- HLGT – Cardiac arrhythmias
- SOC – Cardiac disorders

**Example 2.**

- LLT – Influenza
- HLT – Influenza viral infections
- HLGT – Viral infectious disorders
- SOC – Infections and infestations

*Source*: Data from 1. MedDRA 2013, *Understanding MedDRA: The Medical Dictionary for Regulatory Activities*, viewed March 29, 2014, https://www.meddra.org/sites/default/files/main_page_slideshow/meddra2013.pdf, 2. MedDRA 2014, *Introductory Guide, MedDRA Version 17.0*, viewed March 29, 2014, http://www.meddra.org/sites/default/files/guidance/file/intguide_17_0_english.pdf, 3. MedDRA 2010, *Coding with MedDRA*, viewed March 29, 2014, http://www.ich.org/fileadmin/Public_Web_Site/Training/GCG_-_Endorsed_Training_Events/ASEAN_MedDRA_March_2010/DAy_1/JHarrison_Coding_with_MedDRA_ASEAN_Workshop.pdf

## 6.6 ROLE OF REGULATORY AUTHORITIES

Government bodies have on occasions accelerated clinical trials against advice from researchers, in response to public demands (Exhibit 6.16). The climate today is that due diligence regarding safety has to be performed before the drug is administered to human subjects and that clinical trial applications have to be submitted with due notice to the regulatory authorities before the trial commences.

Regulatory authorities play an important and active role to ensure regulatory compliance in the conduct of a clinical trial. Agencies such as FDA inspect clinical studies. An inspection of a trial may reveal that the protocol is not being followed strictly; the investigator may not be involved with the project as much as is expected; there may be a lack of patient care; changes to the protocol may not have been relayed to the IRB, and so on. In such cases, corrective actions have to be implemented immediately and FDA satisfied before the trial can continue. Deficiencies found are reported on Form 583.

## 6.7 GENE THERAPY CLINICAL TRIAL

As genomic research progresses, the possibility of replacing a person's faulty genes with normal genes becomes a reality (refer to Chapter 4). Currently, there are many ethical and scientific issues facing gene therapy.

For a gene therapy clinical trial, FDA requires that the IND be filed as for normal drug trials. However, there are more stringent requirements on the source and tests being carried out on the gene to be inserted into the subject. There is also the need for closer monitoring, from both the investigator and FDA. In addition, FDA has been

**Exhibit 6.16 Polio Vaccine Trial**

In the 1950s, Dr. Jonas Salk and Dr. Albert Sabin from the University of Pittsburgh in the United States worked on polio vaccines. Salk used inactivated polio virus, whereas Sabin developed a live form of polio virus.

Scientists differed as to which method provided the better vaccine. Both Salk and Sabin agreed that more tests were needed before a mass vaccination program could begin.

The National Foundation, which funded the research, and the American public wanted a mass vaccination urgently. The average incidence of polio in the United States in 1949–1953 was 25.7 cases per 1,00,000 children. The National Foundation ordered 27 million doses of the Salk vaccine for a trial, and close to 1 million children were vaccinated (7,49,236 children from Grades 1, 2, and 3 were offered vaccine, and 4,01,974 completed the trial).

The trial was one of the greatest triumphs in medical history. Church bells rang across the country when the trial results were announced. Within 5 years, polio was wiped out from the United States.

*Source*: Data from Meier, P, *The Biggest Public Health Experiment Ever: The 1954 Field Trial of the Salk Poliomyelitis Vaccine*, viewed March 31, 2014, http://www.stat.luc.edu/StatisticsfortheSciences/MeierPolio.htm

conducting safety symposia to educate the investigator on the safety issues of gene therapy. Exhibit 6.17 shows the number of gene therapy trials being conducted up until June 2012.

**Exhibit 6.17 Gene Therapy Trials**

As of June 2012, there were more than 1,800 gene therapy clinical trials completed. The geographical distributions of gene therapy trials in percentage are as below:

| Country | Distribution (%) |
|---|---|
| United States | 63.7 |
| United Kingdom | 11.0 |
| Germany | 4.4 |
| France | 2.9 |
| Switzerland | 2.7 |
| Netherlands | 1.7 |
| Australia | 1.6 |
| Belgium | 1.5 |
| China | 1.4 |
| Canada | 1.3 |
| Other countries | 7.8 |

In terms of diseases targeted, the percentage distribution is as shown below:

| Diseases Targeted | Distribution (%) |
|---|---|
| Cancer (p53, BRCA-1, Fus-1) | 64.4 |
| Monogenic diseases (cystic fibrosis, SCID, chronic granulomatous disease) | 8.7 |
| Cardiovascular diseases (myocardial ischemia, lower limb ischemia) | 8.4 |
| Infectious diseases | 8.0 |
| Nurological diseases | 2.0 |
| Ocular diseases | 1.5 |
| Inflammatory diseases | 0.7 |
| Other diseases | 1.4 |
| Gene marking | 2.7 |
| Healthy volunteers | 2.3 |

As for stages of clinical trials completed, the table below summarizes it:

| Clinical Phases Completed | Distribution (%) |
|---|---|
| Phase I | 59.6 |
| Phase I/II | 19.0 |
| Phase II | 16.7 |
| Phase II/III | 0.9 |
| Phase III | 3.6 |
| Phase IV | 0.1 |
| Single subject | 0.2 |

Refer to Exhibit 4.16 for more information on gene therapy trials.

*Source*: Ginn, SL et al. 2013, Gene therapy clinical trials worldwide to 2012: an update, *The Journal of Gene Medicine*, 15, pp. 65–77. Reproduced with permission of John Wiley &Sons Ltd.

## 6.8 ADAPTIVE CLINICAL TRIAL

The clinical trials explained thus far required that parameters such as sample size, dosage, endpoints are prespecified at the planning stage before the commencement of the trials. Clinical trials are then conducted to gather information about these parameters as planned without deviations. Adaptive clinical trial, however, allows for modifications to trial parameters as more information is accumulated on a continuous basis as the trial progresses.

FDA defines adaptive clinical trial as "a study that includes a prospectively planned opportunity for modification of one or more specified aspects of the study design and

hypotheses based on analysis of data (usually interim data) from subjects in the study". The term prospective means that the adaptation was planned (and details specified) before data were examined in an unblinded manner by any personnel involved in planning the revision.

According to FDA guidelines, an adaptive clinical trial can involve:

- Interim evaluations to stop or to adjust patient accrual
- Interim evaluations to assess stopping the trial early for success, futility, or harm
- Reversing the hypothesis of noninferiority to superiority or vice versa
- Dropping certain study group/s or doses or adjusting doses
- Modification of the randomization rate to increase the probability that a patient is allocated to the most appropriate group/s.

The aim for adaptive clinical trial is to conduct the trial more efficiently as data are analyzed in a progressive manner. Instead of hypothesis testing, Bayesian statistical analyses with many simulations are used. The impact of unblinding during the study can be reduced when only a small group unrelated to the investigator/s or subjects is privy to the data. In general, adaptive clinical trials are more acceptable in the exploratory stage (Phase II) compared to the confirmatory stage (Phase III).

## 6.9 META-ANALYSIS

Meta-analysis is a statistical approach that analyzes the combined results from multiple, independent clinical studies. This even applies to those trials that have different designs and conducted for different purposes. The end result is that a common statistical approach comparing different studies can provide a single outcome for the safety or effectiveness of treatment. The advantages of meta-analysis are that it can provide:

- Generalization of results to a larger population
- Improvement to accuracy of results
- Quantification to inconsistencies of results.

As part of its effort to enhance regulatory science, FDA has conducted a public meeting in late 2013 on "Meta-Analyses of Randomized Controlled Clinical Trials for the Evaluation of Risk to Support Regulatory Decisions." The objective is to receive feedback from the industry and healthcare organizations on scientific approaches for the conduct and assessment of meta-analyses to evaluate safety risks associated with the use of human drugs or biological products. Such safety risks evaluations will impact on FDA's view on the regulatory approval of drugs or certain classes of drugs.

The Cochrane Collaboration is an independent nonprofit organization that performs meta-analyses and systematic reviews of clinical trial data from different studies. The aim is to provide evidence-based information for healthcare professionals and patients to make informed decisions. The topics reviewed may include general safety of certain class of drugs or the effectiveness of a particular type of vaccines.

To conclude this chapter it is important to note that clinical trial success relies not just on the efficacy of the drug alone, but also on safety (side effects) aspects, how the trial is designed and conducted, and the groups of patients targeted. Exhibit 6.18 provides some insights into the extensive efforts put on clinical trials; it also reports on trial failures, showing that the road to success is fraught with uncertainties.

## 6.10 CASE STUDY #6.1

### 6.10.1 Plavix and Gardasil

This case study presents the clinical trials performed for Plavix (an antiplatelet) and Gardasil (a vaccine against cervical cancer).

***Plavix:*** Two trials were carried out to determine efficacy: the CAPRIE and CURE studies.

CAPRIE was a 19,185-patient, 304-center, international, randomized, double-blind, parallel-group trial comparing Plavix (75 mg daily) with aspirin (325 mg daily). The outcome was to compare the first occurrence of new ischemic stroke, new myocardial infarction, or other vascular death. The results are tabulated below:

| | Plavix | Aspirin |
|---|---|---|
| Patients | 9599 | 9586 |
| Ischemic stroke | 438 (4.6%) | 461 (4.8%) |
| Myocardial infarction | 275 (2.9%) | 333 (3.5%) |
| Other vascular death | 226 (2.4%) | 226 (2.4%) |
| Total | 939 (9.8%) | 1020 (10.6%) |

Plavix resulted in overall reduction of outcome events.

The CURE study involved 12,562 patients randomized to receive Plavix (300 mg loading dose followed by 75 mg daily) or placebo and were treated for up to a year. Patient also received aspirin or other standard treatment such as heparin. The results showed that Plavix had 20% relative risk reduction compared with placebo (582 cases of cardiovascular death, myocardial infarction, or stroke) versus 719 cases for placebo.

***Gardasil:*** Four placebo-controlled, double-blind, randomized Phase II and Phase III trials were conducted to evaluate the efficacy. Cervical intraepithelial neoplasia (CIN) and adenocarcinoma *in situ* (AIS), vulvar intraepithelial neoplasia (VIN), and genital warts were used as the surrogate markers.

The two Phase II trials were Protocol 005 ($N = 2391$) and Protocol 007 ($N = 551$). The Phase III studies were FUTURE I (Protocol 013, $N = 5442$) and FUTURE II (Protocol 015, $N = 12{,}157$). Altogether, 20,541 women from 16 to 26 years were enrolled. Subjects were given Gardasil without prescreening for the presence of HPV infection.

Gardasil was efficacious in reducing the episodes of CIN and AIS, as shown in the table below:

**Exhibit 6.18 Reflections on Clinical Trials**

The enormity of clinical trials is staggering. EMA reported that in the period from 2005 to 2011, there were:

- 8,97,891 patients in pivotal trials (38.11% in Europe, 34.05% in North America, 2.58% in Africa, 9.36% in Middle East/Asia Pacific, 4.44% in CIS, 9.36% in Latin America, 2.1% in others)
- 70,291 clinical trial sites in about 106 countries
- 265 GCP inspections
- 485 new MAA applications plus line extensions

In an analysis by the Center for Medicines Research, it reported success of Phase II clinical trials have fallen from 28% (2006–2007) to 18% (2008–2009). The types of failures in Phase II clinical trials were because of:

- Insufficient efficacy (51%)
- Strategic reasons (29%) – targets, inadequate differentiation from more advanced drugs
- Safety reasons (19%)

Of those that failed, 68% were in four therapeutic areas: alimentary/metabolism, cancer, cardiovascular, and neuroscience.

For Phase III the success rate is about 50% in the period 2007–2010. The failures were in:

- Cancer (28%)
- Nervous system (18%)
- Alimentary and/or metabolism, including diabetes and obesity (13%)
- Anti-infectives (13%)

Almost 90% of the failures were because of either lack of efficacy (66%) or safety issues (21%). The efficacy failures can be divided into projects that failed to show a statistically significant improvement versus placebo (32%), an active control (5%) or as an add-on therapy (29%). The primary reason for failure is because of drugs with novel mechanisms in cancer and neurodegeneration.

*Source*: Data from 1. European Medicines Agency 2012, *EMA Update Clinical Trials*, viewed March 31, 2014, http://www.ambafrance-uk.org/IMG/pdf/201210-EMA-Update_Clinical_Trials_-_Sweeney.pdf, 2. Arrowsmith, J 2011, 'Trial watch: Phase II failures: 2008-2010', *Nature Reviews Drug Discovery*, 10, pp. 328–329, 3. Arrowsmith, J 2011, 'Trial watch: Phase III and submission failures: 2007-2010', *Nature Reviews Drug Discovery*, 10, p. 87.

| | Gardasil | | Placebo | | % Efficacy |
|---|---|---|---|---|---|
| Population | *N* | No. of Cases | *N* | No. of cases | (95% CI) |
| HPV 16- or 18-related CIN or AIS | | | | | |
| Protocol 005 | 755 | 0 | 750 | 12 | 100.0 |
| Protocol 007 | 231 | 0 | 230 | 1 | 100.0 |
| FUTURE I | 2201 | 0 | 2222 | 36 | 100.0 |
| FUTURE II | 5306 | 0 | 5262 | 63 | 96.9 |
| Combined protocols | 8493 | 0 | 8464 | 112 | 98.2 |
| HPV 6-, 11-, 16-, 18-related CIN or AIS | | | | | |
| Protocol 007 | 235 | 0 | 233 | 3 | 100.0 |
| FUTURE I | 2241 | 0 | 2258 | 77 | 100.0 |
| FUTURE II | 5388 | 4 | 5245 | 145 | 93.8 |
| Combined protocols | 7864 | 4 | 7865 | 225 | 96.0 |
| HPB 6-, 11-, 16-, or 18-related Genital Warts | | | | | |
| Protocol 007 | 235 | 0 | 233 | 3 | 100.0 |
| FUTURE I | 2261 | 0 | 2279 | 58 | 100.0 |
| FUTURE II | 5404 | 1 | 5390 | 132 | 98.5 |
| Combined protocols | 7900 | 1 | 7902 | 193 | 99.0 |

The analysis of results for prophylactic efficacy is shown in the table below, regardless of whether the women were HPV-naive or not:

| | | Gardasil or HPV 16L1 VLP vaccine | | Placebo | | % Reduction (95% CI) |
|---|---|---|---|---|---|---|
| Endpoints | Analysis | *N* | Cases | *N* | Cases | |
| HPV 16- or 18-related CIN or AIS | Prophylactic efficacy | 9346 | 4 | 9407 | 155 | 97.4 |
| | HPV 16- and/or 18-positive on Day 1 | 2870 | 142 | 2898 | 148 | – |
| | Women regardless of current or prior exposure to HPV 16 or 18 | 9836 | 146 | 99,904 | 303 | 51.8 |
| HPV 16- or 18-related VIN | Prophylactic efficacy | 8642 | 1 | 8673 | 34 | 97.0 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | HPV 16- and/or 18-positive on Day 1 | 1880 | 8 | 1876 | 4 | – |
| | Women regardless of current or prior exposure to HPV 16 or 18 | 8955 | 9 | 8968 | 38 | 76.3 |
| HPV 6-, 11-, 16-, 18-related CIN or AIS | Prophylactic efficacy | 8630 | 16 | 8680 | 309 | 94.8 |
| | HPV 16- and/or 18-positive on Day 1 | 2466 | 186 | 2437 | 213 | – |
| | Women regardless of current or prior exposure to HPV 16 or 18 | 8819 | 202 | 8854 | 522 | 61.5 |
| HPV 6-, 11-, 16- or 18-related Genital Warts | Prophylactic efficacy | 8761 | 10 | 8792 | 252 | 96.0 |
| | HPV 16- and/or 18-positive on Day 1 | 2501 | 51 | 2475 | 55 | – |
| | General population impact | 8955 | 61 | 8968 | 307 | 80.3 |

The immune response of Gardasil was evaluated in 8,915 women from 18 to 26 years old (Gardasil $N = 4666$, Placebo $N = 4249$) and 2,054 adolescents from 9 to 17 years old (Gardasil $N = 1471$, Placebo $N = 583$). Overall, more than 99.5% of the subjects were seropositive with antibodies against HPV 6, HPV 11, HPV 16, and HPV 18. The table below shows the levels of antibodies with and without Gardasil vaccination.

| | Gardasil | | Aluminum Containing Placebo | |
|---|---|---|---|---|
| Study Time | *N* | Antibody Titer (geometric mean) mMU/mL | *N* | Antibody Titer (geometric mean) mMU/mL |
| *Anti-HPV 6* | | | | |
| Month 07 | 208 | 282.2 | 198 | 4.6 |
| Month 24 | 192 | 93.7 | 188 | 4.6 |
| Month 36 | 183 | 93.8 | 184 | 5.1 |
| *Anti-HPV 11* | | | | |
| Month 07 | 208 | 696.5 | 198 | 4.1 |
| Month 24 | 190 | 97.1 | 188 | 4.2 |
| Month 36 | 174 | 91.7 | 180 | 4.4 |
| *Anti-HPV 16* | | | | |
| Month 07 | 193 | 3889.0 | 185 | 6.5 |
| Month 24 | 174 | 393.0 | 175 | 6.8 |
| Month 36 | 176 | 507.3 | 170 | 7.7 |

| | | | | |
|---|---|---|---|---|
| *Anti-HPV 18* | | | | |
| Month 07 | 219 | 801.2 | 209 | 4.6 |
| Month 24 | 204 | 59.9 | 199 | 4.6 |
| Month 36 | 196 | 59.7 | 193 | 4.8 |

*Source*: 1. Food and Drug Administration 2010, *Plavix Highlights of Prescribing Information*, viewed Mar 31, 2014, http://www.accessdata.fda.gov/drugsatfda_docs/label/2010/020839s042lbl.pdf; 2. Food and Drug Administration 2008, *Gardasil Highlights of Prescribing Information*, viewed Mar 31, 2014, http://www.fda.gov/downloads/biologicsbloodvaccines/vaccines/approvedproducts/ucm125879.pdf

## 6.11 CASE STUDY #6.2

### 6.11.1 Design Considerations of a Bipolar Disorder Trial

This case study examines the rationale undertaken by a clinical trial team when designing a bipolar disorder trial.

The factors considered were:

- Selection of study medications – (i) Second generation antipsychotics – quetiapine, the most widely prescribed antipsychotics for bipolar disorder, (ii) Classic mood stabilizer – lithium, an inexpensive classic mood stabilizer to be used as a comparator
- Other potential medications – Quetiapine and lithium are often prescribed with other adjunct medications for treating bipolar disorder. Study team decided to allow for adjunct medications, with clinicians to monitor symptoms and side effects, and blood concentration of medications if necessary.
- Selection of study outcomes – Bipolar disorder is a complex disorder. Study team decided to prioritize assessing side effects and cost–benefit ratios as outcome measures
- Consideration for participant eligibility – The team included participants with comorbid substance abuse and anxiety disorders and individuals with a history of medication nonadherence but excluded those that require crisis care (e.g., needing urgent inpatient hospitalization or alcohol/substance detoxification)
- Trial design option – A single-blinded trial design was adopted (the raters were blinded to minimize bias). Clinicians were allowed adjustments to both the randomized treatment and adjunct medications.

The team aimed for the following hypotheses:

- Participants with lithium and adjunct medication will have a more favorable benefit relative to harm over 6 months compared to the quetiapine and adjunct medication group

- Participants with quetiapine and adjunct medication will have more adjustments to medications over 6 months compared to those in the lithium and adjunct medication group
- Participants with quetiapine and adjunct medication will have higher cardiovascular risks over 6 months compared to those in the lithium and adjunct medication group.

Statistical analysis:

- The statistical analysis will be a two-tailed alpha level of 0.05.

Note: Seroquel is a quetiapine fumarate approved for the treatment of schizophrenia and bipolar disorder (Appendix 3).

*Source*: Nierenberg, AA 2014, 'Clinical and Health Outcomes Initiative in Comparative Effectiveness for Bipolar Disorder (Bipolar CHOICE): A pragmatic trial of complex treatment for a complex disorder', *Clinical Trials*, 11, pp. 114–127.

## 6.12 SUMMARY OF IMPORTANT POINTS

1. Clinical trials are conducted to test the effects of new drug candidates in humans. There are four phases to clinical trials:
   - Phase I – Safety study, 20–80 subjects, open labeled
   - Phase II – Safety and efficacy studies, 50–500 subjects, randomized, double-blinded
   - Phase III – Pivotal studies, multisite, 100s–1000s subjects, randomized, double-blinded
   - Phase IV – Postmarketing approval trial to monitor drug safety and efficacy at large.
2. Regulatory authorities stipulate the need for ethical principles to be observed when conducting clinical trials. Clinical trials should never be conducted to gain knowledge *per se*. They should be based on risk–benefit considerations, informed consent, and respect for human individuals; furthermore, subjects should be protected without being taken advantage of.
3. Clinical trials are conducted according to GCP. There should be a protocol that states the reason for the clinical trial, how it is to be conducted, the number of people to be included, eligibility criteria, medical tests, and observations to be made and information to be collected. Clinical trial protocol must be approved by IRB/IEC before commencement.
4. Statistical analysis is an integral part of clinical trial. A clinical trial protocol includes information on statistical parameters that the trial is expected to be based on and methods for the analysis of data.
5. An investigator, not an employee of the sponsor, is appointed to be responsible for the conduct of a trial. Appropriate quality system is followed and deviations to trial protocols are reported. Serious adverse events have to be reported to regulatory authorities within specified time.

## 6.13 REVIEW QUESTIONS

1. Explain the reasons for ethical considerations before a clinical trial is conducted.
2. Discuss the use of biomarkers in clinical trials.
3. Describe the term "protocol" and list the parameters to be included in the document.
4. Explain randomization and justify the requirement for randomization and double-blinding in clinical trials.
5. An investigator is designing a clinical trial to test a cholesterol-lowering drug. She wants to compare the drug with placebo with a 95% confidence level, that is, $\alpha$ is 0.05. She also limits the false-negative to 10%, that is, $\beta$ is 0.10. From literature she knows the variability of cholesterol has a standard deviation of 50 mg/dL. How many people must she recruit in the study to demonstrate a 20 mg/dL difference between the drug and placebo?
6. Distinguish the various phases of clinical trials, I–IV. Provide a reason for conducting Phase IV trials.
7. Briefly explain GLP as applied to clinical trials.
8. Why is it necessary to regulate clinical trials?

## 6.14 BRIEF ANSWERS AND EXPLANATIONS

1. Because of problems in some early clinical trials where subjects were taken advantage of, regulatory authorities require that clinical subjects be treated fairly. Ethical considerations should be undertaken to safeguard subject's safety and well-being.
2. For some diseases it is not possible to measure the effect of the drug on trial directly, or the desired direct outcome may require a long time to eventuate. Biomarkers provide more convenient and timely signals in response to the trial drug, and they can be measured at various time points to indicate the progressive treatment reaction.
3. Refer to Section 6.4.4 and Exhibit 6.10.
4. Refer to Section 6.4.7 and Exhibit 6.12 to explain randomization and the techniques used. Randomization and double-blinding are necessary to prevent bias in data collection so that statistical analysis on the basis of normal distribution can be used to evaluate the trial results.
5. We use the following equation (from Exhibit 6.14) to calculate the total number of subjects to be recruited:

$$2N = \frac{4(Z_\alpha + Z_\beta)^2\sigma^2}{\delta^2}$$

Using the standard normal distribution table, $Z_\alpha = Z_{0.05} = 1.96$; $Z_\beta = Z_{0.1} = 1.65$. Substituting into the equation, we have

$$2N = \frac{4(1.96 + 1.65)^2 50^2}{20^2}$$

$$2N = 326$$

Hence $N = 163$, the number for each group.
In reality to account for dropouts and noncompliances, more subjects are normally recruited.

6. Refer to Section 6.3 to describe the phases of clinical trial. Phase IV trials are necessary to maintain a close watch on the efficacy and adverse events of an approved drug when it is administered to the population at large. For example, even a small percentage of adverse events in Phase III trial for several thousand people may translate to a substantial number when a drug is made available to millions of people. A case in point is Vioxx and Bextra (Section 2.10).
7. By following GLP, clinical trials are conducted according to the procedures in the protocol, data are collected and verified as intended, and deviations to procedures are addressed. The aim is to ensure data from clinical trials are valid and conclusions drawn are correct.
8. The regulation of clinical trials is to make certain that first and foremost subjects' welfare is not compromised. The need for review and approval by IRB/IEC provides an independent party to decide the need and procedure contemplated for the clinical trials. The regulatory authorities also ensure that steps are taken to guarantee trials are conducted ethically.

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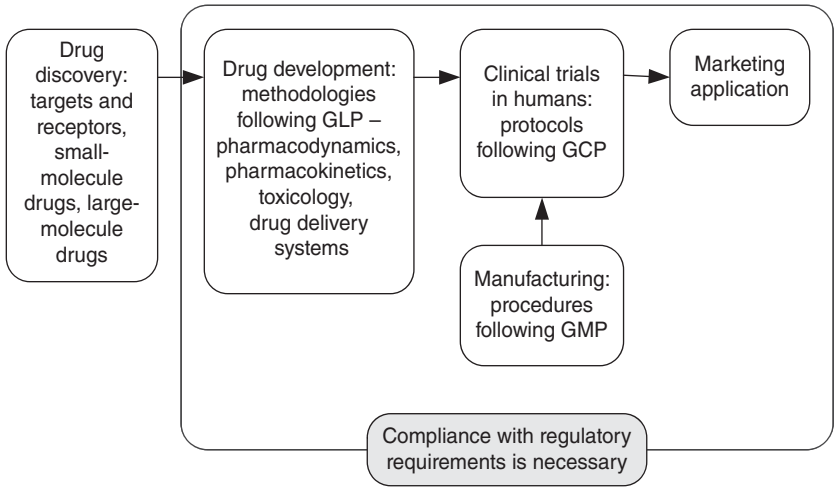
# CHAPTER 7

# REGULATORY AUTHORITIES




## 7.1 ROLE OF REGULATORY AUTHORITIES

All of us want the drugs that are prescribed for us to be safe and effective to treat our ailments. It is the role of public regulatory authorities to ensure that pharmaceutical companies comply with regulations. There are legislations that require drugs to

*Drugs: From Discovery to Approval*, Third Edition. Rick Ng.

be developed, tested, trialed, and manufactured in accordance with guidelines so that they are safe and patients' well-being is protected. There have been several occasions when drugs were not safe and people's health has been compromised; there were times when unscrupulous people or firms wrongly or carelessly manufactured drugs; children or vulnerable people have been recruited to clinical trials without consent, and insufficient tests were carried out on some drugs during development, leading to unimagined damage (refer to Exhibit 7.1 for an account of the thalidomide tragedy).

Regulatory authorities perform the watchdog role to ensure the following:

- Animal studies to test for toxicology comply with Good Laboratory Practice (GLP)
- Clinical trials to determine safety and efficacy are performed in accordance with Good Clinical Practice (GCP)
- Drugs are manufactured to the required quality under current Good Manufacturing Practice (cGMP) conditions.

The regulatory authorities also carry out surveillance to ensure that labels and advertising materials are accurate and in accordance with the approved claims. Advertising materials should have clear explanations about the drug, indications, and contraindications, dosage, and frequency of medication.

In this chapter, we explain the regulatory authorities' organization and policies in the major countries. The regulatory process is complicated and lengthy; this is especially the case where major industrialized nations have over the years set up independently their own systems of regulations and controls, which invariably have different requirements to those in other countries. Processes are, however, in place to harmonize the regulatory procedures in the major industrialized countries. In this way, regulatory requirements, technical documents, and review processes are consistent and can be mutually recognized by member countries. Eventually, harmonization will reduce duplicate requirements, reports, and the cost and time for regulatory reviews. This will translate to patients receiving access to new drugs more speedily and at less cost than now.

We follow this up in Chapter 8 to examine more closely the regulatory processes for testing, trialing, and approving a drug for marketing.

**Exhibit 7.1 Thalidomide**

Thalidomide was synthesized in Germany and became available in late 1957. It was prescribed for the treatment of insomnia and nausea in pregnant women.

However, it had not been known that the thalidomide drug molecule could cross the placental barrier and affect fetal development. As a result, thousands of babies were born with crippled extremities, disfigurement, and disabilities. Numerous fetuses were stillborn or died soon after birth.

The drug was banned in early 1962, but by then the lives of many people had been severely affected.

Refer to Exhibit 10.10 for the chemical structure of thalidomide.

## 7.2 US FOOD AND DRUG ADMINISTRATION

The United States Food and Drug Administration (FDA) is an agency within the Department of Health and Human Services. The organizational structure of FDA is as below:

- Office of the Commissioner
- Office of Foods and Veterinary Medicine
- Office of Global Regulatory Operations and Policy
- Office of Medical Products and Tobacco
- Office of Operations.

FDA is required by the US Federal Food, Drug, and Cosmetic Act to regulate drug products in the United States. Its role is to ensure that drugs are developed, manufactured, and marketed in accordance with regulatory requirements so that they are safe and effective. The relevant offices for drug products are the Office of Global Regulatory Operations and Policy and the Office of Medical Products and Tobacco.

The Office of Global Regulatory Operations is responsible for US domestic and international product quality, global collaboration, global data sharing, development and harmonization of standards, field operations, compliance, and enforcement activities. The regulation of drug products is the responsibility of the Office of Medical Products and Tobacco, which comprises the following:

- Center for Drugs Evaluation and Research (CDER)
- Center for Biologics Evaluation and Research (CBER)
- Center for Devices and Radiological Health
- Center for Tobacco Products
- Office of Special Medical Programs.

Exhibit 7.2 presents a brief history of FDA. For the purpose of regulation on drugs, the relevant centers are CDER and CBER.

### 7.2.1 Center for Drug Evaluation and Research (CDER)

CDER oversees the research, development, manufacture, and marketing of synthetic small molecule drugs (refer to Chapter 3 for more details). CDER is also responsible for the regulation of certain biologic therapeutic products. Most of these drugs are large protein-based molecules generated by hybridoma or recombinant DNA technology, which is described in detail in Chapter 4. These products are the following:

- Monoclonal antibodies for *in vivo* use
- Cytokines, growth factors, enzymes, immunomodulators, and thrombolytics
- Proteins intended for therapeutic use that are extracted from animals or microorganisms, including recombinant versions of these products
- Nonvaccine therapeutic immunotherapies.

**Exhibit 7.2 A Brief History of FDA**

The FDA started from a single chemist in the US Department of Agriculture in 1862, with the appointment of Charles M. Wetherill by President Lincoln. By 2013, it had a staff of about 14,000 and a budget of US$4.1 billion. The FDA now has employees from diverse disciplines, namely, chemists, pharmacologists, physicians, microbiologists, veterinarians, pharmacists, and lawyers.

About a third of the agency's employees are stationed outside Washington, DC area in over 150 field offices and laboratories, including 5 regional offices and 20 district offices.

The FDA regulates the following:

- Drugs (e.g., prescriptions, OTCs, generics)
- Biologics (e.g., vaccines, blood products)
- Medical devices (e.g., pacemakers, contact lenses)
- Food (e.g., nutrition, dietary supplements)
- Animal feed and drugs (e.g., livestock, pets)
- Cosmetics (e.g., safety, labeling)
- Radiation-emitting products (e.g., cell phones, lasers)

*Source*: Data from Department of Health and Human Services 2014, Fiscal Year 2015, *Food and Drug Administration*, Justification of Estimates for Appropriations Committees, viewed April 7, 2014, http://www.fda.gov/downloads/AboutFDA/ReportsManualsForms/Reports/BudgetReports/UCM388309.pdf

CDER's involvement starts with Phase I clinical study via the approval of an Investigational New Drug (IND) application. In its review process for the IND, CDER examines the evidence supporting the scientific basis for the drug, checks that nonclinical tests have been performed in compliance with GLP, toxicological studies are acceptable, and the drug has been manufactured in compliance with applicable cGMP regulations. When the clinical trials commence, CDER monitors the conduct of the clinical trials through Phases I, II, and III, on the basis of adherence to GCP. At the conclusion of Phase III trials, marketing applications from sponsor pharmaceutical organizations are evaluated by CDER, relying on scientific data and clinical results. The marketing applications are the following:

- New Drug Applications (NDAs) for small molecule drugs
- Biologics License Applications (BLAs) for therapeutic biologic drugs.

Risks (drugs have potential risks as they interfere with our body functions) and benefits evaluations are undertaken before drugs are approved for marketing. Expert reviews from external personnel scientifically qualified to opine in the therapeutic area of interest are sought from time to time, to ensure that decisions are based on the latest scientific opinions. It also ensures that advertising and marketing of drugs are

in accordance with claims approved. Marketed drugs are monitored for unanticipated health risks. If unexpected health risks or adverse reactions are confirmed, CDER informs the public or, in severe cases, directs the suppliers to remove the drugs from the market. The manufacture of drugs is monitored to ensure compliance with cGMP.

The three categories of drugs regulated by CDER are the following:

- Prescription drugs
- Generic drugs
- Over-the-counter (OTC) drugs.

### 7.2.2 Center for Biologics Evaluation and Research (CBER)

CBER regulates certain nontherapeutic biologics – see Chapter 4, which are not regulated by CDER. These are as follows:

- Allergenics – allergen extracts (from molds, pollens, insects, insect venoms, and animal hair) for diagnosis and treatment; allergen patch tests; antigen skin tests
- Blood and blood products – blood and blood components for transfusion or to be manufactured into drug products (e.g., clotting factors); devices for blood collection, separation, and storage
- Cellular and gene therapy products – cellular immunotherapies; embryonic and adult stem cells; gene therapy to replace faulty or missing genes
- Tissue and tissue products – human cells and tissues, for example, bone, skin, corneas, ligaments, tendons, sperm, and heart valves intended for implantation, transplantation, infusion, or transference into a human recipient
- Vaccines – including cell-based immunotherapeutic products (dendritic cells, natural killer cells, T cells, and tumor cells)
- Xenotransplantation – transplantation of nonhuman cells, tissues, or organs into humans.

These nontherapeutic biologics are complex, large compounds with molecular weights >5 kDa and they are not easily characterized. They are also labile (i.e., heat and shear sensitive) and are very dependent on the manufacturing process parameters and storage conditions.

The regulatory process is the filing of IND for clinical trials. At the conclusion of clinical trials, the sponsor files Biological License Application (BLA) for marketing approval. CBER evaluates a biologic in terms of risk versus benefits before approving it for marketing. The important criteria are identity, purity, safety, and potency.

### 7.2.3 Pertinent FDA Processes and Controls

Drugs (small molecule drugs) are regulated in the United States as required by the Food, Drug, and Cosmetic Act (FDCA) of 1938. Biologics are, however, regulated by the Public Health Service Act (PHSA) of 1944 and the FDCA. This is because the PHSA is concerned with medical products that are less well defined, necessitating more control in the handling and manufacturing processes.

**TABLE 7.1 Selected Regulations from 21 CFR**

| Document Number | Description |
|---|---|
| 21 CFR Part 11 | Electronic Records, Electronic Signatures |
| 21 CFR Part 50 | Protection of Human Subjects |
| 21 CFR Part 56 | Institutional Review Board |
| 21 CFR Part 58 | Good Laboratory Practices for Nonclinical Laboratory Studies |
| 21 CFR Part 202 | Prescription Drug Advertising |
| 21 CFR Part 203 | Prescription Advertising |
| 21 CFR Part 210 | Current Good Manufacturing Practice in Manufacturing, Processing, Packaging or Holding of Drugs; General |
| 21 CFR Part 211 | Current Good Manufacturing Practice for Finished Pharmaceuticals |
| 21 CFR Part 312 | Investigational New Drug Applications |
| 21 CFR Part 314 | Applications for FDA Approval to Market a New Drug |
| 21 CFR Part 600 | Biological Products: General |
| 21 CFR Part 610 | General Biological Products Standards |

The applicable regulations for drugs are codified in Title 21 of the US *Code of Federal Regulations* (CFR). These regulations promulgate FDA's requirement in many aspects of drug clinical research, manufacturing, and marketing. Table 7.1 lists some of these applicable regulations. Readers should note that these regulations are updated from time to time by FDA as a result of new requirements or information.

In addition, FDA publishes Guidelines and Points to Consider (PTCs) documents to guide pharmaceutical organizations in many relevant areas, from testing methodologies, manufacturing requirements, and drug stability information, to filling in of forms and the requisite data.

FDA also carries out inspections on establishments to ensure compliance with regulations. The establishments are laboratories, clinical trial centers, and manufacturing facilities. Further information on establishment inspection is discussed in Chapter 10.

In some circumstances, FDA processes drug reviews under an accelerated scheme. This mechanism is to review and approve drugs speedily for cases that lack effective therapies or in situations of rare diseases. One of the fastest approval times to date is the case of imatinib mesylate (Gleevec, Novartis – Exhibit 7.3) for the treatment of chronic myeloid leukemia (CML); it was approved in less than 3 months after the filing of an NDA with FDA. Another example is the AIDS drug indinavir (Crixivan, Merck), which was approved in a mere 42 days.

## 7.3 EUROPEAN MEDICINES AGENCY

There are several avenues for drug approval in Europe:

- *Centralized procedure:* Under the European Community Regulation 726/2004 and Directive 2004/27/EC, the Centralized Procedure (also known as Community Authorization Procedure) is a single authorization procedure for marketing authorization of medicinal products for the entire European Community. The scope is divided into three parts: mandatory, optional, and generic/hybrid.

**Exhibit 7.3 Imatinib Mesylate (Gleevec)**

CML occurs when there is a translocation of chromosomes 9 and 22 (also called Philadelphia translocation – a chromosomal abnormality). These two different chromosomes break off and reattach on the opposite chromosome. A consequence is that the activity of the Bcr–Abl gene, which encodes the enzyme tyrosine kinase, is turned on all the time. With this heightened activity, high levels of white blood cells are produced in the bone marrow.

Imatinib mesylate is a tyrosine kinase inhibitor (refer to Chapter 2 on receptors). It is used to block the growth of white blood cells.

Imatinib mesylate is manufactured by Novartis. Clinical trials showed that patients had their white blood cells reduced substantially after being treated with Gleevec.

Gleevec was approved by FDA under the accelerated approval regulations for the treatment of CML.

*Source*: Data from Food and Drug Administration 2013, *Gleevec (Imatinib Mesylate) Questions and Answers*, viewed April 7, 2014, http://www.fda.gov/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/ucm110505.htm

Under the mandatory scope, centralized procedure is a "must" for medicinal products of the following categories:

- Medicines derived from biotechnology processes, such as genetic engineering, and include gene therapy, somatic cell therapy, and tissue-engineered products
- Medicines intended for the treatment of HIV/AIDS, cancer, diabetes, neurodegenerative disorders, autoimmune diseases, and viral diseases
- Orphan medicines (medicines used for rare diseases).

Under the optional scope, an applicant can request assessment via the centralized procedure for the following medicinal products:

- A medicinal product containing a new active substance
- A medicinal product that constitutes a significant therapeutic, scientific, or technical innovation.

Under the generic/hybrid scope, a generic/hybrid product of a reference medicinal product authorized via the centralized procedure has "automatic" access to the centralized assessment.

- *Mutual recognition procedure:* A medicine is first authorized by one member state, according to the member state's own national procedure. The applicant can seek further authorizations through a mutual recognition procedure. When there is a dispute between member states on the issue of mutual recognition, the European

Medicines Agency (EMA) is called on to arbitrate, and its decision is binding on the member states.

- *Decentralized procedure:* This is applicable where authorization has not yet been approved in any member state. The applicant may apply for simultaneous authorization in more than one EU member state for medicines that do not fall within the mandatory scope of the centralized procedure.

EMA's key aims, according to the EU Enterprise Directorate-General publication, are to:

- Protect and promote public health by providing safe and effective medicines for human and veterinary use;
- Give patients quick access to innovative new therapy;
- Facilitate the free movements of pharmaceutical products throughout the European Union;
- Improve information for patients and professionals on the correct use of medicinal products;
- Harmonize scientific requirements to optimize pharmaceutical research worldwide.

There are two committees within EMA; they are the following:

- Committee for Medicinal Products for Human Use (CHMP)
- Committee for Medicinal Products for Veterinary Use (CVMP).

For our purposes, the committee for drug approval is the CHMP. Applications are submitted to EMA according to the centralized procedure. The review process is described in Section 8.3. In 2013, the CHMP has provided 67 positive opinions (decisions) on new medicines, including the first two monoclonal antibody biosimilars: Remsima and Inflectra, and the first combined tissue-engineered medicine, Maci (matrix-induced autologous chondrocyte implantation) for the repair of cartilage defects.

European Council Regulation EEC/2309/93 together with Directive 75/319/EEC requires member states to establish a national pharmacovigilance system to collect and evaluate information on adverse reactions to medicinal products and to take appropriate action.

Clinical trial applications are not centralized. Submissions are made through individual member states. Refer to Section 8.3 for details on clinical trial application in Europe.

## 7.4 JAPAN'S PHARMACEUTICALS AND MEDICAL DEVICES AGENCY (PMDA)

The Japanese pharmaceutical market, on a country basis, is the second largest in the world next to the United States. It is larger than the combined markets of the United

Kingdom, France, and Germany. Japan's Pharmaceutical Affairs Law aims to improve public health through regulations ensuring the quality, efficacy, and safety of drugs and medical devices. The Pharmaceuticals and Medical Devices Agency (PMDA) is the Japanese pharmaceutical and medical device regulatory agency. It works with the Ministry of Health, Labor, and Welfare (MHLW), which has the overall responsibility for pharmaceutical affairs in Japan.

PMDA has four main functions:

- Drug and Medical Device Reviews – (i) Performs reviews of marketing authorizations for pharmaceuticals and medical devices, (ii) provides consultations for planning and implementation of clinical trials and preparation of NDA dossiers, (iii) conducts GLP, GCP, GMP, Quality Management Systems (QMS), and Good Postmarketing Surveillance Practice (GPSP) inspections.
- Postmarketing safety – (i) collects, analyzes, and disseminates information related to the quality, efficacy, and safety of pharmaceuticals and medical devices, (ii) provides consultation for consumers about the safe use of pharmaceuticals and medical devices.
- Relief Services for Adverse Health Effects – (i) Provides medical expenses, disability pensions, bereaved family pensions, and so on for people who have suffered from severe illness and disabilities caused by adverse drug reactions of pharmaceuticals or infections from biological products.
- International Programs – (i) Implements the PMDA International Strategic Plan through international regulatory harmonization and bilateral relationship with relevant external parties.

Foreign clinical trial results are acceptable except in areas where there are immunological and ethnic differences between Japanese and foreigners. The ethnic factors are divided into two components: intrinsic factors such as racial factors and physiological differences; and extrinsic factors, which include cultural and environmental issues. In these cases, PMDA may require that some bridging comparative clinical trials be performed with dose-ranging protocols. This will enable absorption, distribution, metabolism, and excretion studies to be carried out on Japanese and provide better dosage and indication for the Japanese people. PMDA also requires that application be accompanied with one year of real-time stability data and that sterility test results be included.

Standard processing period for drug approval according to PMDA is as follows:

- 1 year for review
- 1 year for applicant response
- Total 2 years for approval.

All NDAs are expected to be in the Common Technical Document (CTD) format according to International Conference on Harmonization (ICH) guidelines (refer to Sections 7.11 and 8.9). Priority reviews are applicable for orphan drugs and those drugs for the treatment of serious illnesses. A restricted approval system has been

implemented for emergency drugs to prevent spread of diseases. In this case, the standard review procedure is not applicable.

## 7.5 CHINA FOOD AND DRUG ADMINISTRATION

China's pharmaceutical market is growing at a very fast pace. The current data show that the total market is forecast to surpass US$100 billion by 2015. The Chinese government maintains price control on imported drugs. With China's entry into the World Trade Organization (WTO), tariffs have been reduced from 20% to 6.5%. The projection is that the market size will expand by more than 15% annually, and China will be the world's largest market by 2020.

The regulation of drugs in China is under the jurisdiction of the China Food and Drug Administration (CFDA). CFDA is under the control of the State Council. Through the Drug Administration Law of the People's Republic of China, regulations are instituted for the control of clinical trials, registration, distribution, and marketing surveillance of new, generic, and OTC drugs (Exhibit 7.4). It also controls GMP manufacturing compliance, monitors adverse events, and prosecutes illicit, fraudulent, and unlicensed drug manufacturers. There are also strict controls on the advertising of drugs; these prohibit the use of certain words, phrases, and unsubstantiated or unscientific claims.

Drugs are classified into several categories. These are synthetic drugs, traditional Chinese Medicine (TCM), and biological products. CFDA stipulates compliance to GMP for medical products, GCP for clinical trials, and GLP for nonclinical drug safety research. Foreign drugs are required to have import registration. Foreign drug manufacturers and distributors file for examination and registration of their products with relevant data and documents. Clinical trials may need to be conducted on the basis of evaluation by CFDA (refer to Section 8.5).

Among the many departments of CFDA (Exhibit 7.5), the relevant departments for drugs are the following:

- Department of Drug and Cosmetics Registration (DDCR), which performs drug registration and supervision of good practices for nonclinicals, clinicals, and TCM preparations and biological species protection. Within the DDCR, the Division of Pharmaceuticals and the Division of Biological Products manages the regulation for "Western" drugs. TCM is under the Division of Traditional Chinese Medicines and Ethno Medicines.
- Department of Drug and Cosmetics Supervision, which is responsible for overseeing the manufacture, distribution, and postmarket surveillance of drug products.

## 7.6 INDIA'S CENTRAL DRUGS STANDARD CONTROL ORGANIZATION

Increasingly, India is becoming an important player in drug manufacture, in particular, the production of generics. Many of India's generics are now found in all parts of the world, challenging the dominance once held by the large pharmaceutical companies in Western countries.

**Exhibit 7.4 Clinical Trials and Selected Drugs Approved in China**

China offers a large pool of treatment to naive patients for clinical trials. There are 1.3 billion people, of which 50% are urbanized. More than 15 cities have populations of more than 5 million people. Clinical trials are one-third the cost of that in the United States, and recruitments are expected to be rapid because of high enrollment rate. However, complicating factors are slower regulatory processes, limited qualified central laboratories for testing, and restriction of export of blood and serum samples outside of China for testing.

Unlike in the United States and Europe, however, only certain research centers and hospitals are especially designated by the CFDA for the conduct of clinical trials.

In October 2003, the China authority approved the world's first gene therapy – Gendicine (a recombinant human adenovirus type 5 mediated delivery of p53 gene) for the treatment of head and neck cancer. In 2005, another head and neck cancer drug, Oncorine (a recombinant oncolytic adenovirus type 5), was approved. In the same year, another recombinant human endostatin, Endostar, was approved for the treatment of small-cell lung cancer.

The China regulatory framework is evolving and more stringent regulations in the approval process are implemented in recent years, requiring that (i) approval of drug licenses are based on collective decisions, (ii) drug evaluators are made public and held accountable for their decisions, and (iii) there should be no potential conflict of interest in the evaluators in reviewing the application.

*Source*: Data from 1. Jia, H 2007, 'China syndrome – a regulatory framework in meltdown', *Nature Biotechnology*, 25, pp. 835–837; 2. Yan, L and Hugo P 2013, *Current Trends for Conducting Clinical Trials in China*, viewed April 7, 2014, http://xtalks.com/archives/ondemand/xto700.wmv

Under India's Drug and Cosmetics Act, the central government of India, through the Central Drugs Standard Control Organization (CDSCO), is responsible for the approval of new drugs, clinical trials, maintenance of the standard of drugs, jurisdiction of importation of foreign drugs, approval of manufacturing licenses, and coordination of the activities of the State Drug Control Organizations. The central government is also responsible for the testing of drugs by the Central Drugs Labs, whereas the state authorities are responsible for the regulation of the manufacture, sales, and distribution of drugs.

Schedule Y of the Drugs and Cosmetics Rules sets up the requirements for clinical trials and that of Schedule M for GMP compliance system.

## 7.7 AUSTRALIA'S THERAPEUTIC GOODS ADMINISTRATION

Australia's Therapeutic Goods Administration (TGA) has perhaps one of the most progressive and comprehensive regulatory systems in the world. Under the Therapeutic

**Exhibit 7.5 Divisions of Pharmaceuticals and Biological Products, Department of Drug and Cosmetics Registration**

**Division of Pharmaceuticals**

- Draft and revise national standards and research guidelines of pharmaceuticals
- Evaluate and approve new drugs
- Approve and reregister controlled drugs
- Evaluate and approve clinical trials
- Approve and regulate pharmaceutical preparations dispensed by provincial medical institutions.

**Division of Biological Products**

- Draft and revise national standards and research guidelines of biological products
- Evaluate and approve new biological products
- Evaluate and approve clinical trials of biological products
- Regulate and supervise the national release of a lot of biological products.

**Department of Drug and Cosmetics Registration**

- Accept drug application and issue certificate for new drug, generic drug, imported drug, protected TCM products, and packaging material for drugs.

*Source*: Data from China Food and Drug Administration 2014, *Department of Drug and Cosmetics Registration*, viewed April 7, 2014, http://eng.sfda.gov.cn/WS03/CL0791/

Goods Act, TGA regulates prescription medicines, OTC medicines, complementary medicines, and medical devices. The roles of TGA in medicines are:

- Premarket evaluation and approval of registered products intended for supply in Australia
- Development, maintenance, and monitoring of the systems for the listing of medicines
- Licensing of manufacturers in accordance with international standards of Good Manufacturing Practice (GMP)
- Postmarket monitoring, through sampling, adverse event reporting, surveillance activities, and response to public inquiries
- Assessment of medicines for export.

All medicines in Australia are listed or registered with the Australian Register of Therapeutic Goods (ARTG), except specifically exempted, and are provided with unique numbers in the database. Listed medicines are considered to be of lower risk and are self-selected by consumers. These medicines bear the "AUST L" numbers. In contrast, registered medicines belong to "low-risk" category, which are of the OTC type, or "high-risk" medicines, which require prescription. Registered medicines have the "AUST R" numbers. Complementary medicines, such as alternative or traditional medicines, are either listed or registered depending on ingredients and claims.

Section 8.7 explains the clinical trial and drug approval processes in Australia.

## 7.8 CANADA'S HEALTH CANADA

All drugs sold in Canada must be authorized by Health Canada, which has several directorates: the Therapeutic Products Directorate (TPD) reviews and authorizes new pharmaceuticals and medical devices, the Biologics and Genetic Therapies Directorate (BGTD) evaluates biological and radiopharmaceutical drugs, and the Natural Health Products Directorate (NHPD) regulates natural health products such as vitamins and health supplements. For postmarket surveillance, the Marketed Health Products Directorate (MHPD) monitors adverse events and investigates complaints and problem reports.

## 7.9 OTHER REGULATORY AUTHORITIES

Table 7.2 shows the regulatory authorities in selected countries. A summary of the health systems, both public and private in selected countries, is given in Appendix 10. It shows the %GDP each country spends on healthcare, per capita health expenditure, number of hospital beds and doctors/10,000 population.

## 7.10 AUTHORITIES OTHER THAN DRUG REGULATORY AGENCIES

Although pharmaceutical organizations have to comply with the requirements of regulatory agencies, there are other authorities that control the manufacturing and marketing of drugs. For example, in the United States these are:

- State health authorities
- Occupational Safety and Health Administration (OSHA)
- Environmental Protection Agency (EPA)
- Local regulatory bodies.

Compliance with all these authorities would assist in smoother paths toward approval of drugs for manufacturing and marketing.

**TABLE 7.2 Selected International Regulatory Authorities**

| Country | Regulatory Authority |
|---|---|
| Argentina | National Administration of Drugs, Foods, and Medical Technology |
| Brazil | National Health Surveillance Agency |
| Chile | National Institute of Public Health |
| Denmark | Danish Health and Medicines Authority |
| Egypt | Egypt Drug Authority |
| Finland | Finnish Medicines Agency |
| France | National Agency for the Safety of Medicine and Health Products |
| Germany | Federal Institute of Drugs and Medical Devices |
| Greece | National Organization for Medicines |
| Indonesia | National Agency for Drug and Food Control |
| Israel | Ministry of Health |
| Italy | Italian Medicines Agency |
| Jamaica | Ministry of Health |
| Kenya | Ministry of Health |
| South Korea | Food and Drug Administration |
| Malaysia | National Pharmaceutical Control Bureau |
| Mexico | Ministry of Health |
| The Netherlands | Medicines Evaluation Board |
| New Zealand | Medicines and Medical Devices Safety Authority |
| Norway | Norwegian Medicines Agency |
| Philippines | Food and Drug Administration |
| Russia | Ministry of Health |
| Singapore | Health Sciences Authority |
| South Africa | Department of Health |
| Spain | Spanish Agency for Medicines and Health Products Drug Agency |
| Sweden | Medical Products Agency |
| Switzerland | International Office for Control of Medicaments |
| Taiwan | Taiwan Food and Drug Agency |
| Thailand | Food and Drug Administration |
| United Kingdom | Medicines and Healthcare Products Regulatory Agency |
| Zimbabwe | Ministry of Health |

## 7.11 INTERNATIONAL CONFERENCE ON HARMONIZATION

Specific plans for the formation of the ICH were conceived at the WHO International Conference of Drug Regulatory Authorities (ICDRA) in Paris in 1989. In April 1990, ICH was formed in Brussels, with the aim of formulating a joint regulatory–industry initiative on international harmonization of drug regulations. ICH is composed of representatives from the regulatory agencies and industry associations of the United States, Europe, and Japan. The ICH Steering Committee meets at least twice a year, with the location rotating among the three regions. It is charged with the responsibilities to prepare harmonized guidelines that can be accepted by each region.

There are four major categories of guidelines. They are topics on Quality, Safety, Efficacy, and Multidisciplinary. A description of the ICH guidelines is provided in

**Exhibit 7.6 ICH Guidelines**

ICH guidelines are divided into four major categories.

**Current Status of Harmonization:**

- Quality: 11 topic headings – Stability, Analytical Validation, Impurities, Pharmacopeias, Quality of Biotechnological Products, Specifications, GMP, Pharmaceutical Development, Quality Risk Management, Pharmaceutical Quality System, Development and Manufacture of Drug Substances. A total of 47 documents.
- Safety: 10 topic headings – Carcinogenicity Studies, Genotoxicity Studies, Toxicokinetics and Pharmacokinetics, Toxicity Testing, Reproductive Toxicology, Biotechnological Products, Pharmacology Studies, Immunotoxicology Studies, Nonclinical Evaluation for Anticancer Pharmaceuticals, Photosafety Evaluations. A total of 15 documents.
- Efficacy: 9 topic headings – Clinical Safety, Clinical Study Reports, Dose–Response Studies, Ethnic Factors, GCP, Clinical Trials, Clinical Evaluation by Therapeutic Category, Clinical Evaluation, Pharmacogenomics: A total of 26 documents.
- Multidisciplinary: 8 cross-cutting topics that do not fit uniquely into one of the above categories –
  - M1 – Medical Terminology (MedDRA)
  - M2 – Electronic Standards for Transmission of Regulatory Information (ESTRI)
  - M3 – Nonclinical Safety Studies
  - M4 – The Common Technical Document (CTD)
  - M5 – Data Elements and Standards for Drug Dictionaries
  - M6 – Gene Therapy
  - M7 – Genotoxic Impurities
  - M8 – Electronic Common Technical Document (eCTD).

Exhibit 7.6. Further details of the Quality Guidelines are presented in Exhibit 9.4, and those for Safety in Exhibit 5.6, and Efficacy in Table 6.1. For the Multidisciplinary topics, M1 – Medical Terminology (MedDRA) – is described in Exhibit 6.15 and M3 – The CTD – is discussed in Section 8.9.

## 7.12 WORLD HEALTH ORGANIZATION

The World Health Organization (WHO) is a specialized agency of the United Nations. There are 194 member states as on April 2014. WHO is headquartered in Europe with

regional offices in Africa, the Americas, the Eastern Mediterranean, South East Asia, and the Western Pacific. WHO is not a regulatory agency; its functions are the following:

- To give worldwide guidance in the field of health
- To set global standards for health
- To cooperate with governments in strengthening national health programs
- To develop and transfer appropriate health technology, information, and standards.

WHO works with regulatory authorities in member states in setting up policies and training programs to ensure drugs are safe, pure, and effective and are being distributed and administered as specified.

## 7.13 PHARMACEUTICAL INSPECTION COOPERATION SCHEME

The Pharmaceutical Inspection Cooperation Scheme (PIC/S) was formed in 1995 to enhance the work set up under the Pharmaceutical Inspection Convention (PIC) in 1970. The mission of PIC/S is the following:

- To lead the international development, implementation, and maintenance of harmonized GMP standards and quality systems of inspectorates in the field of medicinal products.

There are 44 participating authorities from Argentina, Australia, Austria, Belgium, Canada, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Indonesia, Ireland, Israel, Italy, Latvia, Liechtenstein, Lithuania, Malaysia, Malta, New Zealand, Norway, Poland, Portugal, Romania, Singapore, Slovak Republic, Slovenia, South Africa, Spain, Sweden, Switzerland, Taiwan, The Netherlands, Ukraine, United Kingdom, and the United States. Inspection of pharmaceutical facilities by one member is mutually recognized by another member to streamline regulatory inspection processes. The partners/observers to PIC/S are European Directorate for the Quality of Healthcare (EDQM), EMA, United Nations International Children's Emergency Fund (UNICEF), and WHO.

## 7.14 CASE STUDY # 7.1

### 7.14.1 International Health Regulations (IHR 2005)

This case study examines the WHO report on the International Health Regulations.

In the early years of the twenty-first century, we have witnessed the spread of diseases quickly from one country to another, whether via human beings in the case of Severe Acute Respiratory Syndrome (SARS) and more recently Middle East Respiratory Syndrome (MERS) or through migrating livestock as evidenced by the proliferation of avian influenza across national borders, in addition to the cases of Ebola outbreak in

Africa. These situations necessitated prompt and skillful control at early stages by more than one or a few countries alone, and in concerted effort to control the spread.

On August 23, 2007, WHO issued its World Health Report for 2007, which highlighted the international spread of disease. This report entitled: *A Safer Future: Global Public Health Security in the 21st Century*, tables six key recommendations for global public health security:

- Full implementation of the revised International Health Regulations (IHR 2005) by all countries
- Global cooperation in surveillance and outbreak alert and response
- Open sharing of knowledge, technologies, and materials, including viruses and other laboratory samples, necessary to optimize and secure global public health
- Global responsibility for capacity building within the public health infrastructure of all countries
- Cross-sector collaboration within governments
- Increased global and national resources for training, surveillance, laboratory capacity, response networks, and prevention campaigns.

It is under these circumstances of SARS and avian flu threats, together with the emergence of Ebola and other viruses, that prompted WHO and the member states to update the IHR in 2005. The realization is for member states to organize proactive measures to curb the spread of diseases.

An excerpt of the International Health Regulations (2005) is presented in the following:

> The IHR (1969) addressed only four diseases: cholera, plague, yellow fever, and smallpox by focusing on border controls and passive notification and control measures.
>
> In contrast, the IHR (2005), which has been enforced since June 15, 2007, has an expanded scope that covers existing, new, and reemerging diseases, including emergencies caused by noninfectious disease agents. It is an international law that helps countries working together to save lives and livelihoods caused by the international spread of diseases and other health risks. The aim is to prevent, protect against, control and respond to the international spread of disease while avoiding unnecessary interference with international traffic and trade. The IHR (2005) are also designed to reduce the risk of disease spread at international airports, ports, and ground crossings.
>
> The IHR (2005) requires member states to notify WHO of all events that may constitute a public health emergency of international concern and to respond to requests for verification of information regarding such events. This will enable WHO to ensure appropriate technical collaboration for effective prevention of such emergencies or containment of outbreaks and, under certain defined circumstances, inform other states of public health risks where action is necessary on their part.
>
> The IHR (2005) have been agreed on by consensus among WHO member states as a balance between their sovereign rights and shared commitment to prevent the international spread of disease. Although the IHR (2005) do not include an enforcement mechanism *per se* for states that fail to comply with its provisions, the potential consequences of

noncompliance are themselves a powerful compliance tool. Perhaps the best incentives for compliance are "peer pressure" and public knowledge. With today's electronic media, nothing can be hidden for very long. States do not want to be isolated. The consequences of noncompliance may include a tarnished international image, increased morbidity/mortality of affected populations, unilateral travel and trade restrictions, economic and social disruption and public outrage. Working together and with WHO to control a public health event and to accurately communicate how the problem is being addressed helps to protect against unjustified measures being adopted unilaterally by other states.

The key obligations for member states and WHO are as in the following:

***Member States:***

- To designate a National IHR Focal Point
- To assess events occurring in their territory and to notify WHO of all events that may constitute a public health emergency of international concern
- To respond to requests for verification of information regarding events that may constitute a public health emergency of international concern
- To respond to public health risks that may spread internationally
- To develop, strengthen, and maintain the capacity to detect, report, and respond to public health events
- To provide routine facilities, services, inspections, and control activities at designated international airports, ports, and ground crossings to prevent the international spread of disease
- To report to WHO evidence of a public health risk identified outside their territory, which may cause international disease spread, manifested by exported/imported human cases, vectors carrying infection or contamination, contaminated goods
- To respond appropriately to WHO-recommended measures
- To collaborate with other state parties and with WHO on IHR (2005) implementation.

***WHO:***

- Designating WHO IHR Contact Points at the headquarters or the regional level
- Conducting global public health surveillance and assessment of significant public health events and disseminating public health information to states, as appropriate
- Offering technical assistance to states in their response to public health risks and emergencies of international concern
- Supporting states in their efforts to assess their existing national public health structures and resources, as well as to develop and strengthen the core public health capacities for surveillance and response, and at designated points of entry
- Determining whether or not a particular event notified by a state under the Regulations constitutes a public health emergency of international concern, with advice from external experts if required
- Developing and recommending the critical health measures for implementation by state parties

- Monitoring the implementation of IHR (2005) and updating guidelines so that they remain scientifically valid and consistent with changing requirements.

*Source*: 1. World Health Organization 2005, *International Health Regulations,* viewed April 4, 2014, http://www.who.int/topics/international_health_regulations/en/; 2. World health Organization 2008, *International Health Regulations (2005)*, 2nd edn., http://www.who.int/ihr/publications/9789241596664/en/

## 7.15 CASE STUDY # 7.2

### 7.15.1 FDA's Pathway to Global Product Safety and Quality

This case study discusses FDA's initiative on global product safety and quality.

Many of the products, from food to cosmetics, drugs, and medical devices that Americans consume are supplied by other countries. About 80% of the active ingredients for drug manufacture in the United States are produced outside the country. With globalization, there are many factors that affect the world economy: rise of emerging markets, scarcity of natural resources and increasing flow of capital, information, and goods across national borders. This has put enormous pressure on FDA to regulate drug substances and drug products being imported into and exported out of the United States. As FDA's role is to promote and protect the health of the American people, it has developed a strategy to face these challenges and released *A Special Report – Pathway to Global Product Safety and Quality* in 2011.

The major headings for this report are presented in the following:

- Ten years from now, the world will be very different from as it is today
  - The great rebalancing
  - The productivity imperative
  - The global grid
  - Pricing the planet
  - Government and the market place
- The coming changes will make FDA's product safety and quality responsibilities formidable and more global in the years ahead
  - Global changes will have significant implications for the manufacturers and products that FDA regulates
    - Increasing pressure to reduce costs and increase productivity
    - Greater government influence in healthcare markets
    - Growing globalization of production of FDA-regulated products leading to growth in imports
    - Changing nature of risk in global supply chains
    - Increased risk of counterfeiting and other fraud
- FDA must substantially change its operating model to address the challenges of the future
  - There has been little change to the fundamental operating model for regulators over the past several decades

- Current international efforts at addressing these challenges are not sufficient
- Several of FDA's foreign counterparts have begun to implement innovative solutions to address the global safety challenges facing FDA

- FDA is pursuing a strategy with four primary components to address the coming challenges
  - FDA will partner with foreign counterparts to create global coalitions of regulators focused on ensuring and improving global product safety
  - FDA will work to build a global data information system and network and proactively share data with peers
  - FDA will expand its capabilities in intelligence gathering and use, with an increased focus on risk analytics and thoroughly modernized IT capabilities
  - FDA will effectively allocate agency resources on the basis of risk, leveraging the combined efforts of government, industry, and public and private sector third parties
- Implementing the pathway to global product safety and quality
  - Establishing the framework and approach for broader data sharing and use of third parties
  - Establishing the Global Coalitions of Regulators

*Source*: Food and Drug Administration 2011, *A Special Report – Pathway to Global Product Safety and Quality,* viewed *Apr 5, 2014, http://www.fda.gov/downloads/AboutFDA/CentersOffices/OfficeofGlobalRegulatoryOperationsandPolicy/GlobalProductPathway/UCM262528.pdf*

## 7.16 SUMMARY OF IMPORTANT POINTS

1. The major regulatory agencies worldwide are the following:
   - FDA of the United States
   - EMA of European Union
   - Pharmaceuticals and Medical Devices Agency (PMDA) of Japan
   - CFDA of China
   - CDSCO of India
   - TGA of Australia
   - Health Canada of Canada.
2. At FDA the Center for Drug Evaluation and Research (CDER) is responsible for the approval of small molecule drugs and therapeutic biologics while nontherapeutic biologics and blood products are under the jurisdiction of the Center for Biologics Evaluation and Research (CBER).
3. Drug approval at EMA is by the Committee for Medicinal Products for Human Use (CHMP).
4. The Pharmaceutical and Medical Device Agency (PMDA) is responsible for drug approval in Japan.

5. In China, the Department of Drug and Cosmetics Registration (DDCR) manages drug approval through the Division of Pharmaceuticals for "Western drugs," the Division of Biological Products for biologics, and the Division of TCM for traditional Chinese Medicine.
6. The other nonregulatory authorities related to drugs are (i) the ICH, which harmonizes regulations for the United States, European Union, and Japan, (ii) the WHO, which sets global guidance and standards on health matters and coordinates international health activities, and (iii) the PIC/S, which sets up mutual recognition on GMP inspections.

## 7.17 REVIEW QUESTIONS

1. Distinguish the different responsibilities of CDER and CBER.
2. Explain the formation of EMA and its drug approval procedure.
3. How does the MHLW of Japan treat foreign clinical trial data in its approval process?
4. Describe the mechanism for the approval of drugs in China by CFDA.
5. Outline the four categories of harmonized documents prepared by ICH.
6. Describe the functions served by PIC/S.
7. Provide a summary of the International Health Regulations (2005) and the roles for member states and WHO in the control of spread of diseases.

## 7.18 BRIEF ANSWERS AND EXPLANATIONS

1. Refer to Sections 7.2.1 and 7.2.2 about CDER and CBER. It should be understood that small and large molecule drugs are legislated differently: the former under the FDCA and the latter under the PHSA.
2. EMA is an agency set up by the European Union to evaluate and approve drugs for European countries under the centralized procedure for all biopharmaceuticals, new drugs for specific diseases, and orphan drugs (Section 7.3 and Exhibit 8.4). The other two routes to drug approval in Europe are the mutual recognition procedure and decentralized procedure. EMA may arbitrate if disputes arise on drug approval decisions via the mutual recognition procedure.
3. Foreign data are acceptable to Japan's authority, but there may be additional information with regard to immunological responses and ethnic factors required to gain approval (Section 7.4).
4. Drugs are separated into three categories in China: "Western drugs" are reviewed by the Division of Pharmaceuticals, biologics by the Division of Biological Products, and traditional Chinese medicine by the Division of Traditional Chinese Medicines and Ethno Medicines (Section 7.5). The approval process is described in Section 8.5.

5. Refer to Section 7.11 and Exhibit 7.6.
6. PIC/S is a cooperative entity for member countries to work on harmonization of documents and procedures and mutual recognition processes with respect to GMP inspections being performed.
7. Refer to Section 7.14.

## 7.19 FURTHER READING

Central Drugs Standard Control Organization, India website, http://www.cdsco.nic.in/forms/contentpage1.aspx?lid=1424

China Food and Drug Administration website, http://eng.sfda.gov.cn/WS03/CL0755/

Deloitte 2011, *The Next Phase: Opportunities in China's Pharmaceuticals Market*, viewed April 3, 2014, https://www.deloitte.com/assets/Dcom-Germany/Local%20Assets/Documents/03_CountryServices/2012/CSG_Studie_Pharmaceutical_China_190112.pdf

European Agency for the Evaluation of Medicinal Products website, http://www.emea.eu.int/

Food and Drug Administration website, http://www.fda.gov/

Food and Drug Administration, Center for Biologics Evaluation and Research website, http://www.fda.gov/cber/

Food and Drug Administration, Center for Drugs Evaluation and Research website, http://www.fda.gov/cder/

Health Canada website, http://www.hc-sc.gc.ca/index-eng.php

International Conference on Harmonization website, http://www.ifpma.org/ ich1.html

Japan Pharmaceutical Manufacturers Association 2012, *Pharmaceutical Administration and Regulations in Japan*, viewed April 3, 2014, http://www.jpma.or.jp/english/parj/pdf/2012.pdf

Mantus, D and Pisano, DJ (eds.) 2014, *FDA Regulatory Affairs*, 3rd edn., CRC Press, Boca Raton, FL.

Ministry of Health, Labor and Welfare, Japan website, http://www.mhlw.go.jp/english/index.html

Pharmaceuticals and Medical Devices, Japan website, http://www.pmda.go.jp/english/

Pharmaceutical Inspection Cooperation Scheme website, http://www.picscheme.org/

Therapeutic Goods Administration, Australia website, http://www.tga.gov.au

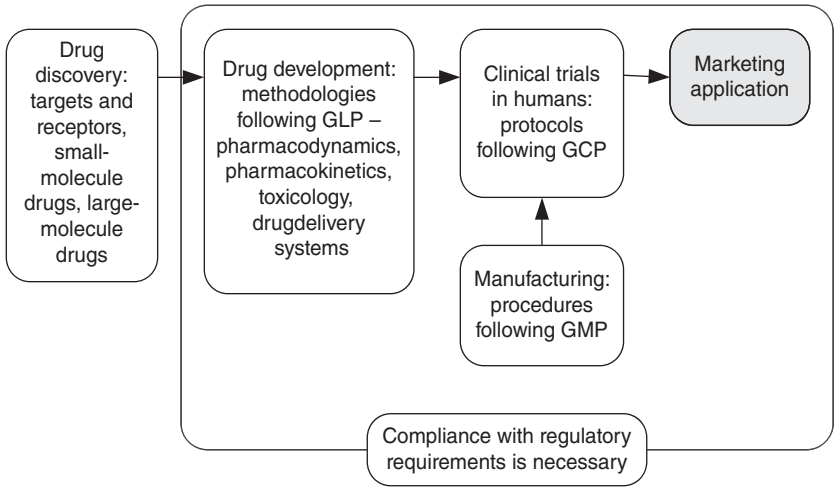
Vatsan, RS et al. 2013, 'Regulation of immunotherapeutic products for cancer and FDA's role in product development and clinical evaluation', *Journal of ImmunoTherapy of Cancer*, 1:5, doi:10.1186/2051-1426-1-5

World Health Organization website, http://www.who.int/en/

World Health Organization 2013, *The World Health Report 2013*, viewed April 4, 2014, http://apps.who.int/iris/bitstream/10665/85761/2/9789240690837_eng.pdf?ua=1

# CHAPTER 8

# REGULATORY APPLICATIONS




## 8.1 INTRODUCTION

There are very few regulatory requirements that stipulate how an organization or institution should conduct drug discovery. In general, organizations and institutions are

*Drugs: From Discovery to Approval*, Third Edition. Rick Ng.

relatively unencumbered on the methods and techniques they adopt to discover new drugs, except in the case of gene therapy and stem cell research, where the regulations are specific and ethical limits set. However, as drugs move along the pipeline from discovery to preclinical and clinical trials and marketing authorization there are strict procedures to follow.

In Chapter 5, we discussed the use of animals in preclinical studies. The applicable regulatory requirement is Good Laboratory Practice (GLP). In Chapter 6, we discussed clinical trials in humans. Here Good Clinical Practice (GCP) is required. Further along the pipeline, assuming that the drug shows efficacy with acceptable adverse events in the clinical trials, the drug will be registered and manufactured in compliance with Good Manufacturing Practice (GMP) for commercial sale. The processes for all these steps are governed by regulatory authorities as discussed in Chapter 7.

The United States Food and Drug Administration (FDA) has one of the most comprehensive and transparent regulatory systems in the world. In this chapter, we base most of our discussion on the FDA system. The aim in this Chapter is to introduce the processes for regulatory approvals. Before any new drug is trialed on human subjects, an Investigational New Drug (IND) application has to be filed. At the conclusion of clinical trials, the marketing approval for a drug is filed using a New Drug Application (NDA) for small molecule drugs or a Biologics License Application (BLA) for protein-based drugs. In the case of a generic drug, an Abbreviated New Drug Application (ANDA) is filed. Other regulatory processes for Europe, Japan, China, India, Australia, and Canada are introduced in later sections of this chapter.

## 8.2 UNITED STATES

### 8.2.1 Drug Development Process

Figure 8.1 shows the drug development processes and the applicable regulatory steps. Each item in the diagram is progressively explained in the following sections. Before a drug is administered to humans, FDA requires that preclinical research on animals be carried out. The information is necessary to assess the safety level of the drug. On the basis of this information, clinical trials on humans can be designed. The trial protocol will consider the safe dose, methods for dose ranging, route of drug administration, and toxicity effects.

### 8.2.2 Investigational New Drug

IND is an application to FDA to seek permission for a human clinical trial to be conducted. An IND application is detailed under 21 CFR Part 312. The process for an IND is summarized in Figure 8.2.

An organization or institution, called a sponsor, is responsible for submitting the IND application. The relevant authorities are the Center for Drug Evaluation and Research (CDER) for small molecule drugs and specified biologics and the Center for Biologics Evaluation and Research (CBER) for traditional biologics (refer to Sections 7.2.1 and 7.2.2). A pre-IND meeting can be arranged with FDA to discuss a number of issues:

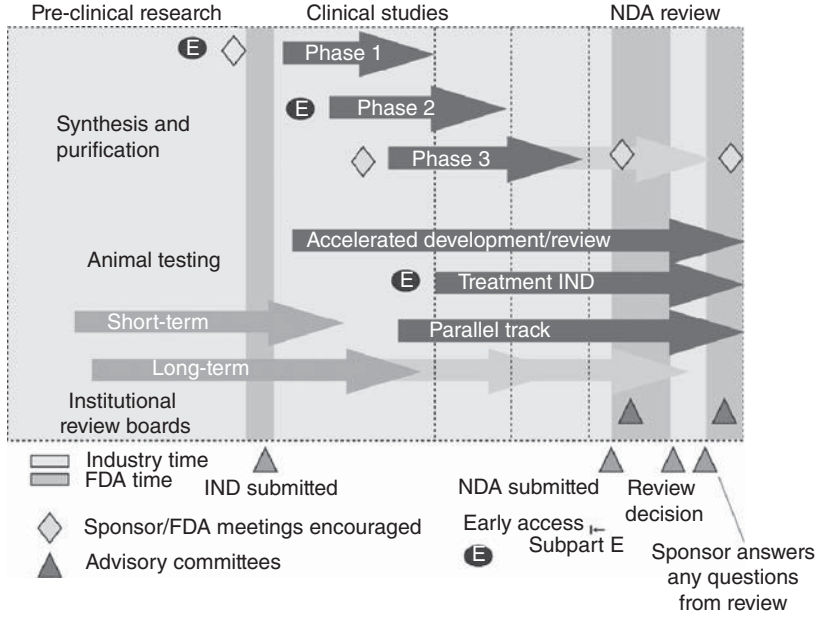



**Figure 8.1** Drug development process. (*Source*: Food and Drug Administration 2012, '*New Drug Development and Review Process*', viewed April 14, 2014, http://www.fda.gov/drugs/developmentapprovalprocess/smallbusinessassistance/ucm053131.htm.)

- The design of animal research, which is required to lend support to the clinical studies
- The intended protocol for conducting the clinical trial
- Discussion on chemistry, manufacturing, and control of the investigational drug.

Such a meeting will help the sponsor to organize animal research, gather data, and design the clinical protocol on the basis of suggestions by FDA.

An IND is submitted on Form 1571. The materials to submit to FDA are stated in Section 13 of Page 2 of Form 1571. These are:

1. Form 1571 *[21CFR 312.23(a)(1)]*
2. Table of Contents *[21 CFR 312.23(a)(2)]*
3. Introductory Statement *[21 CFR 312.23(a)(3)]*
4. General Investigational Plan *[21 CFR 312.23(a)(3)]*
5. Investigator's Brochure *[21 CFR 312.23(a)(5)]*
6. Protocols *[21 CFR 312.23(a)(6)]*
   Study Protocols *[21 CFR 312.23(a)(6)]*
   Investigator data *[21 CFR 312.23(a)(6)(iii)(b)]*
   Facilities data *[21 CFR 312.23(a) (6)(iii)(b)]*
   Institutional Review Board data *[21 CFR 312.23(a)(6)(iii)(b)]*

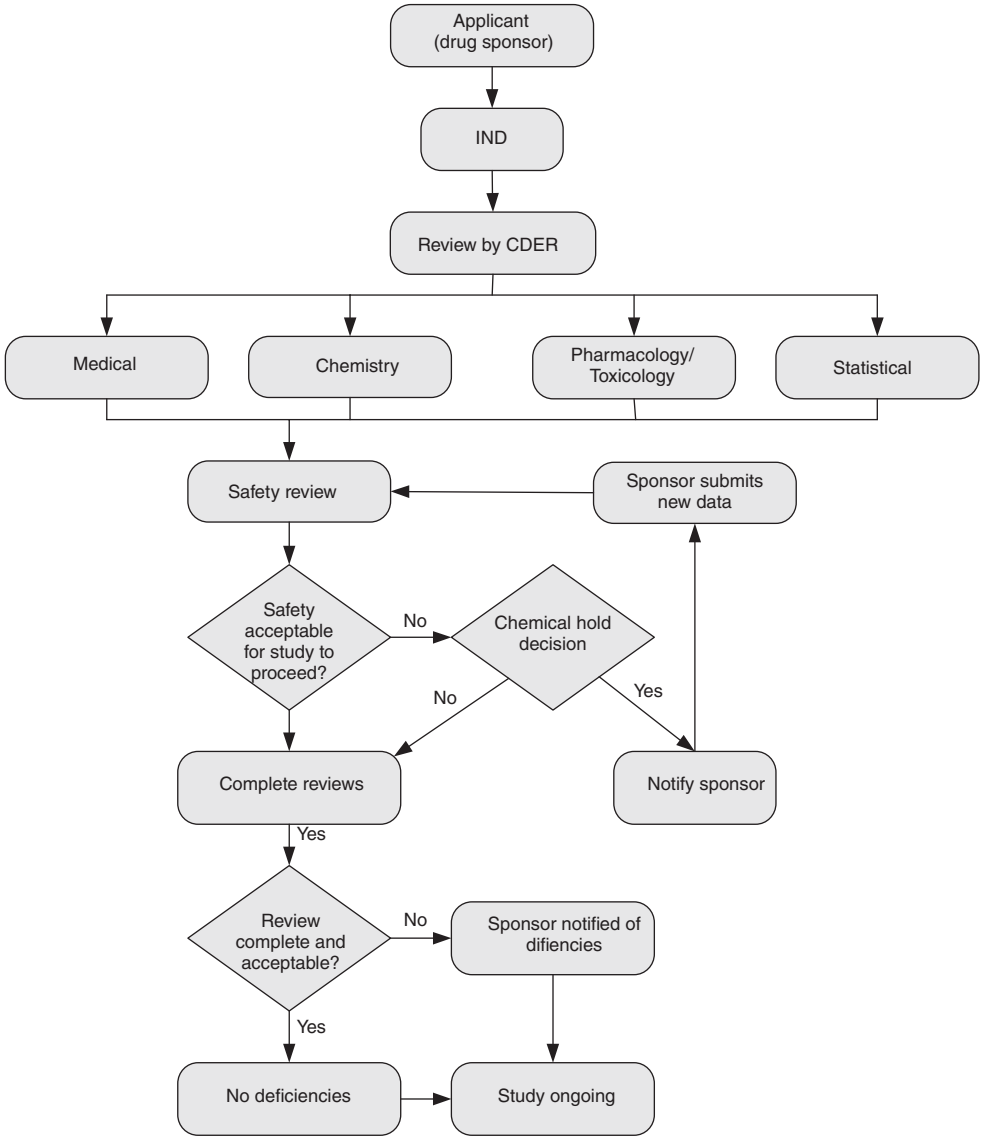



**Figure 8.2** The IND process. (*Source*: Food and Drug Administration 1998, '*The CDER Handbook*', April 14, 2014, http://www.fda.gov/downloads/AboutFDA/CentersOffices/CDER/UCM198415.pdf.)

7. Chemistry, Manufacturing, and Control data *[21 CFR 312.23(a)(7)]*
   Environmental Assessment or claim for exclusion *[21 CFR 312.23(a)(7)(iv)(e)]*
8. Pharmacology and Toxicology data *[21 CFR 312.23(a)(8)]*
9. Previous Human Experience *[21 CFR 312.23(a)(9)]*

10. Additional Information *[21 CFR 312.23(a)(10)]*
11. Biosimilar User Fee Cover Sheet *(Form FDA 3792)*
12. Clinical Trials Certification of Compliance *(Form FDA 3674)*.

Items 1–3 and 10 are self-explanatory and will not be discussed further. Items 4–6 and 9 on Investigational Plan, Investigator's Brochure, Protocols, and Human Experience are covered in Chapter 6, and Item 8, Pharmacology and Toxicity data, are discussed in Chapter 5. Item 11 is a self-generated invoice to determine the fee required for biosimilar biological products. Item 12 is to certify that all applicable requirements of 42 USC § 282(j) (the Public Health Service Act) have been met, and the appropriate National Clinical Trial (NCT) numbers have been provided if available. We concentrate our discussion on Item 7 in this chapter.

***Chemistry, manufacturing, and control***: As stated in 21 CFR Part 312, the chemistry, manufacturing, and control (CMC) information is to "describe the composition, manufacture, and controls of the drug substance and the drug product … sufficient information is required to be submitted to assure the proper identification, quality, purity and strength of the investigational drug … "

FDA has various guidelines pertaining to the requirements of the data to be presented in the CMC for different drugs. In general, the CMC describes the drug, its chemistry, and characterization. Other requirements are the manufacturing processes, quality control testing and storage, stability, and labeling. We highlight in Table 8.1 an example of the necessary information to be provided for a vaccine CMC according to the contents presented in *Guidance for Industry: Content and Format of Chemistry, Manufacturing and Controls Information and Establishment Description Information for a Vaccine or Related Product* (CBER, January 1999).

The sponsor has to explain how the drug substance and drug product are to be manufactured, tested, and stored. The important criterion is to ensure that it is safe for the subjects of the clinical trials. The CMC is a "living" document; it is updated as the clinical trials proceed from Phase I to Phases II and III and eventually to a licensed product. In essence, the CMC describes the adherence to GMP for the manufacture of the trial drug. The subject of GMP is described in Chapters 9 and 10.

***IND review***: Following submission of Form 1571, FDA has 30 days to review the application. The topics reviewed are medical, chemistry, pharmacology, toxicology, and statistics. Medical review focuses on the design of the clinical trial protocol, risk–benefit issues for the trial subjects, and supporting safety data from preclinical research. Chemistry review is based on the CMC to determine that appropriate controls are in place to manufacture, test, package, and label the drug for the trial. Pharmacological and toxicological review considers the mechanism of drug action, absorption, distribution, metabolism, and excretion (ADME), organs targeted or affected by the drug, and toxicological studies, including acute toxicity doses. Statistical review examines the design of the protocol with respect to subject numbers, doses, biomarkers, or indicators to demonstrate that sufficient data will be gathered for meaningful statistical analysis of the outcomes.

Within 30 days, FDA informs the sponsor if a clinical hold is imposed. Without FDA objections, the trial may then commence. In certain cases, there may be additional information that FDA requires the sponsor to submit, in which case the trial is put on hold

**TABLE 8.1 Content and Format of Chemistry, Manufacturing, and Controls Information**

| Drug Substance | Drug Product |
|---|---|
| A. Description and characterization<br>1. Description<br>2. Characterization<br>a. Physicochemical characterization<br>b. Biological activity<br>B. Manufacturer<br>1. Identification<br>2. Floor diagram(s)<br>3. Manufacture of other products<br>4. Contamination precautions<br>C. Method of manufacture<br>1. Raw materials<br>2. Flow charts<br>3. Detailed description<br>a. Animal sources<br>b. Virus sources<br>c. Cellular sources (microbial cells, animal cells, genetic constructs, cell bank system, cell growth, and harvesting)<br>d. Purification and downstream processing (inactivation, purification, stability processing, detoxification)<br>e. Synthetic drug substance (synthetic peptides, conjugates, and modified drug substance)<br>4. Batch records<br>D. Process controls<br>1. In-process controls<br>2. Process validation<br>a. Propagation<br>b. Harvest<br>c. Inactivation<br>d. Purification<br>e. Microbiology<br>3. Control of bioburden<br>E. Manufacturing consistency<br>1. Reference standards<br>2. Release testing | A. Description and characterization<br>1. Composition<br>a. Drug substance(s)<br>b. Excipient<br>c. Adjuvant<br>d. Preservative<br>2. Specifications and analytical methods for drug product ingredients<br>a. Description<br>b. Identity<br>c. Purity and impurities<br>d. Potency<br>B. Manufacturing and facilities<br>C. Manufacturing methods<br>D. Drug product specifications<br>1. Sampling procedures<br>2. Specifications and methods<br>3. Validation results<br>E. Container and closure system<br>F. Microbiology<br>G. Lyophilization<br>H. Drug product stability<br>1. Stability protocol<br>2. Stability data<br>3. Stability program |

(*continued*)

**TABLE 8.1 (*Continued*)**

| Drug Substance | Drug Product |
|---|---|
| F. Drug substance specifications | |
| 1. Specifications | |
| 2. Impurities profile | |
| G. Reprocessing | |
| H. Container and closure system | |
| I. Drug substance stability | |

*Source*: Adapted from Food and Drug Administration 2014, *Guidance for Industry: Content and Format of Chemistry, Manufacturing and Controls Information and Establishment Description Information for a Vaccine or Related Product*, viewed April 14, 2014, http://www.fda.gov/biologicsbloodvaccines/guidancecompliance regulatoryinformation/guidances/vaccines/ucm076612.htm.

until all queries are satisfactorily answered. If FDA considers the information provided does not support the conduct of a trial or subjects may be at unacceptable risk in a trial, the clinical hold is not lifted.

***Phases I, II, and III trials***: An IND may be submitted for one or more phases of the clinical trials. In general, once the original IND is filed and recognized by FDA, amendments are filed to the IND, for example, to obtain permission to advance to additional trials, to report safety information, to report changes in manufacturing, and to provide annual reports. At any stage of the trial, FDA has the authority to put clinical hold on the trial until deficiencies or safety issues are resolved. The sponsor can request meetings with FDA at various stages as below:

- *End of Phase I meeting*: After completing Phase I, sponsor meets with FDA to discuss results of the trial and agree on plan for Phase II studies
- *End of Phase II/pre-Phase III meeting*: The meeting will evaluate the data obtained from Phase II studies. If the results are encouraging, Phase III is planned to gather further confirmation of the safety and efficacy of the drug. A more extensive protocol may need to be devised.
- *Pre-NDA/BLA meeting*: This meeting is to prepare for the filing of the NDA (for small molecule drug) or BLA (for protein-based drug). Results from Phase III are discussed. These data should support the safety and efficacy of the drug. A meeting at this stage can help to facilitate FDA review process by providing the necessary data when the NDA or BLA is submitted.

***Other review mechanisms***: Although most drugs go through all the stages of Phases I, II, and III, there are special mechanisms in place to expedite development and approval of certain drugs. These mechanisms include:

- *Expedited programs*: They are divided into Fast Track, Breakthrough Therapy, Accelerated Approval, and Priority Review as shown in Table 8.2. There are different criteria involved for each program, but the common criterion among them

**TABLE 8.2 FDA's Expedited Programs**

| | Fast Track | Breakthrough Therapy | Accelerated Approval | Priority Review |
|---|---|---|---|---|
| Qualifying criteria | A drug that is intended to treat a serious condition<br>AND nonclinical or clinical data demonstrate the potential to address unmet medical needs<br>OR<br>A drug that has been designated as a qualified infectious disease product | A drug that is intended to treat a serious condition<br>AND preliminary clinical evidence indicates that the drug may demonstrate substantial improvement on a clinically significant endpoint(s) over available therapies | A drug that treats a serious condition<br>AND generally provides meaningful advantage over available therapies<br>AND demonstrates an effect on a surrogate endpoint that is reasonably likely to predict clinical benefit or on a clinical endpoint that can be measured earlier than an effect on irreversible morbidity or mortality (IMM) that is reasonably likely to predict an effect on IMM or other clinical benefit (i.e., an intermediate clinical endpoint) | An application (original or efficacy supplement) for a drug that treats a serious condition<br>AND if approved, would provide a significant improvement in safety or effectiveness<br>OR any supplement that proposes a labeling change pursuant to a report on a pediatric study under 505A<br>OR an application for a drug that has been designated as a qualified infectious disease product<br>OR any application or supplement for a drug submitted with a priority review voucher |

*Source*: Food and Drug Administration 2013, *Guidance for Industry: Expedited Programs for Serious Conditions – Drugs and Biologics*, viewed April 21, 2014, http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm358301.pdf.

is that the drug is intended for the treatment of serious conditions and an expedited program will help to bring these drugs to the patients in shorter timeframes. Refer to Exhibit 8.1 on Imbruvica and Sovaldi, two drugs approved under the expedited programs.

- *Treatment investigational new drugs* (*Treatment IND*): FDA allows certain drugs to be administered to patients who have life-threatening illnesses that will lead to death without suitable treatment. To qualify for Treatment IND the drug has to be under investigation concurrently in a controlled clinical trial and that the sponsor is actively pursuing marketing approval of the drug.
- *Parallel track*: Some patients do not fulfill the inclusion criteria to be enrolled in clinical trials, but their conditions qualify them to be treated in parallel with an

**Exhibit 8.1 Imbruvica and Sovaldi**

*Imbruvica*

In November 2013, FDA granted Imbruvica (ibrutinib) accelerated approval to treat patients with mantle cell lymphoma, a rare and aggressive type of blood cancer, if those patients received at least one prior therapy.

Imbruvica works by blocking the enzyme that allows cancer cells to grow and divide.

In February 2014, FDA approved the expanded use of Imbruvica for chronic lymphocytic leukemia (CLL) patients who have received at least one previous therapy. CLL is a rare blood and bone marrow disease that usually gets worse slowly over time, causing a gradual increase in white blood cells called B lymphocytes, or B cells.

(Refer also to Exhibit 11.2).

*Source*: Data from Food and Drug Administration 2014, FDA New Release: FDA approves Imbruvica to treat chronic lymphocytic leukemia, viewed April 20, 2014, http://www.fda.gov/newsevents/newsroom/pressannouncements/ucm385764.htm.

*Sovaldi*

In December 2013, FDA approved Sovaldi (sofosbuvir) to treat chronic hepatitis C virus (HCV) infection under the breakthrough therapy designation. Sovaldi is the first drug that has demonstrated safety and efficacy to treat certain types of HCV infection without the need for coadministration of interferon.

Hepatitis C is a viral disease that causes inflammation of the liver that can lead to diminished liver function or liver failure.

Sovaldi is a nucleotide analog inhibitor that blocks a specific protein needed by the HCV to replicate. Sovaldi is to be used as a component of a combination antiviral treatment regimen for chronic HCV infection.

*Source*: Food and Drug Administration 2013, *FDA News Release: FDA approves Sovaldi for chronic hepatitis C*, viewed April 20, 2014, http://www.fda.gov/newsevents/newsroom/pressannouncements/ucm377888.htm.

ongoing clinical trial. AIDS patients are an example for this group. This Parallel Track policy is now superseded by the Treatment IND mechanism.

### 8.2.3 New Drug Application/Biologics License Application

At the conclusion of the Phase III clinical trial, if the results demonstrate that the drug is safe and efficacious over existing treatment drugs, an application is made to FDA to seek approval for marketing the drug. An NDA or a BLA is filed. NDA is covered under the Federal Food, Drug and Cosmetic Act, Section 505 while BLA is mandated by the Public Health Service Act, Section 351. The process for filing and reviewing of the NDA/BLA is presented in Figure 8.3. The application is submitted using Form 356h (Figure 8.4).

Form 356h is a harmonized form, and a sponsor can use it for NDA, BLA, and ANDA (refer to Section 8.2.5). Page 1 of the form is for Applicant Information, Product Description, and Application Information. Pages 2 and 3 require Establishment Information and the provision of a number of items to substantiate the application. The items to be submitted under Form 356h are:

1. Index
2. Labeling
3. Summary
4. Chemistry section
   - Chemistry, manufacturing, and controls information
   - Samples
   - Methods validation package
5. Nonclinical pharmacology and toxicology section
6. Human pharmacokinetics and bioavailability section
7. Clinical microbiology
8. Clinical data section
9. Safety update report
10. Statistical section
11. Case report tabulations
12. Case report forms
13. Patent information on any patent that claims the drug
14. A patent certification on any patent that claims the drug
15. Establishment description
16. Debarment certification
17. Field copy certification
18. User fee cover sheet
19. Financial disclosure information
20. Other

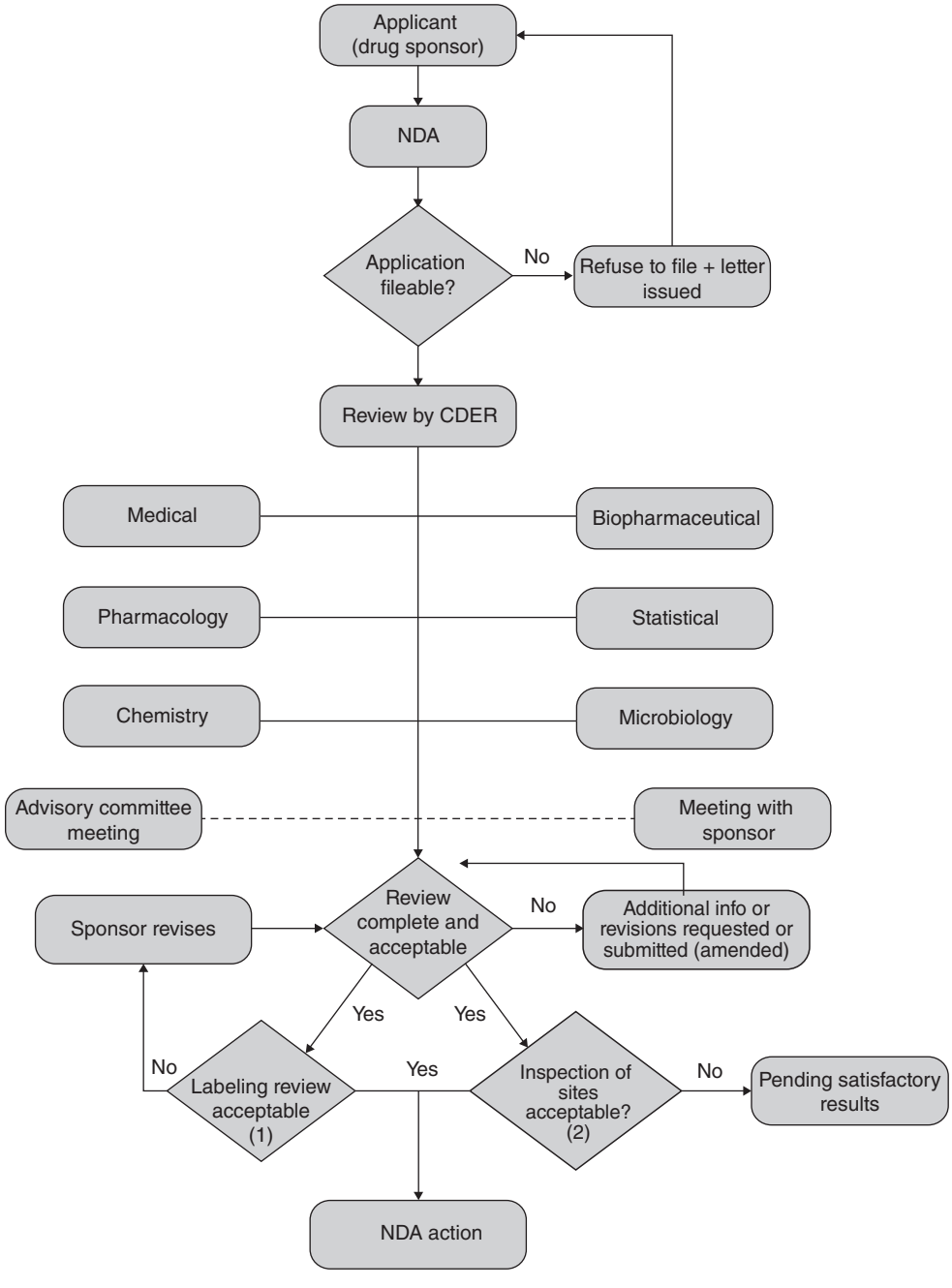



**Figure 8.3** The NDA process. (*Source*: Food and Drug Administration 1998, '*The CDER Handbook*', April 14, 2014, http://www.fda.gov/downloads/AboutFDA/CentersOffices/CDER/UCM198415.pdf.)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

APPLICATION TO MARKET A NEW OR ABBREVITED NEW DRUG OR BIOLOGOC FOR HUMAN USE

*(Title 21, Code of Federal Regulations, Parts 314 & 601)*

Form Approved: OMB NO. 0910-0338
Expiration Date: January 31, 2017
*See PRA Statement on page 3.*

1. Date of Submission *(mm/dd/yyyy)*

APPLICAT INFORMATION

2. Name of Applicant

3. Telephone Number *(Include contry code if applicable and are code)*

4. Fnasimile (FAX) Number *(Include contry code if applicable and area code)*

5. Applicant Address

Address 1 *(Street address, P.O. box, company name c/o)*

Address 2 *(Apartment, suite, unit, building, floor, etc.)*

City | State/Provience/Region | ZIP or Postal Code | Country

Email Address

U.S. License Number if Previously issued

6. Authorized U.S.Agent *(Required for non-U.S.Applicants)*

Authorized U.S.Agent Name

Address 1 *(Street address, P.O. box, company name c/o)*

Address 2 *(Apartment, suite, unit, building, floor, etc.)*

City | State

ZIP Code

Telephone Number *(Include area code)*

FAX Number *(Include area code)*

Email Address

PRODUCT DESCRIPTION

7. NDA, ANDA, or BLA Application Number

8. Supplement Number *(If applicable)*

9. Established Name *(e.g., proper name, USP/USAN name)*

10. Proprietary Name *(e.g., proper name, USP/USAN name)*

11. Chemical/Biochemical/Blood Product Name *(If any)*

12. Dosage Form

13. Strengths

14. Route of Administration

15. Proposed Indication for Use

Is this indication for a rae disease (prevalance <200,000 in U.S.)? ☐ Yes ☐ No

Does this product have an FDA Orphan Designation for this indication? ☐ Yes ☐ No

If yes, provide the Orphan Designation number forn this indication:

Contain. Page for #15

APPLICATION INFORMATION

16. Application Type *(Select one)* ☐ New Drug Application (NDA) ☐ Biologics License Application (BLA) ☐ Abbreviated New Drug Application (ANDA)

17. If an NDA, identify the type ☐ 505 (b)(1) ☐ 505 (b)(2)

18. If BLA, identify the type ☐ 351 (a) ☐ 351 (k)

19. If a 35(k), identify the biological refernce product tha the basic for the submission.
Name of Biologic: _____ Holder of Licensed Application: _____

20. If an ANDA, or 505(b)(2), identify the listed drug product that is the basis for the submission.
Name of Drug: _____ Application Number of Relied Upon Product: _____
Indicate Patent Certification(s): ☐ P1 ☐ P2 ☐ P3 ☐ P4 ☐ Section viii - MOU ☐ Statement of no relevent Patents

21. Submission *(Select one)* ☐ Original ☐ Labeling Suppliment ☐ CMC Supplement ☐ Efficacy Supplement ☐ Annual Report ☐ Product Correspondence ☐ REMS Supplement ☐ Postmarketing Requirements or Commitments ☐ Periodic Safety Report ☐ Other *(Specify)* _____

**Figure 8.4** Form 356 h (Page 1). (*Source*: Food and Drug Administration 2014, *Application to Market a New Drug, Biologic or an Antibiotic Drug for Human Use*, viewed April 21, 2014, http://www.fda.gov/downloads/AboutFDA/ReportsManualsForms/Forms/UCM082348.pdf.)

The submission of Form 356h is the culmination of all the work and effort that has been put into discovering, developing, and trialing the drug. The information submitted is substantial, with many volumes prepared for separate sections; literally, truckloads of documents are delivered to FDA. However, the submission can now be streamlined through electronic means, for example, via the electronic Common Technical Document system (eCTD – refer to Sections 7.11 and 8.9). By 2016, FDA requires that all NDA, BLA, and ANDA submissions to be in eCTD. Instructions for electronic submission are detailed in the FDA document *Regulations and Instructions for Submitting Drug Applications Electronically*. It should be noted that every new drug in the United States has been approved via the NDA process since 1938, although there have been changes to the requirements for submission over the years. Before the introduction of BLA in 1998, biologics were approved under two separate submissions of Product License Application (PLA) and Establishment License Application (ELA).

Details for the required information to be submitted with Form 356h are stated in 21 CFR Part 314 for small molecule drugs and 21 CFR Part 601 for protein-based drugs. We select a few key items for discussion.

***Index***: The index of Form 356h sets out how the extensive numbers of documents are to be referenced. A well-organized index system is important for the reviewers to search for the required information. This will assist to expedite the review process, without the necessity for FDA to stop the review time clock to seek clarification.

***Labeling***: Labeling is reviewed following requirements of 21 CFR Part 201. The requirements are as listed in Table 8.3.

***Summary***: The summary presents the case for the drug's approval. It includes discussion on the drug's mechanism of action, its effect on animals, results of clinical trials, manufacturing and tests methods, its stability and proposed dosage, and treatment protocol. The summary may run into hundreds of pages. It is one of the few documents being read by all the different reviewers; as such, a good summary will assist with the review process.

***Chemistry section***: This is the CMC with updated information pertaining to the chemistry, manufacturing, and control of the drug. FDA recognizes that manufacturing processes and test methods go through various stages of optimization and refinement as the drugs are produced for Phases I and II clinical trials. By the Phase III stage, however, all the manufacturing processes are expected to be defined and test methods validated. A detailed explanation of the drug manufacturing processes is presented in Chapter 10. Some pertinent data are given below:

- *Drug molecule*: chemical composition, physical and chemical characteristics, and specifications.
- *Raw materials*: list of all materials used, specifications and tests for these raw materials.
- *Equipment*: list of equipment used, validation of the equipment, validated methods for cleaning, and procedures for contamination control.
- *Analytical methods*: validations are performed to assure that the analytical methods are appropriate for the tests.
- *Manufacturing processes*: flow charts for production steps, controls of contamination, removal of impurities, purification steps, in-process tests, and batch records.

**TABLE 8.3 Review of Labeling**

| Item | Explanatory Notes |
|---|---|
| Description | Proprietary and established name of drug; dosage form; ingredients; chemical name; and structural formula. |
| Clinical pharmacology | Summary of the actions of the drug in humans; *in vitro* and *in vivo* actions in animals if pertinent to human therapeutics; pharmacokinetics. |
| Indications and usage | Description of use of drug in the treatment, prevention, or diagnosis of a recognized disease or condition. |
| Contraindications | Description of situations in which the drug should not be used because the risk of use clearly outweighs any possible benefit. |
| Warnings | Description of serious adverse reactions and potential safety hazards, subsequent limitation in use, and steps that should be taken if they occur. Certain contraindications or serious warnings, particularly those that may lead to death or injury, may be required by FDA to be presented in a box. |
| Precautions | Information regarding any special care to be exercised for the safe and effective use of the drug. Includes general precautions and information for patients on drug interactions, carcinogenesis/mutagenesis, pregnancy rating, labor and delivery, nursing mothers, and pediatric use. |
| Adverse reactions | Description of undesirable effect(s) reasonably associated with the proper use of the drug. |
| Drug abuse/dependence | Description of types of abuse that can occur with the drug and the adverse reactions pertinent to them. |
| Over dosage | Description of the signs, symptoms, and laboratory findings of acute over dosage and the general principles of treatment. |
| Dosage/administration | Recommendation for usage dose, usual dosage range, and, if appropriate, upper limit beyond which safety and effectiveness have not been established. |
| How to be supplied | Information on the available dosage forms to which the labeling applies. |

*Source*: Adapted from Food and Drug Administration 2013, *Code of Federal Regulations Title 21, Part 201*, Subpart B, Sec. 201.57, Specific requirements on content and format of labeling for human prescription drug and biological products described in 201.56(b) (1), viewed April 21, 2014, http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?fr=201.57.

- *Facility*: controls on equipment, calibration policies, security of access, maintenance of clean environment, flow of materials, equipment, and products.
- *Drug stability*: data to substantiate the stability of the drug for storage and transportation.
- *Product release criteria*: specifications, test methods, storage, and shipping conditions.

For biopharmaceuticals, further information is required. Listed below are some examples:

- *Cell line*: source, species, history, characteristics, cloning methods, vectors used, genotype, and phenotype of host cell system.
- *Cell bank*: controls for working and master cell banks.
- *Assays*: validated methods of analysis, for example, ELISA for MAb, QPCR for residual DNA and potency assays for vaccines.
- *Production*: culture medium used, cell culture and fermentation techniques, in-process controls, purification steps, and cleaning of chromatographic columns and matrices.

The CMC details all the manufacturing steps and controls being introduced, to ensure that the drug product is pure, consistent, safe, and effective. The sponsor has to demonstrate that the manufacturing facility is set up and complies with GMP regulations for the production of the drug when it is approved. FDA has the right to obtain samples from the sponsor for evaluation and test.

The CMC can be submitted in the format as detailed in Module 3 of the CTD (refer to Section 8.9).

***Nonclinical pharmacology and toxicology section***: This Section is to present data in addition to that included in the IND. Long-term toxicology data are required. The sponsor is also expected to provide study results of the drug on reproduction and effects on fetuses. Module 4 of the CTD sets out the format for the presentation of nonclinical data (refer to Section 8.9).

***Clinical results***: Items 6–12 of Form 356h are all related to the clinical results. These are perhaps the most important sections of the submission to demonstrate the safety and efficacy of the drug for treating the target disease. Detailed analyses of clinical data are presented to support the application. Some of these analyses are:

- Kinetics studies to show the ADME mechanisms on target organs and tissues
- For anti-infective agents, *in vivo* and *in vitro* tests and the effects of the drug on the microorganisms have to be reported
- Description of the statistical model adopted for analyses
- Statistical analyses of results from the clinical trials, showing statistical power of the test
- Comparison of the therapeutic index and safety data
- Report on adverse events, incapacity and death, if any, and investigation of the cause.

Module 5 of the CTD sets out the format for the presentation of clinical data (refer to Section 8.9).

***Drug Master File***: As stated by FDA, the Drug Master File (DMF) is submitted to FDA to provide confidential information relating to the facilities and manufacturing processes and techniques for producing the drug material. It is, however, not required by law or FDA regulations that a DMF be submitted accompanying the IND or NDA/BLA applications. In reality, however, most organizations prepare and submit the DMF with their applications.

The FDA *Guideline for Drug Master Files* (21 CFR Part 314.420) consists of the following sections:

- Contents
- Definitions
- Types of Drug Master Files
  - Type I: Not applicable. Provision removed by FDA
  - Type II: Drug substance, drug substance intermediate, and materials used in their preparation, or drug product
  - Type III: Packaging materials
  - Type IV: Excipient, colorant, flavor, essence, or materials used in their preparation
  - Type V: Facilities for production, contract manufacturing facilities, and testing facilities
- Authorization to Refer to a Drug Master File
- Processing and Reviewing Policies
- Holder Obligations
- Closure of a Drug Master File

The Type V DMF enables confidential information to be submitted to FDA, for example, a contract manufacturing facility may provide proprietary information to FDA without divulging it to the sponsor client. FDA reviews the DMF, but the DMF is never approved or disapproved. The holder of the DMF is notified of deficiencies for rectification. It is the holder's responsibility to update the DMF on an annual basis.

***NDA/BLA review***: Review is undertaken by FDA staff from different offices within CDER and CBER. These staff members are trained physicians, statisticians, chemists, biologists, pharmacologists, and other scientists. FDA may consult with external review committees and experts (Advisory Committees), but is not bound by their recommendations.

Since the introduction of the Prescription Drug User Fee Act (PDUFA, see Review Outcome below) in 1992, FDA has set a target time for the review of NDA/BLA. In general, the review time for the standard NDA/BLA is 12 months, including FDA time and sponsor time to respond to deficiencies. The target for priority expedited NDA/BLA is 6 months. Table 8.4 shows the review time of the NDA/BLA application. Drugs are eligible for priority review if they show significant improvement compared with marketed products in the treatment, diagnosis, or prevention of a disease.

For a new facility being set up to manufacture a drug under NDA/BLA, FDA is likely to perform a preapproval inspection (PAI) or prelicensing inspection for biologics to ensure the facility has adequate procedures and controls to manufacture the drug under GMP according to *Compliance Program Guide 7346.832*. For an existing facility already manufacturing the drug, with the NDA/BLA for the extension of treatment indications or other non-manufacturing-related matters, FDA may waive the PAI.

When the NDA/BLA is approved, the sponsor has the license to market the drug. It is the sponsor's responsibility to inform FDA of adverse events or any unexpected

**TABLE 8.4 Approval Times for NDAs and BLAs**

| | Priority | | | Standard | | |
|---|---|---|---|---|---|---|
| Year | Number Approved | Median FDA Review Time (Months) | Median Total Approval Time (Months) | Number Approved | Median FDA Review Time (Months) | Median Total Approval Time (Months) |
| 1993 | 19 | 16.3 | 20.5 | 51 | 20.8 | 26.9 |
| 1994 | 16 | 13.9 | 14.0 | 45 | 16.8 | 21.0 |
| 1995 | 16 | 7.9 | 7.9 | 67 | 16.2 | 18.7 |
| 1996 | 29 | 7.8 | 7.8 | 102 | 15.1 | 17.8 |
| 1997 | 20 | 6.3 | 6.4 | 101 | 14.7 | 15.0 |
| 1998 | 25 | 62 | 6.4 | 65 | 12.0 | 12.0 |
| 1999 | 28 | 6.1 | 6.1 | 55 | 12.0 | 13.8 |
| 2000 | 20 | 6.0 | 6.0 | 78 | 12.0 | 12.0 |
| 2001 | 10 | 6.0 | 6.0 | 56 | 12.0 | 14.0 |
| 2002 | 11 | 13.8 | 19.1 | 67 | 12.7 | 15.3 |
| 2003 | 14 | 7.7 | 7.7 | 58 | 11.9 | 15.4 |
| 2004* | 29 | 6.0 | 6.0 | 89 | 11.8 | 12.7 |
| 2005* | 22 | 6.0 | 6.0 | 59 | 11.8 | 13.1 |
| 2006* | 21 | 6.0 | 6.0 | 80 | 12.0 | 13.0 |
| 2007* | 23 | 6.0 | 6.0 | 55 | 10.2 | 10.4 |
| 2008* | 18 | 6.0 | 6.0 | 70 | 13.0 | 13.1 |

*Source*: Food and Drug Administration 2009, *CDER Approval Times for Priority and Standard NDAs and BLAs (1993-2008)*, viewed April 14, 2014, http://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/DrugandBiologicApprovalReports/UCM123957.pdf.

*Include BLAs transferred from CBER to CDER.

findings. FDA has the responsibility to safeguard the public's health. It monitors adverse events, advertising, and manufacturing in accordance with GMP.

***Review outcome***: The outcomes from the review can be classified into three categories:

- *Not approvable letter*: application cannot be approved and deficiencies are detailed.
- *Approvable letter*: deficiencies are minor and can be corrected or supplementary information has to be provided. Eventually, the drug is approved after deficiencies are resolved.
- *Approval letter*: the drug is approved. An example of the approval news for Alprolix, a recombinant coagulation factor IX for adults and children who have hemophilia B to prevent or reduce the frequency of bleeding, is presented in Exhibit 8.2.

On the basis of the PDUFA, FDA collects fees from applicants to expedite the review and approval processes under strict guidelines. The PDUFA fees for the fiscal year 2014 (October 1, 2013–September 30, 2014) are shown in Table 8.5.

**Exhibit 8.2 Alprolix (Factor IX) Approval News**

*FDA approves first long-acting recombinant coagulation Factor IX concentrate for patients with Hemophilia B*

The US Food and Drug Administration today approved Alprolix, Coagulation Factor IX (Recombinant), Fc Fusion Protein, for use in adults and children who have Hemophilia B. Alprolix is the first Hemophilia B treatment designed to require less frequent injections when used to prevent or reduce the frequency of bleeding.

Alprolix is approved to help control and prevent bleeding episodes, manage bleeding during surgical procedures, and prevent or reduce the frequency of bleeding episodes (prophylaxis). Alprolix consists of the Factor IX molecule linked to a protein fragment, Fc, which is found in antibodies. This makes the product last longer in circulation.

"The approval of this product provides another therapeutic option for the treatment and prevention of bleeding in patients with Hemophilia B," said Karen Midthun, M.D., director of the FDA's Center for Biologics Evaluation and Research.

Hemophilia B is an inherited sex-linked, blood-clotting disorder, which primarily affects males, and is caused by defects in the Factor IX gene. Hemophilia B affects about 3,300 people in the United States. People with Hemophilia B can experience repeated episodes of potentially serious bleeding, mainly into the joints, which can be destroyed by the bleeding.

The safety and efficacy of Alprolix were evaluated in a multicenter clinical trial that compared each of two prophylactic treatment regimens to on-demand treatment. A total of 123 individuals with severe Hemophilia B, ages 12–71, were followed for up to a year and a half. The studies demonstrated the effectiveness of Alprolix in the prevention and treatment of bleeding episodes and during perioperative management of patients undergoing a surgical procedure. No safety concerns were identified in this trial.

Alprolix received orphan-drug designation for this use by the FDA because it is intended for the treatment of a rare disease or condition.

Alprolix is manufactured by Biogen Idec, Inc., Cambridge, MA.

*Source*: Data from Food and Drug Administration 2013, *FDA News Release: FDA approves Sovaldi for chronic hepatitis C*, viewed April 20, 2014. http://www.fda.gov/newsevents/newsroom/pressannouncements/ucm377888.htm.

### 8.2.4 Orphan Drugs

Drugs are designated as orphan drugs for those diseases with patient population of less than 200,000 in the United States. FDA has a special provision for the development, approval, and marketing of orphan drugs (refer to 21 CFR Part 316). The Orphan Drug Act provides incentives to organizations to research, develop, and test drugs that have limited commercial returns because of the small size of the patient group. In return for

**TABLE 8.5 PDUFA Fees for FY 2014**

| Application | Fee (US$) |
|---|---|
| Applications requiring clinical data | 2,169,100 |
| Applications not requiring clinical data | 1,084,550 |
| Establishments | 554,600 |
| Products | 104,060 |

*Source*: Food and Drug Administration 2013, *Establishment of Prescription Drug User Fee Rates for Fiscal Year 2014*, viewed April 10, 2014, http://www.gpo.gov/fdsys/pkg/FR-2013-08-02/pdf/2013-18624.pdf.

the commercial risks undertaken, there is assistance in the forms of NDA fee waivers, tax credits for clinical research, and grants for the research. FDA also provides market exclusivity (monopoly) to the organization to market the drug for 7 years.

### 8.2.5 Generics

A generic drug is defined as a drug that is equivalent to a prescription drug that has been approved by FDA, but for which the patent validity has expired. An ANDA approval is required (Figure 8.5).

There is no requirement to provide preclinical or clinical data to demonstrate safety and efficacy of generic drug. However, the review is based on bioequivalence and manufacturing control information. The sponsor provides data to establish that the generic drug is equivalent to the off-patent prescription drug in terms of chemistry, dosage, bioavailability, ADME characteristics, and toxicology. Information on manufacturing and control is submitted to demonstrate that production of generics complies with GMP.

### 8.2.6 Over-the-Counter Drugs

The approval process for over-the-counter (OTC) drugs is presented in Figure 8.6. Some of the important points are (i) the review of labeling to ensure it is clear and understandable by consumers, and (ii) public comment on the listing of the OTC drug. Monographs are prepared for OTC drugs; they list the raw materials used in the drug, dosage, indications of use, and labeling information.

### 8.2.7 Debarred Persons

It should be noted that the US legislation excludes persons who have been debarred from being involved in drug product application as noted below:

> "Section 306(k) of the Federal Food, Drug, and Cosmetic Act (the Act) (21 U.S.C. 335a(k)), as amended by the Generic Drug Enforcement Act of 1992 (GDEA), requires that drug product applicants certify that they did not and will not use in any capacity the services of any debarred persons in connection with a drug product application. If the application is an abbreviated new drug application (ANDA), it must also include a list of all convictions described under Section 306(a) and (b) of the Act (21 U.S.C. 335a(a) and (b)) that occurred within the previous 5 years and were committed by the applicant or affiliated persons responsible for the development or submission of the ANDA."

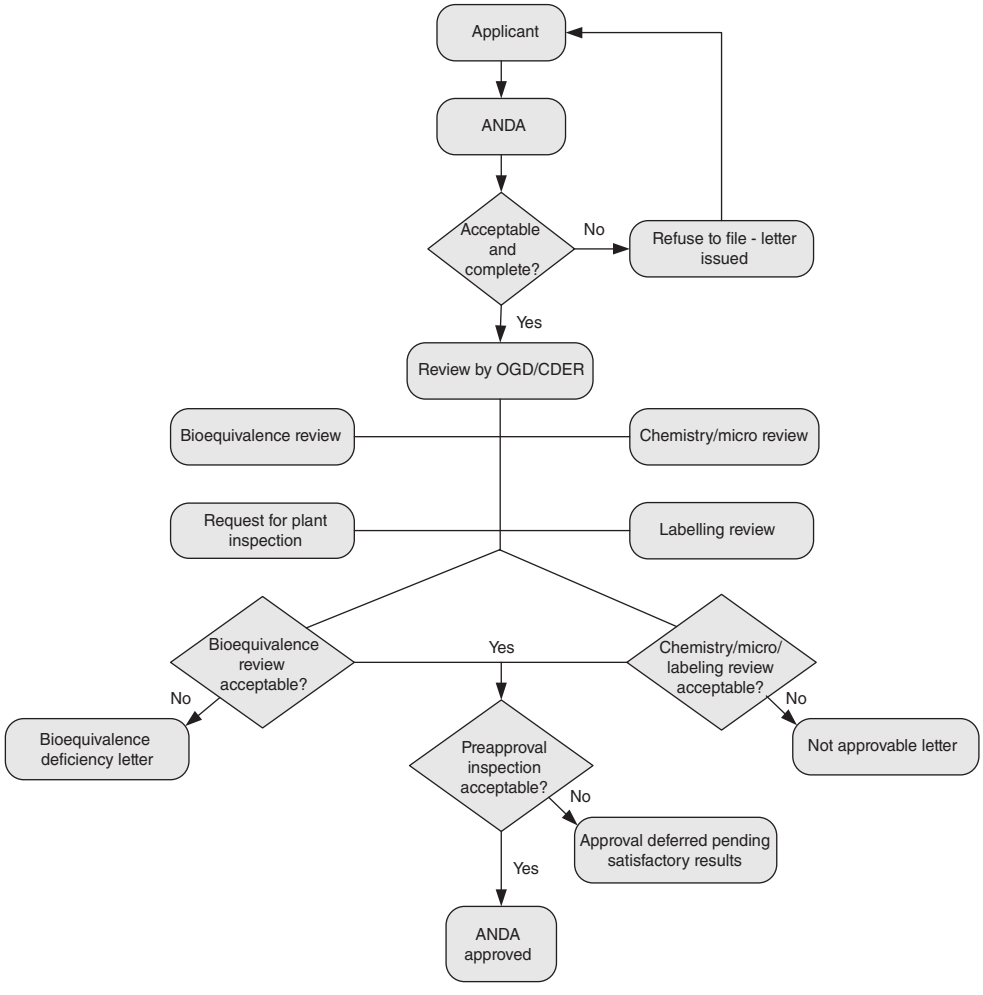



**Figure 8.5** Approval process for generics. (*Source*: Food and Drug Administration 1998, '*The CDER Handbook*', April 14, 2014, http://www.fda.gov/downloads/AboutFDA/CentersOffices/CDER/UCM198415.pdf.)

## 8.3 EUROPEAN UNION

Similar to the US requirements, there are two regulatory steps to go through before a drug is approved to be marketed in the European Union. These two steps are clinical trial application (CTA) and marketing authorization application (MAA). There are 28 member states in the European Union (as of April 2014); clinical trial applications are approved at the member state level, whereas marketing authorization applications are approved at both the member state and centralized levels.

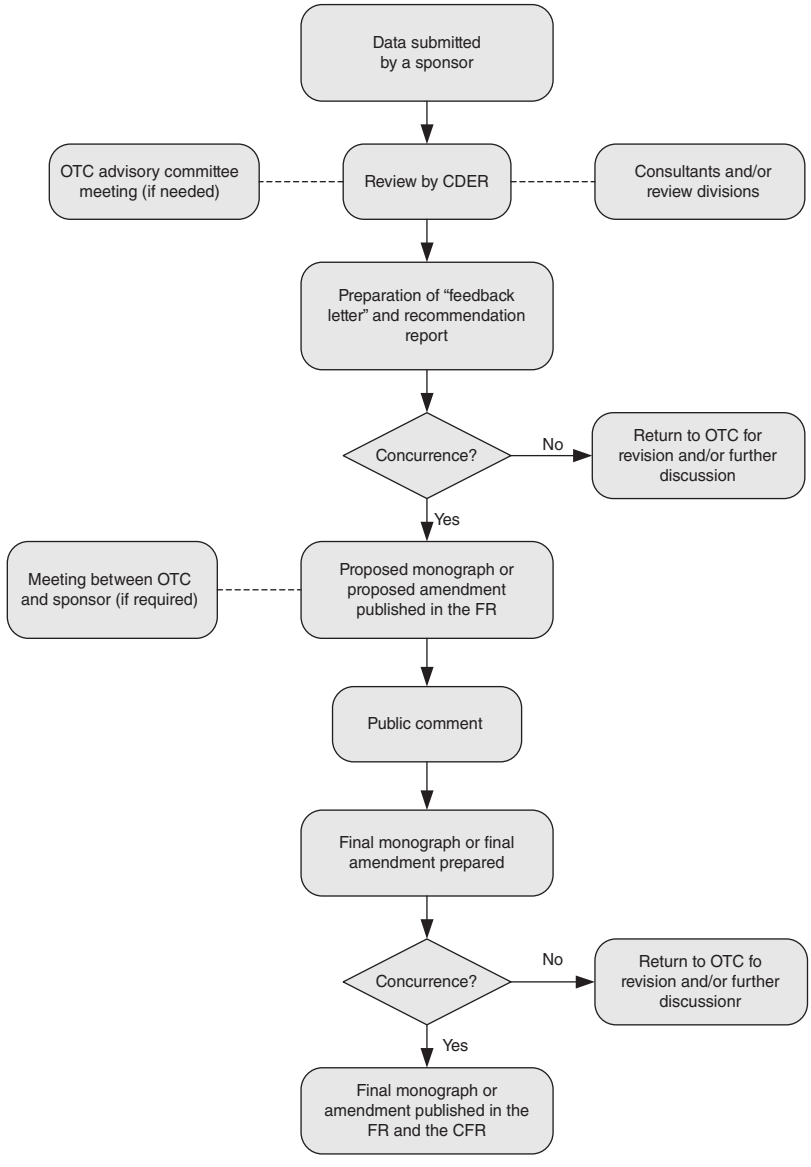



**Figure 8.6** Approval process for OTC drugs. (*Source*: Food and Drug Administration 1998, '*The CDER Handbook*', April 14, 2014, http://www.fda.gov/downloads/AboutFDA/CentersOffices/CDER/UCM198415.pdf.)

### 8.3.1 Clinical Trial Application

EU Directives 2001/20/EC, 2001/83/EC, and 2005/28/EC set out the new rules and regulations for the conduct and approval of clinical trials in the European Union. In addition, clinical trials have to follow GCP and adherence to the Declaration of Helsinki

(refer to Section 6.2.7). Authorization of clinical trials is the responsibility of member states.

A sponsor submits a CTA to the Competent Authority (a term used in EU for the regulatory authority) in each member state where the trials are to be conducted. The competent authority has 60 days (90 days for gene therapy) to review and approve or reject the application. Application is in prescribed forms and covers the proposed clinical trial protocol, manufacturing and quality controls on the drug, and supporting data, such as (i) chemical, pharmaceutical, and biological data, (ii) nonclinical pharmacological and toxicological data, and (iii) clinical data and previous human experience. The supporting data are submitted in the CTD format (refer to Sections 7.11 and 8.9).

Most of the information sought is similar to FDA's IND requirements. One major difference is that a Qualified Person (QP) (typically a chemist, biologist, or pharmacist who has the knowledge and experience working in pharmaceutical manufacturing operations) has to certify that the investigational medicinal product (IMP) is manufactured according to GMP. The competent authority has the right to inspect the manufacturing facility for GMP compliance, the preclinical facility for GLP compliance, and the clinical trial sites for GCP compliance.

In the United Kingdom, clinical trial applications are submitted to the Medicines and Healthcare Products Regulatory Agency (MHRA). There are two schemes for CTA: Clinical Trial Notification (CTN) and Clinical Trial Authorization (CTA) schemes.

***Clinical Trial Notification (CTN)***: The CTN scheme is for trials that involve medicinal products already licensed in any EU member state:

- Trials that relate to the licensed range of indications, dosage, and form, or
- Trials that involve off-label use supported by sufficient published evidence and/or guidelines.

MHRA provides acknowledgment of the application, and trial may proceed 14 days after submission if no objections are raised. The information required for the CTN is:

- Cover letter
- CTN form
- Clinical protocol
- Summary of product characteristics (SmPC)
- Justification for the absence of labeling
- Justification for the absence of a manufacturer's authorization.

***Clinical Trial Authorization (CTA)***: The CTA scheme is applicable to new chemical entities (NCEs) or new biological entities (NBEs). The requisite documents are:

- Cover letter
- CTA form
- Protocol
- Investigator's brochure

- Investigational Medicinal Product Dossier (IMPD) or simplified IMPD – similar to CMC data
- Noninvestigational Medicinal Product Dossier (NIMPD) (if required)
- Scientific advice from any member state or the European Medicines Agency (EMA, if available)
- EMA decision (for pediatric investigation, if applicable)
- Content of labeling
- Proof of payment
- Manufacturer's authorization or importer's authorization plus QP declaration on GMP.

Application is assessed within 30 days. The outcomes are as follows:

- Acceptance of the CTA
- Acceptance of the trial application subject to certain conditions
- Nonacceptance of the trial application.

### 8.3.2 Marketing Authorization

Following successful clinical trials, the sponsor has to apply for authorization to market the drug in Europe. Depending on the type of the drug product and the intended market, there are four different types of marketing authorization applications (Figure 8.7).

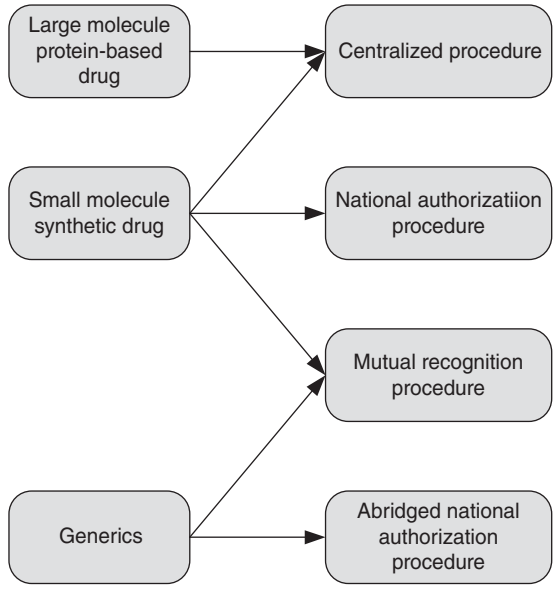



**Figure 8.7** Marketing authorization procedures.

**Exhibit 8.3 Drug Products According to EU Regulation 726/2004**

Medicinal products developed by:

- Recombinant DNA technology
- Expression of proteins in prokaryotes and eukaryotes cells
- Hybridoma and monoclonal antibody methods.

Medicinal products with a new active substance for the treatment of:

- AIDS
- Cancer
- Neurodegenerative disorder
- Diabetes
- Autoimmune diseases and other immune dysfunctions
- Viral diseases.

Orphan medicinal products pursuant to Regulation (EC) 141/2000

***Centralized Procedure***: This procedure, according to Regulation 726/2004 and Directive 2004/27/EC, is for drugs developed using biotechnology processes, novel drugs for specific treatments, and orphan drugs (refer to Section 7.3 and Exhibit 8.3). The marketing authorization is for the entire European Union. The process for the Centralized Procedure is summarized in Figure 8.8.

An application is submitted to the European Medicines Agency (EMA). EMA evaluates the application and forwards its opinion (positive or negative for the granting of a marketing authorization) to the European Commission. The opinion is supported by the European Public Assessment Report (EPAR) generated by EMA, which summarizes the scientific analyses and discussions during the evaluation process. The European Commission consults the relevant committees before granting the marketing authorization. The process takes up to 210 days.

***Mutual Recognition Procedure***: The Mutual Recognition Procedure is stated in Council Directive 93/39/EEC. In essence, once a drug is approved for marketing authorization by one member state, the company can apply for marketing authorization in other member states through the mutual recognition procedure in place since 1998.

Identical applications are submitted to those member states where marketing authorizations are sought. The first member state that reviews the application is called the "Reference Member State." It notifies other states, called "Concerned Member States." Concerned member states may suspend their own evaluations to await assessment by the reference member state. The decision of the reference member state is forwarded to the concerned member states. If the concerned member states reject mutual recognition, the matter is referred to the Committee for Medicinal Products for Human Use

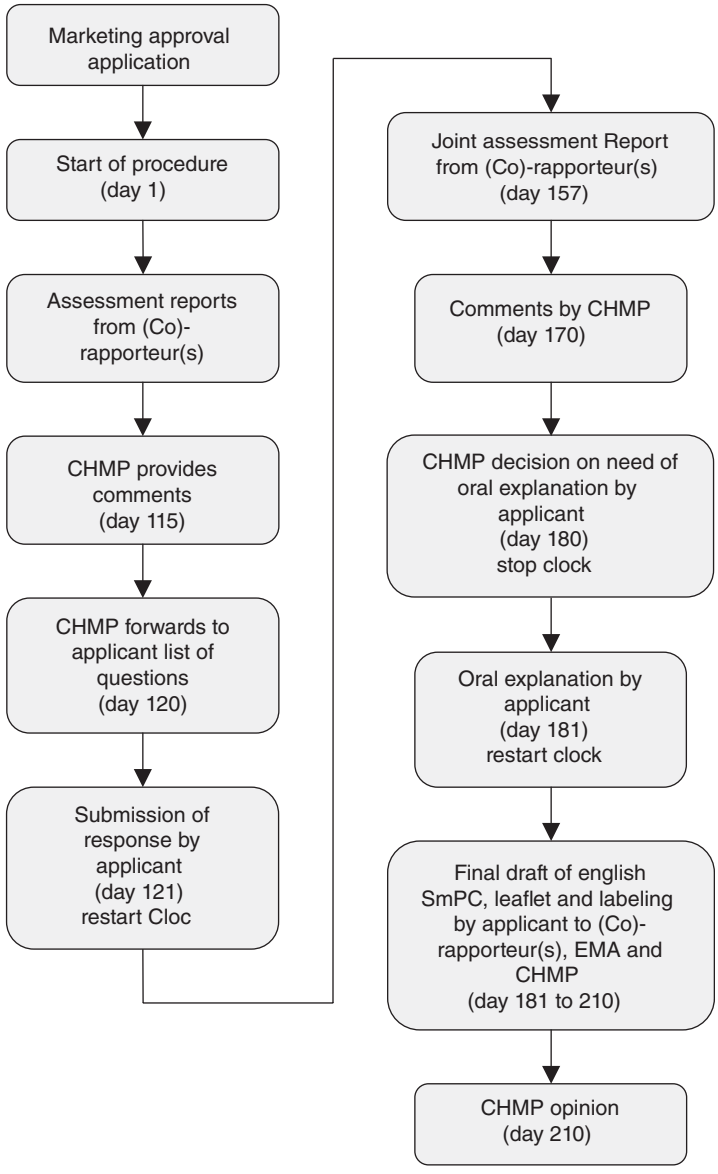



**Figure 8.8** Centralized procedure. *Note:* Rapporteur – a person appointed to oversee the procedure, CHMP – Committee for Medicinal Products for Human Use

(CHMP) of EMA for arbitration. EMA forwards its opinion to the European Commission, which makes the final decision. Altogether, the decision process may take up to 300 days if there is no objection, and 600 days when objections are raised.

***National Authorization Procedure***: To obtain marketing authorization in a country, the application must be submitted to the competent authority of that member state in its own language. For national authorizations in more than one country, submissions have

to be sent to each country in its own language. In many ways, the national authorization system has been superseded by the centralized and mutual recognition procedures.

***Abridged National Authorization Procedure***: This procedure is for generics, and there is no necessity to provide preclinical or clinical results. However, evidence of bioavailability and bioequivalence, and GMP manufacturing compliance has to be submitted. If the applicant has an abridged approval from a member state, the mutual recognition procedure can be used.

***Submission Details for Centralized Procedure***: This procedure was effective from 1995 with further amendments effective from November 2005. The pharmaceutical company (sponsor) wishing to apply for marketing authorization via the Centralized Procedure notifies EMA at least 7 months before submission of its intention and expected date of application. This notification is required to be accompanied by a number of items, for example:

- Draft SmPC
- Justification for evaluation under the Centralized Procedure
- Proposed European Drug master File (EDMF, refer to Exhibit 8.4)
- Manufacturing location.

EMA reviews the presubmission and informs the sponsor of its decision regarding the intended marketing application. A Rapporteur and Corapporteur (refer to Figure 8.8), whose roles are to coordinate the evaluation of the application, are appointed by EMA 3 months before the submission.

Submission for marketing authorization is in a prescribed format. It is accompanied by the payment of fees (Table 8.6). Details for the submission dossier are described later.

There are two stages for the centralized procedure. The first stage is divided into the primary evaluation phase and secondary evaluation phase. In the primary evaluation phase, the rapporteur and corapporteur coordinate the evaluation within EMA and communicate with the applicant with a list of questions. The secondary evaluation commences after the receipt of response to the questions. EMA has the right to request drug samples for testing. EMA may also perform preauthorization inspection of the drug manufacturing facility to ensure compliance to GMP. At the end of stage 1, EMA sends its opinion, positive or negative, to the European Commission for decision making, which is the second stage. Documents in 11 languages are sent by EMA to the European Commission. The European Commission checks to ensure the marketing authorization complies with European Union law and formalizes the EMA decision into a decision for the entire European Union.

***Preparation of Marketing Authorization Application Dossier***: The application dossier is prepared in accordance with the Common Technical Document (CTD) format as detailed in Section 8.9.

***Approval of Marketing Authorization***: Assessment of the application by the Committee for Medicinal Products for Human Use (CHMP) is published initially as a Summary of Opinion – positive or negative. After the granting of a Marketing Authorization by the European Commission, a more detailed report is published as the European Public Assessment Report (EPAR).

**Exhibit 8.4 European DMF**

The DMF is used for the following active substances:

- New active substances
- Existing active substances not described in the *European Pharmacopoeia* but described in the pharmacopoeia of a member state

Existing active substances, not described in the *European Pharmacopoeia* or the pharmacopoeia of a member state.

The DMF consists of a confidential part and a nonconfidential part. The confidential part is to protect valuable intellectual property or "know-how" of the active substance manufacturer.

| Content of the DMF | Restricted Part (Expert Report), Confidential | Applicants Part (Expert Report), NonConfidential |
|---|---|---|
| Names and sites of active substance manufacturer | + | + |
| Specification and routine test | | + |
| Nomenclature | | + |
| Description | | + |
| Previous use in medicinal products | + | |
| Manufacturing method | + | + |
| Brief outline (flow chart) | | |
| Detailed description | | |
| Quality control during manufacture | + | |
| Process validation and evaluation of data | + | |
| Development chemistry | | + |
| Evidence of structure | | + |
| Potential isomerism | | + |
| Physicochemical characterization | | |
| Analytical validation | | + |
| Impurities | | + |
| Batch analysis | | + |
| Stability | | + |

The EPAR shows the scientific conclusion reached by CHMP at the end of the centralized evaluation process. It is available to the public, with commercial confidential information deleted. The EPAR gives a summary of the reasons for the CHMP opinion in favor of granting a marketing authorization for a specific medicinal product. It results from the Committee's review of the documentation submitted by the applicant, and from subsequent discussions held during CHMP meetings. The EPAR is updated throughout the authorization period as changes to the original terms and conditions of the authorization (i.e., variations, pharmacovigilance issues, specific obligations) are made. The EPAR also contains a summary written in a manner that is understandable to the public.

**TABLE 8.6 EMA Application Fees, 2014**

| Application | Fee (Euro) |
|---|---|
| Full application | 278,500 |
| Marketing application (not full dossier) | 180,000 |
| Extension of a marketing authorization | 83,600 |
| Variation to a marketing authorization | |
| Type IIA | 3,000 |
| Type IIB | 7,000 |
| Type II Level I | 83,600 |
| Type II Level II | 62,700 |
| Type II Level III | 20,900 |
| Scientific advice | 83,600 |
| Inspection | 20,900 |

*Source*: European Medicines Agency 2014, *Explanatory note on fees payable to the European Medicines Agency*, viewed April 12, 2014, http://www.ema.europa.eu/docs/en_GB/document_library/Other/2014/04/WC500164415.pdf. Reproduced with permission of the European Medicines Agency.

***Accelerated Assessment***: EU has introduced the accelerated assessment in November 2005. The aim is to speed up the regulatory procedure to enable patients access to new medicines. The EMA review time for accelerated assessment is 150 days. See Exhibit 8.5 for the approval of Soliris (eculizumab), an antibody for the reduction of hemolysis – destruction of red blood cells in patients with rare blood disorder. Soliris was the first medicinal product assessed under the accelerated scheme by EMA and granted a positive opinion in 147 days.

Orphan drug legislation in EU was introduced in 2001. By definition, orphan drugs are reserved for the treatment of rare diseases. In EU the orphan status is defined by prevalence as diseases that affect not more than five people per 10,000 in the general population. Because of its rarity certain regulatory requirements are eased. For example, it is acceptable for clinical studies to be statistically underpowered because of the fact that few patients are available. But the clinical studies must still demonstrate statistical significance. Orphan drugs automatically qualified for accelerated assessment via the Centralized Procedure. The EU grants market exclusivity (refer to Section 11.15) of 10 years for orphan drugs, together with fee reductions, tax incentives, and support of R&D activities.

## 8.4 JAPAN

Drug approval processes go through IND and NDA procedures in Japan. The Pharmaceutical and Medical Device Agency (PMDA) provides technical consultation services for clinical trials. There are four types of consultations: before IND, at end of Phase II studies, before NDA, and consultation on individual protocols.

Japan has adopted the ICH GCP guidelines for clinical trials since 1997. It upholds the Helsinki Declaration to ensure that the rights, welfare, and privacy of subjects are protected in clinical trials. Japan accepts foreign clinical trials, but bridging trials may

**Exhibit 8.5 Soliris**

Soliris is for the treatment of Paroxysmal Nocturnal Hemoglobinuria (PNH). PNH is a chronic disease where a patient's oxygen-carrying red blood cells are missing the normally present complement inhibitors (refer to Section 4.9, Trastuzumab for the definition of the complement). The cells are therefore abnormally fragile and inadvertently destroyed by normal complement activation.

PNH is caused by a mutation in certain types of adult blood cells. Because of this mutation, certain types of proteins, including complement inhibitors, are unable to attach to the surface of the cell, as is normally the case. More specifically, the PNH mutation prevents the assembly of a fatty tail, known as a glycosyl-phosphatidylinositol (GPI) anchor, a necessary step in surface attachment of some proteins.

Consequently, proteins with this GPI anchor are diminished or absent, two of which are crucial in protecting blood cells from inappropriate complement destruction. Without these two protective proteins, PNH red blood cells, in particular, are easily burst by complement, resulting in low red blood cell count (anemia), fatigue, bouts of dark colored urine, and various other complications.

Soliris is a protein-based drug that specifically blocks cleavage of the C5 component of the complement system, thereby preventing the final stages of complement activation.

*Source*: Data from European Medicines Agency 2007, *European Medicines Agency concludes first accelerated assessment for a medicine for human use*, viewed April 20, 2014, http://www.ema.europa.eu/ema/index.jsp?curl=pages/news_and_events/news/2009/12/news_detail_000689.jsp&mid=WC0b01ac058004d5c1

need to be performed to take into consideration effects of ethnic factors. The ICH guideline *E5 – Ethnic Factors in the Acceptability of Foreign Clinical Data* recommends a framework for evaluating the impact of ethnic factors on a drug's effect and dosage. It describes the characteristics of foreign data that will enable these data to be extrapolated to a different population.

ICH E5 has been implemented in Japan. Some of the drug characteristics that may be influenced by ethnic factors are:

- Pharmacokinetics
- Pharmacodynamics
- Dosage
- Metabolism
- Potential for protein binding
- Potential for drug–drug, drug–diet, and drug–disease interactions.

Following satisfactory results from the bridging trials, an NDA for marketing authorization can be filed. An NDA submitted to the MHLW is reviewed by PMDA. The CTD

format (refer to Exhibit 7.11 and Section 8.9) for submission is mandatory in Japan since 2003. Modules 1 and 2 are to be in Japanese. Other modules can be written in English. PMDA personnel have the authority to inspect the drug manufacturing facility and clinical trial sites to assess compliance to GMP and GCP, respectively. In the process PMDA consults the Pharmaceutical Affairs and Food Sanitation Council (PAFSC). Results of the review are forwarded to the Pharmaceutical and Food Safety Bureau (PFSB), which prepares the final approval through the minister of MHLW. Figure 8.9 shows the drug approval process in Japan. The procedure for the manufacturing and distribution of drugs for overseas manufacturers is presented in Figure 8.10.

Similar to the United States and EU, Japan has priority reviews and incentives on drugs for serious diseases and orphan drugs. In Japan orphan drugs are those that target diseases with a prevalence of less than 50,000 patients.

## 8.5 CHINA

In China two regulatory processes exist: one for imported products and the other for locally manufactured products. For imported drugs, the registration package includes:

- Application form
- Technical data
- CMC
- Nonclinical pharmacology and toxicology
- PK/PE, local clinical data
- Labeling
- Samples from three different batches.

The regulations regarding the registration of imported drugs, both synthetic and protein-based, are complex, with several different levels of reviews. We discuss below (Figure 8.11) the application for registration to import a "Western" drug into China.

A CTA on the prescribed form is submitted to the Department of Drug and Cosmetics Registration (DDCR) of the China Food and Drug Administration (CFDA). The information to be provided is similar to that for United States and EU. Generally, the information comprises: Summary and data for CMC, pharmacology, and toxicology. China follows the CTD format for submission. The DDCR evaluates completeness of document and then forwards it to the Center for Drug Evaluation (CDE) for technical review. External experts may be consulted, and the CDE compiles a technical report for the DDCR.

The National Institute for the Control of Pharmaceutical & Biological Products (NICPBP) performs tests on the drug samples submitted. On the basis of the test results and report from the CDE, the DDCR approves the conduct of clinical trials at accredited clinical research centers (Figure 8.12). The overall approval timeline may take almost 1 year to complete.

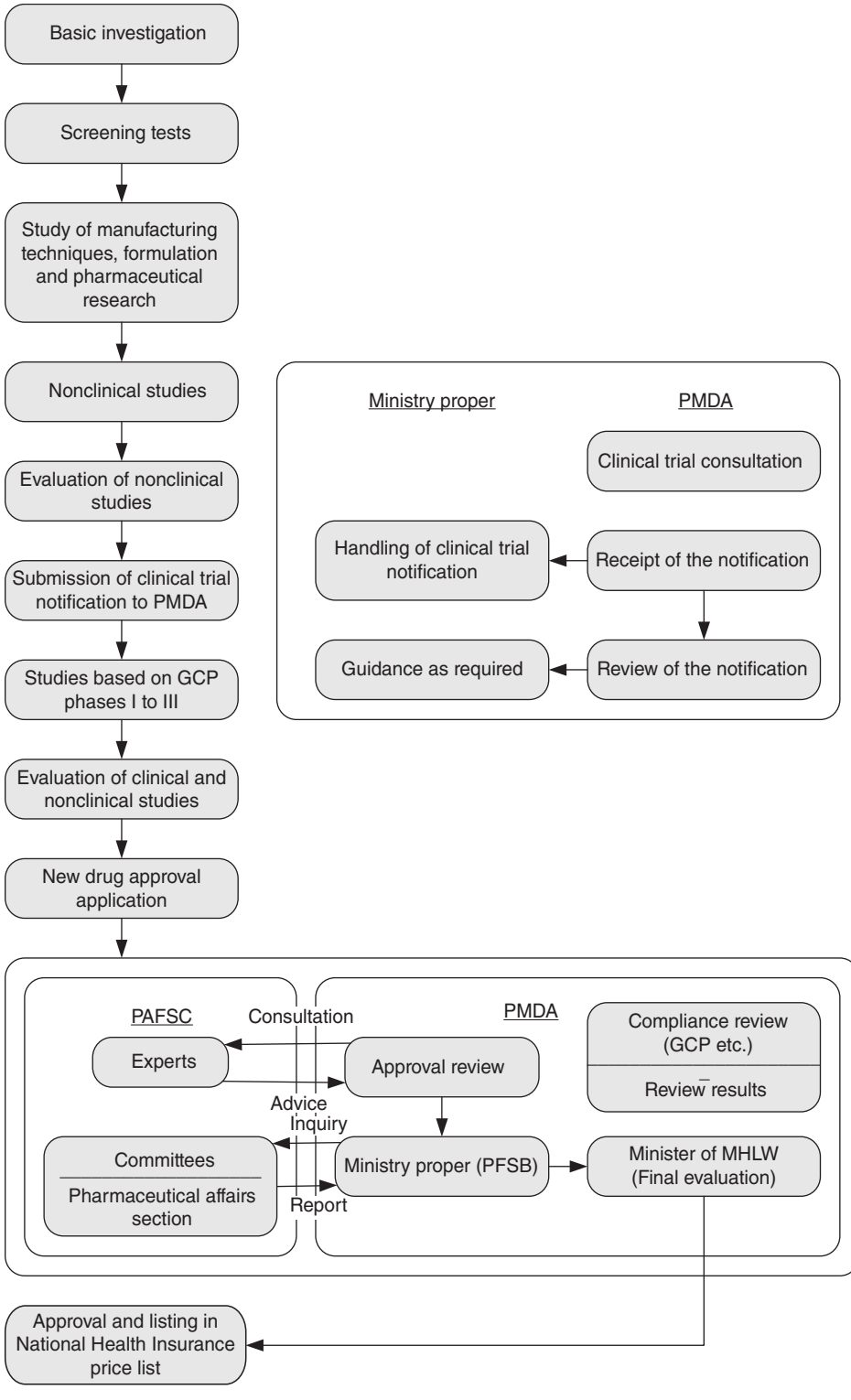



**Figure 8.9** Drug approval process in Japan.

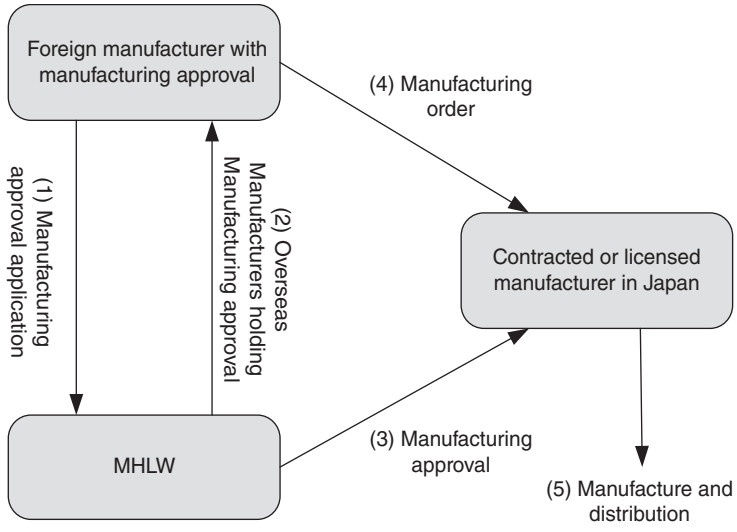



**Figure 8.10** Manufacturing and distribution of drugs for overseas manufacturers.

At the conclusion of clinical trials, the results are evaluated by the CDE, which submits a report to the DDCR. On the basis of the report, the DDCR makes a final recommendation to the Director of CFDA for approval to import the drug into China. The overall process may take 1–2 years.

Almost all new drugs entering the country must go through domestic testing in some form. The Chinese government approved 50–60 hospitals and medical centers where trials can be performed; and study must be conducted in at least three sites.

To ensure innovation and availability of new drugs to meet medical needs, China has implemented Special Evaluation and Approval Procedures for certain categories of drugs:

- New drug materials and active ingredients (including their preparations) that have not been marketed in China
- Chemical drug substances and their preparations, and/or biological products that have not been marketed in China or outside China
- New drugs for the treatment of AIDS, cancer, and orphan diseases
- New drugs for the treatment of diseases that have no effective therapy.

For generic drugs, application is filed with the provincial FDA, which will inspect the manufacturing facility for GMP compliance. Recommendations are provided by the provincial FDA to CFDA. CFDA reviews the submitted data and recommendations and makes the final decision.

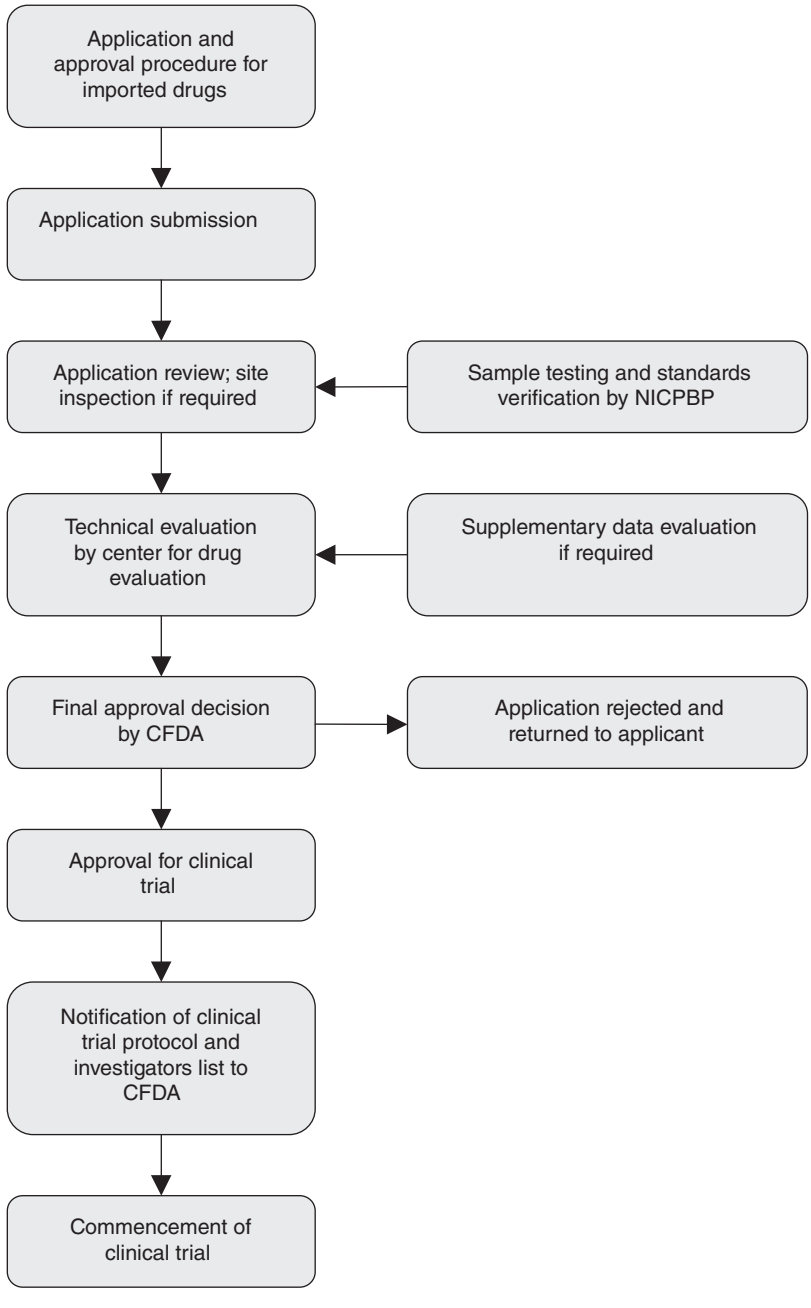



**Figure 8.11** Imported "Western" drug approval process in China.

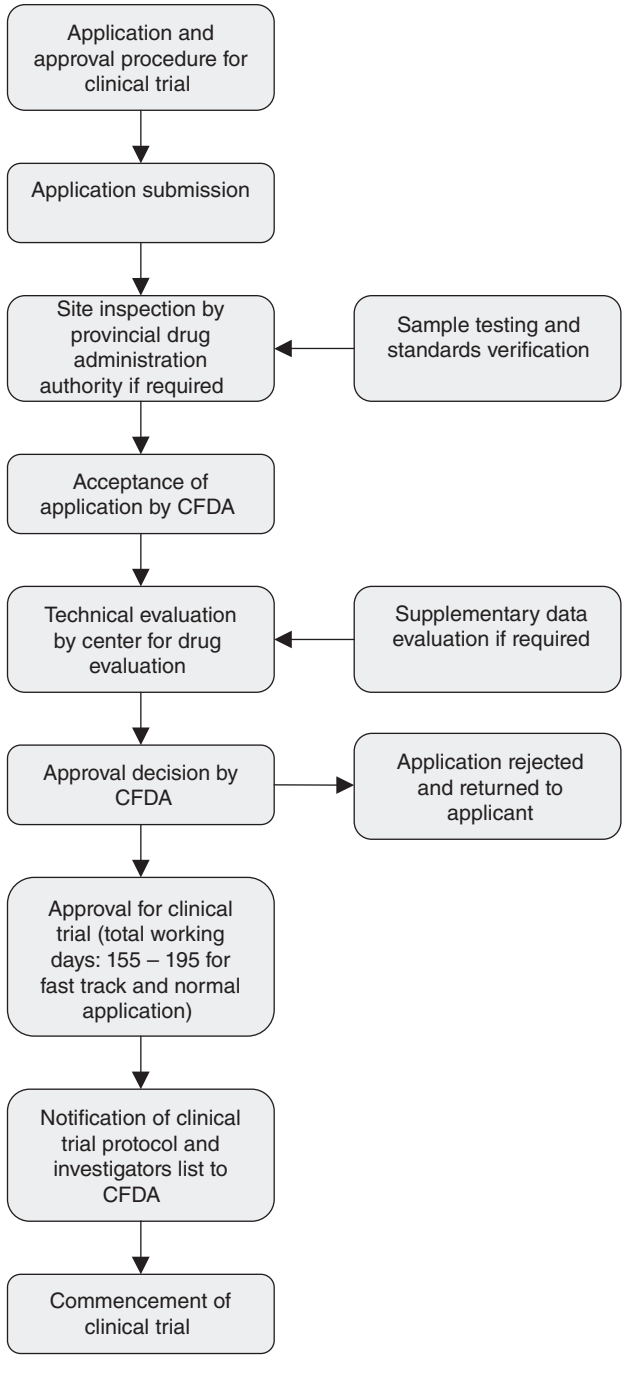



**Figure 8.12** Clinical trial process in China.

## 8.6 INDIA

Application for the importation and manufacture of new drugs or to undertake clinical trial is made on Form 44 to the Central Drugs Standard Control Organization (CDSCO) together with the appropriate fees. Data to be submitted according to Schedule Y are:

- Chemical and pharmaceutical information
- Animal pharmacology
- Animal toxicology
- Human/clinical pharmacology (Phase I)
- Exploratory clinical trials (Phase II)
- Confirmatory clinical trials (Phase III, including published review articles)
- Bioavailability, dissolution, and stability study data
- Regulatory status in other countries
- Marketing information
- Proposed product monograph
- Drafts of labels and cartons
- Application for test license.

Local clinical trial may be waived by the licensing authority in the interest of public good, in which case data from preclinical studies are to be evaluated. The approval for import permission is given in Form 45 or 45A, clinical trial on Form 46 and/or 46A, and new bulk drug substance on Form 54a.

## 8.7 AUSTRALIA

All drugs to be imported into, supplied in, or exported from Australia must be included in the Australian Register of Therapeutic Goods (ARTG). The sponsoring company for the drug must apply to TGA to show the safety and efficacy of the drug before it can be accepted on ARTG. Submissions of data are based on the CTD format. The review process is depicted in Figure 8.13.

For clinical trials, two systems exist: the CTN Scheme and the Clinical Trial Exemption (CTX) Scheme. CTN relies on approval by local research institution whereas CTX is approved by TGA. Figure 8.14 shows these two schemes.

In 2013, the fees payable for various applications are tabulated in Table 8.7.

## 8.8 CANADA

CTA has to be submitted to Health Canada seeking permission to conduct clinical trials. The submission should include information regarding drug characteristics, test data, animal studies, and clinical protocol. A clinical trial may be stopped either when it is shown to be unsafe or dramatic benefits are obtained. The approval process may be

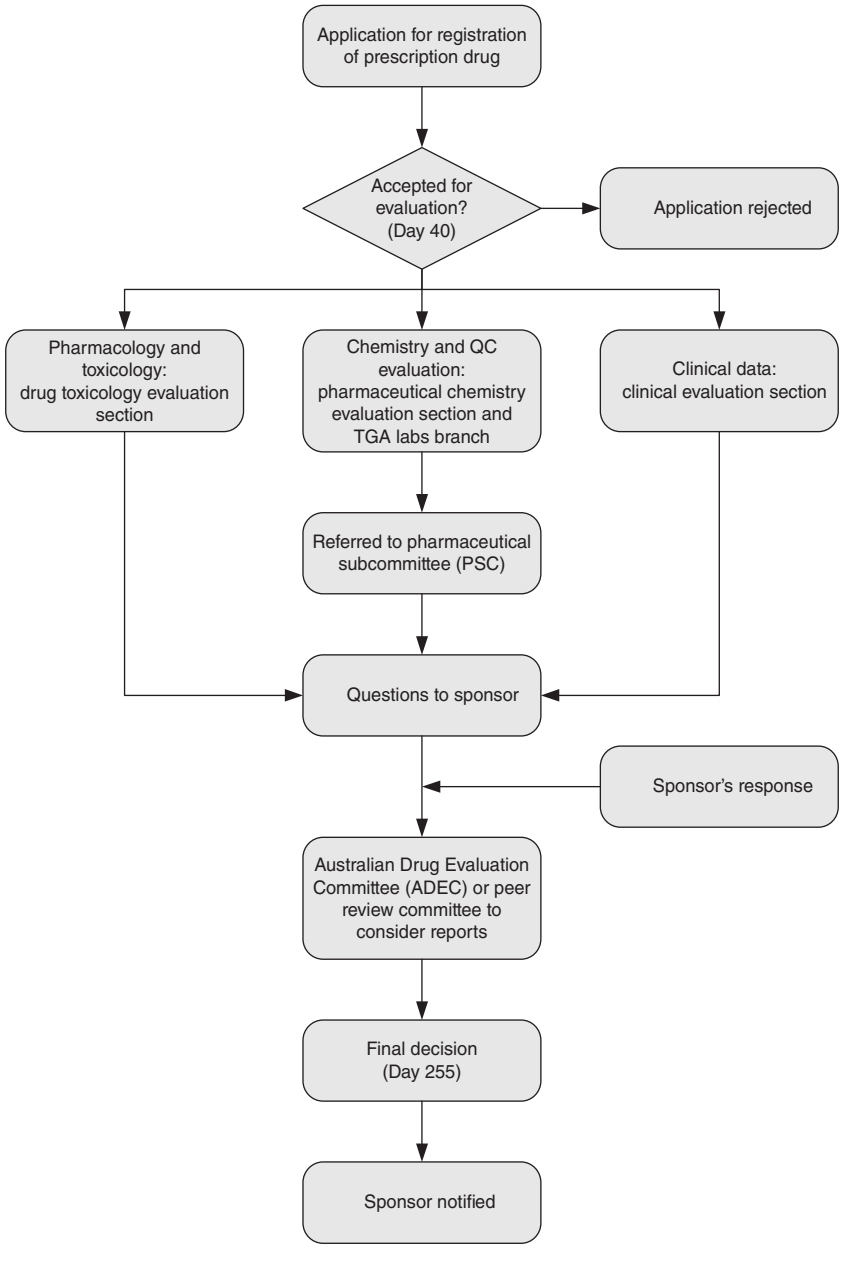



**Figure 8.13** Drug approval process in Australia.

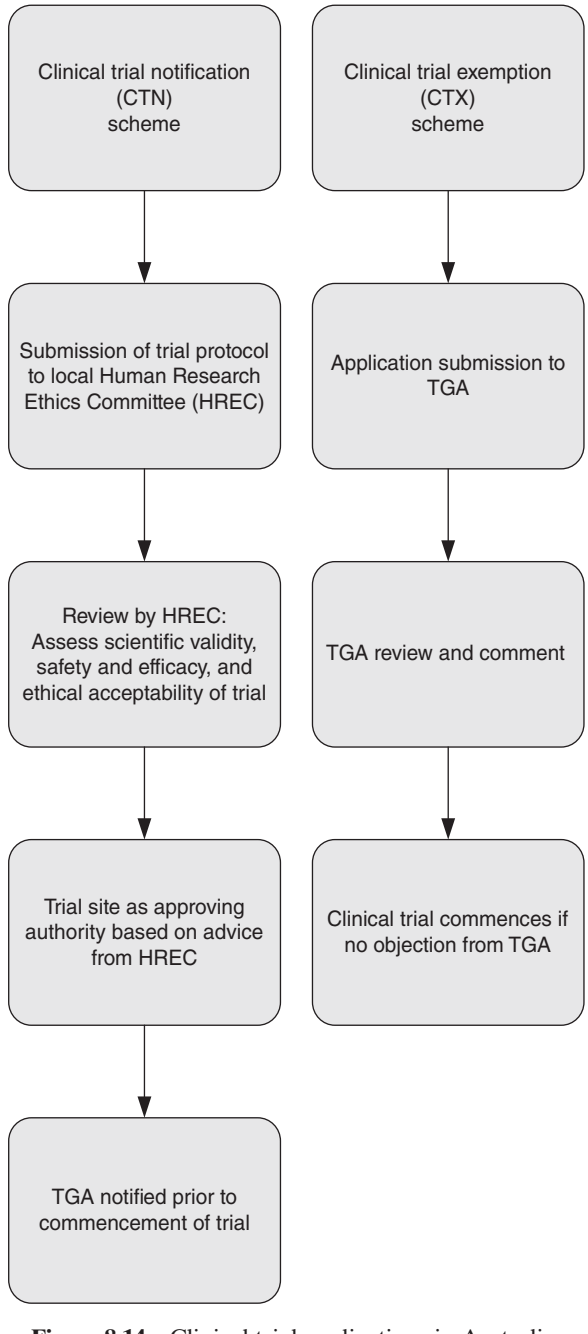



**Figure 8.14** Clinical trial applications in Australia.

**TABLE 8.7 TGA Fees and Charges, 2014**

| Prescription Medicines | Application Fee (A$) | Evaluation Fee (A$) |
|---|---|---|
| NCE | 44,200 | 177,200 |
| Extensions of indications | 26,300 | 105,300 |
| New generics | 17,000 | 67,600 |
| Clinical trial CTX 30 days | 1,595 | |
| Clinical trial CTX 50 days | 19,900 | |
| CTN | 330 | |

*Source*: *Summary of fees and charges at 1 July 2014*, Therapeutic Goods Administration. Reproduced with permission of the Australian Government http://www.tga.gov.au/summary-fees-and-charges-1-july-2014.

fast-tracked if a drug is shown to have substantial benefits, such as for the treatment of life-threatening or severely debilitating conditions.

After a drug has demonstrated its efficacy in Phase III, a New Drug Submission (NDS) may be submitted. The information submitted includes preclinical, clinical, chemistry, and manufacturing data in CTD format for Health Canada to evaluate. Samples may be required for testing and assessment. Generic manufacturers are required to submit abbreviated NDS, showing bioequivalence to established drugs. There is a Priority Review that allows for expedited review of drugs for life-threatening or severely debilitating conditions.

Approval for a drug to be marketed in Canada is in the form of a Notice of Compliance (NOC); and a Drug Identification Number (DIN) is issued. Drugs deemed not to have shown sufficient safety and efficacy are not approved, and a Notice of Noncompliance is issued.

## 8.9 CASE STUDY #8.1

### 8.9.1 The Common Technical Document (CTD), ICH Guideline M4

The CTD was prepared by ICH and implemented in July 2003. It is an assembly of a set of format-based documents for the submission of nonclinical, clinical, and manufacturing information to the regulatory authorities for marketing approval to license a drug for sale. Figure 8.15 shows the CTD structure – there are five modules:

- Module 1 for regional administrative information specific to each country
- Module 2 on summaries of quality, nonclinical, and clinical data
- Module 3 on quality information (manufacturing)
- Module 4 on safety studies (nonclinical)
- Module 5 on efficacy studies (clinical).

It should be noted that the country-specific process of marketing application and the review procedures, for example, NDA/BLA in United States, MAA via the Centralized Procedure in EU, and NDA in Japan are not affected by the CTD. The CTD provides

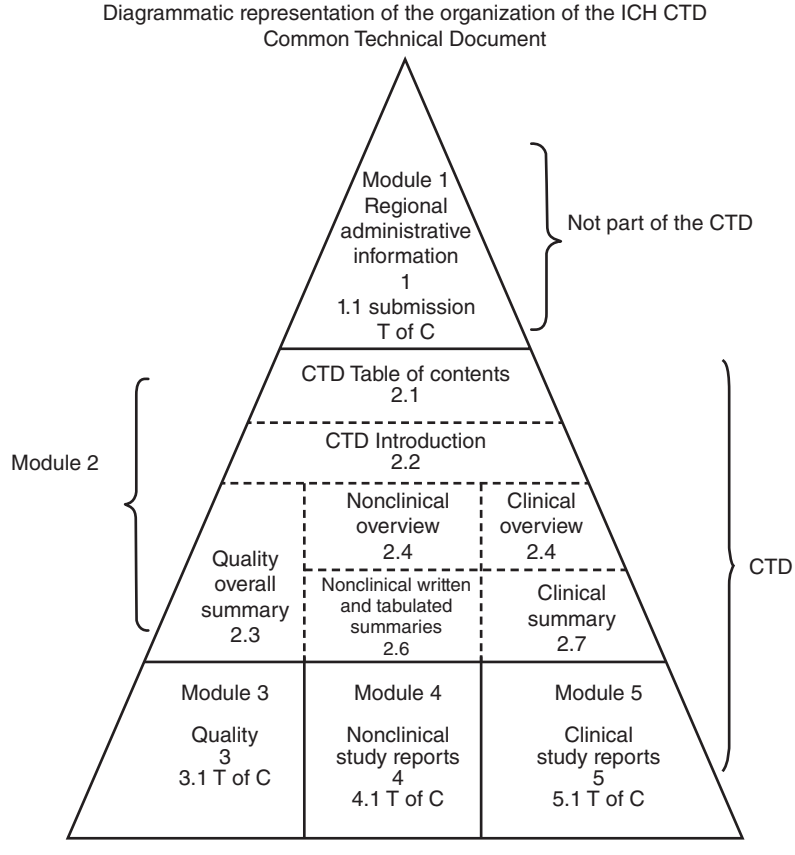



**Figure 8.15** Common technical document. (*Source*: International Conference on Harmonization 2014, *M4: The Common Technical Document*, viewed April 21, 2014, http://www.ich.org/products/ctd.html. Reproduced with permission of ICH.)

a standard format for the preparation and submission of technical information. It is recognized by United States, EU, Japan, and now most regulatory authorities around the world. The CTD is an international harmonized set of documents that help to reduce cost and accelerate approval time to bring new and innovative drugs to the patients.

When filing for marketing approval the specific information to be provided in each of the module is as below:

- Module 1 contains the administrative information and prescribing information. This module contains documents specific to each region; for example, application forms or the proposed label for use in the region. The content and format of this module can be specified by the relevant regulatory authorities.
- Module 2 is for the CTD summaries. It includes description of the drug, its pharmacologic class, mode of action, and proposed clinical use. There are seven sections as shown in Table 8.8.

**TABLE 8.8 Module 2: Common Technical Document Summaries**

| | |
|---|---|
| 2.1 Table of Contents | 2.3.P.2 Pharmaceutical Development |
| 2.2 CTD Introduction | 2.3.P.3 Manufacture |
| 2.3 Quality Overall Summary | 2.3.P.4 Control of Excipients |
| 2.3.S Drug Substance | 2.3.P.5 Control of Drug Product |
| 2.3.S.1 General Information | 2.3.P.6 Reference Standards or Materials |
| 2.3.S.2 Manufacture | 2.3.P.7 Container Closure System |
| 2.3.S.3 Characterization | 2.3.P.8 Stability |
| 2.3.S.4 Control of Drug Substance | 2.3.A Appendices |
| 2.3.S.5 Reference Standards or Materials | 2.3.A.1 Facilities and Equipment |
| 2.3.S.6 Container Closure System | 2.3.A.2 Adventitious Agents Safety Evaluation |
| 2.3.S.7 Stability | 2.3.A.3 Excipients |
| 2.3.P Drug Product | 2.3.R Regional Information |
| 2.3.P.1 Description and Composition of the Drug Product | |

*Source*: International Conference on Harmonization 2014, *M4: The Common Document*, viewed April 18, 2014, http://www.ich.org/products/ctd.html. Reproduced with permission of ICH.

- Module 3 is for the provision of quality data. The required information for drug substance and drug product is presented in Table 8.9.
- Module 4 is for nonclinical study reports and these are submitted in the format as shown in Table 8.10.
- Module 5 is the collection of human clinical study reports and related information as prescribed in Table 8.11.

## 8.10 CASE STUDY #8.2

### 8.10.1 FDA and EMA

This case study examines the new drugs approved by FDA and EMA in 2013.

***FDA, Approved Drugs in 2013***

In 2013, FDA received 36 NDA/BLA filings and approved 25 of these as NMEs and 2 biologics. These NMEs and biologics are shown in Table 8.12.

Out of these nine (those with asterisks) are identified as First-in-Class drugs, which mean these drugs use a new and unique mechanism for treating a medical condition. There are nine orphan drugs: Adempas, Gazyva, Gilotrif, Imbruvica, Kynamro, Mekinist, Opsumit, Pomalyst, and Tafinlar. Ten of these NMEs: Gilotrif, Imbruvica, Kadcyla, Mekinist, Olysio, Pomalyst, Sovaldi, Tafinlar, Tivicay, Xofigo were approved under the expedited programs as they have the potential to address unmet medical needs. The drugs considered as breakthrough therapies are Gazyva, Imbruvica, and Sovaldi. Gazyva was approved in 6.3 months, Imbruvica in 4.5 months, and Xofigo in 5.0 months.

***EMA Positive Opinions, 2013***

The Committee for Medicinal Products for Human Use (CHMP) of EMA has provided 62 positive opinions on new medicines to the European Commission for approval. A list of these new medicines is presented in Table 8.13.

Many of these drugs are different to those approved by FDA in the same year. This reflects differences in the timing and strategies of sponsors in their applications for

**TABLE 8.9 Module 3: Quality**

- 3.1 Table of Contents
- 3.2 Body of Data
  - 3.2.S Drug Substance
    - 3.2.S.1 General Information
      - 3.2.S.1.1 Nomenclature
      - 3.2.S.1.2 Structure
      - 3.2.S.1.3 General Properties
    - 3.2.S.2 Manufacture
      - 3.2.S.2.1 Manufacturer(s)
      - 3.2.S.2.2 Description of Manufacturing and Process Controls
      - 3.2.S.2.3 Control of materials
      - 3.2.S.2.4 Controls of Critical Steps and Intermediates
      - 3.2.S.2.5 Process Validation and/or Evaluation
      - 3.2.S.2.6 Manufacturing Process Development
    - 3.2.S.3 Characterization
      - 3.2.S.3.1 Elucidation of Structure and Other Characteristics
      - 3.2.S.3.2 Impurities
    - 3.2.S.4 Control of Drug Substance
      - 3.2.S.4.1 Specification
      - 3.2.S.4.2 Analytical Procedures
      - 3.2.S.4.3 Validation of Analytical Procedures
      - 3.2.S.4.4 Batch Analyses
      - 3.2.S.4.5 Justification of Specification
    - 3.2.S.5 Reference Standards or Materials
    - 3.2.S.6 Container Closure System
    - 3.2.S.7 Stability
      - 3.2.S.7.1 Stability Summary and Conclusions
      - 3.2.S.7.2 Postapproval Stability Protocol and Commitment
      - 3.2.S.7.3 Stability Data
  - 3.2.P Drug Product
    - 3.2.P.1 Description and Composition of Drug Product
    - 3.2.P.2 Pharmaceutical Development
      - 3.2.P.2.1 Components of the Drug Product
        - 3.2.P.2.1.1 Drug Substance
        - 3.2.P.2.1.2 Excipients
      - 3.2.P.2.2 Drug Product
        - 3.2.P.2.2.1 Formulation Development
        - 3.2.P.2.2.2 Overages
        - 3.2.P.2.2.3 Physicochemical and Biological Properties
      - 3.2.P.2.3 Manufacturing Process Development
      - 3.2.P.2.4 Container Closure System
      - 3.2.P.2.5 Microbiological Attributes
      - 3.2.P.2.6 Compatibility
    - 3.2.P.3 Manufacture
      - 3.2.P.3.1 Manufacturer(s)
      - 3.2.P.3.2 Batch Formula
      - 3.2.P.3.3 Description of Manufacturing and Process Controls
      - 3.2.P.3.4 Controls of Critical Steps and Intermediates
      - 3.2.P.3.5 Process Validation and/or Evaluation
    - 3.2.P.4 Control of Excipients
      - 3.2.P.4.1 Specifications
      - 3.2.P.4.2 Analytical Procedures
      - 3.2.P.4.3 Validation of Analytical Procedures
      - 3.2.P.4.4 Justifications of Specifications
      - 3.2.P.4.5 Excipients of Human or Animal Origin
      - 3.2.P.4.6 Novel Excipients
    - 3.2.P.5 Control of Drug Product
      - 3.2.P.5.1 Specifications
      - 3.2.P.5.2 Analytical Procedures
      - 3.2.P.5.3 Validation of Analytical Procedures
      - 3.2.P.5.4 Batch Analysis
      - 3.2.P.5.5 Characterization of Impurities
      - 3.2.P.5.6 Justifications of Specifications
    - 3.2.P.6 Reference Standards or Materials
    - 3.2.P.7 Container Closure System
    - 3.2.P.8 Stability
      - 3.2.P.8.1 Stability Summary and Conclusions
      - 3.2.P.8.2 Postapproval Stability Protocol and Commitment
      - 3.2.P.8.3 Stability Data
  - 3.2.A Appendices
    - 3.2.A.1 Facilities and Equipment
    - 3.2.A.2 Adventitious Agents Safety Evaluation
    - 3.2.A.3 Excipients
  - 3.2.R Regional Information
- 3.3 Literature References

*Source*: International Conference on Harmonization 2014, *M4: The Common Document*, viewed April 18, 2014, http://www.ich.org/products/ctd.html. Reproduced with permission of ICH.

**TABLE 8.10 Module 4: Nonclinical Study Reports**

| Module 4: Nonclinical Study Reports | |
|---|---|
| 4.1 Table of Contents | 4.2.3.3.2 In vivo |
| 4.2 Study Reports | 4.2.3.4 Carcinogenicity |
| 4.2.1 Pharmacology | 4.2.3.4.1 Long-term Studies |
| 4.2.1.1 Primary Pharmacodynamics | 4.2.3.4.2 Short or Medium-term Studies |
| 4.2.1.2 Secondary Pharmacodynamics | 4.2.3.4.3 Other Studies |
| 4.2.1.3 Safety Pharmacology | 4.2.3.5 Reproductive and Developmental Toxicity |
| 4.2.1.4 Pharmacodynamic Drug Interaction | 4.2.3.5.1 Fertility and Early Embryonic Development |
| 4.2.2 Pharmacokinetics | 4.2.3.5.2 Embryo-fetal Development |
| 4.2.2.1 Analytical Methods and Validation Reports | 4.2.3.5.3 Prenatal and Postnatal development |
| 4.2.2.2 Absorption | 4.2.3.5.4 Studies on Offspring |
| 4.2.2.3 Distribution | 4.2.3.6 Local Tolerance |
| 4.2.2.4 Metabolism | 4.2.3.7 Other Toxicity Studies |
| 4.2.2.5 Excretion | 4.2.3.7.1 Antigenicity |
| 4.2.2.6 Pharmacokinetic Drug Interaction | 4.2.3.7.2 Immunotoxicity |
| 4.2.2.7 Other Pharmacokinetic Studies | 4.2.3.7.3 Mechanistic Studies |
| 4.2.3 Toxicology | 4.2.3.7.4 Dependence |
| 4.2.3.1 Single-dose Toxicity | 4.2.3.7.5 Metabolites |
| 4.2.3.2 Repeat-dose Toxicity | 4.2.3.7.6 Impurities |
| 4.2.3.3 Genotoxicity | 4.2.3.7.7 Other Antigenicity |
| 4.2.3.3.1 In vitro | 4.3 Literature References |

*Source*: International Conference on Harmonization 2014, *M4: The Common Document*, viewed April 18, 2014, http://www.ich.org/products/ctd.html. Reproduced with permission of ICH.

marketing approvals in the United States and Europe, respectively. There are also differences in the review criteria by FDA and EMA, as the authorities seek answers and supporting evidence to their own set of rules and requirements. An example is Kynamro, which was approved by FDA but not EMA because EMA was concerned with the high incidence of side effects. Sponsors sometimes choose different names for their drugs to be marketed in different regions, as exemplified by the names Gilotrif in United States and Giotrif in Europe.

## 8.11 SUMMARY OF IMPORTANT POINTS

1. In United States the drug approval processes are:
   - Clinical trial
     IND Application: submit information on the drug, preclinical data (PD, PK, and toxicology), manufacturing procedures, test methods, specifications, contamination controls, and stability data.
   - Marketing approval
     (i) NDA for new small molecular drug: submit information on drug, preclinical data (PD, PK, and toxicology), Chemistry Manufacturing and Control (CMC), clinical data, statistical analysis, safety information, and validation of processes and test methods.
     (ii) BLA: submit information as for NDA.
     (iii) ANDA for generics: submit information on drug, comparability studies, and CMC.

**TABLE 8.11 Module 5: Clinical Study Reports**

| Module 5: Clinical Study Reports |
|---|
| 5.1 Table of Contents |
| 5.2 Tabular Listing of All Clinical Studies |
| 5.3 Clinical Study Reports Tabular Listing of All Clinical Studies |
| 5.3.1 Reports of Biopharmaceutic Studies |
| 5.3.1.1 Bioavailability Study Reports |
| 5.3.1.2 Comparative BA and Bioequivalence Study Reports |
| 5.3.1.3 In Vitro – In Vivo Correlation Study Reports |
| 5.3.1.4 Bioanalytical and Analytical Methods |
| 5.3.2 Pharmacokinetics using Human Biomaterials |
| 5.3.2.1 Plasma Protein Binding Study Reports |
| 5.3.2.2 Hepatic Metabolism and Drug Interaction Study Reports |
| 5.3.2.3 Reports of Studies using Other Human Biomaterials |
| 5.3.3 Reports of Human Pharmacokinetic Studies |
| 5.3.3.1 Healthy Subject PK and Initial Tolerability Study Reports |
| 5.3.3.2 Patient PK and Initial Tolerability Study Reports |
| 5.3.3.3 Intrinsic Factor PK Study Reports |
| 5.3.3.4 Extrinsic Factor PK Study Reports |
| 5.3.3.5 Population PK Study Reports |
| 5.3.4 Reports of Human Pharmacodynamic Studies |
| 5.3.4.1 Healthy Subject PD and PK/PD Study Reports |
| 5.3.4.2 Patient PD and PK/PD Study Reports |
| 5.3.5 Reports of Efficacy and safety Studies |
| 5.3.5.1 Study Reports of Controlled Clinical Studies to Claimed Indication |
| 5.3.5.2 Study Reports of Uncontrolled Clinical Studies |
| 5.3.5.3 Reports of Analyses of data from More then One Study |
| 5.3.5.4 Other Study Reports |
| 5.3.6 Reports of Post-Marketing Experience |
| 5.3.7 Case Report Forms and Individual Patient Listings |
| 5.4 Literature References |

*Source*: International Conference on Harmonization 2014, *M4: The Common Document*, viewed April 18, 2014, http://www.ich.org/products/ctd.html. Reproduced with permission of ICH.

(iv) Orphan drug: submit either NDA or BLA with assistance and provision of exclusivity.

(v) OTC drug: submit raw material list and samples showing labeling, dosage, and indication.

2. In Europe the drug approval processes are:
   - CTA: submit information similar to that for IND, but requirement of QP to certify drug manufacture complies to GMP.
   - Marketing approval
     (i) Centralized Procedure: required for all biopharmaceuticals, specific novel drugs and orphan drugs; approval is for the entire EU; submit information similar to NDA/BLA in CTD format and inclusion of Expert Reports.
     (ii) Mutual Recognition Procedure: application for marketing approval in other member states through a mutual recognition process after approval by one or more member state/s. Dispute is arbitrated by EMA, which makes final determination.

**TABLE 8.12 FDA NMEs/Biologics in 2013**

| New Medicines | Treatment For |
|---|---|
| Adempas* | Adults with two forms of pulmonary hypertension |
| Anoro Ellipta | Once-daily, long-term maintenance treatment of airflow obstruction in patients with chronic obstructive pulmonary disease (COPD) |
| Aptiom | An add-on medication to treat seizures associated with epilepsy |
| Breo Ellipta | Long-term, once-daily, maintenance treatment of airflow obstruction in patients with COPD, including chronic bronchitis and/or emphysema |
| Brintellix | Adults with major depressive disorder |
| Dotarem | For use in magnetic resonance imaging (MRI) of the brain, spine, and associated tissues of patients ages 2 years and older |
| Duavee | Moderate-to-severe hot flashes (vasomotor symptoms) associated with menopause and to prevent osteoporosis after menopause |
| Gazyva | In combination with chlorambucil to treat patients with previously untreated chronic lymphocytic leukemia |
| Gilotrif | Late stage (metastatic) nonsmall cell lung cancer (NSCLC) whose tumors express specific types of epidermal growth factor receptor (EGFR) gene mutations |
| Imbruvica* | Patients with mantle cell lymphoma (MCL), a rare and aggressive type of blood cancer |
| Invokana* | Used with diet and exercise to improve glycemic control in adults with type 2 diabetes |
| Kadcyla* | Patients with HER2-positive, late-stage (metastatic) breast cancer |
| Kynamro* | Patients with a rare type of high cholesterol called homozygous familial hypercholesterolemia |
| Luzu | Topical treatment of interdigital tinea pedis, tinea cruris, and tinea corporis caused by the organisms Trichophyton rubrum and Epidermophyton floccosum in patients 18 years of age and older |
| Lymphoseek | A radioactive diagnostic imaging tool to locate lymph nodes in patients with breast cancer or melanoma |
| Mekinist* | Patients whose tumors express the BRAF V600E or V600K gene mutations |
| Nesina | Improve blood sugar control in adults with type 2 diabetes |
| Olysio | Chronic hepatitis C virus (HCV) infection |
| Opsumit | Adults with pulmonary arterial hypertension (PAH), a chronic, progressive, and debilitating disease that can lead to death or the need for lung transplantation |
| Osphena | Women experiencing moderate-to-severe dyspareunia (pain during sexual intercourse), a symptom of vulvar and vaginal atrophy because of menopause |
| Pomlyst | Patients with multiple myeloma whose disease progressed after being treated with other cancer drugs |
| Sovaldi* | Chronic HCV infection |
| Tefinlar | Patients with melanoma whose tumors express the BRAF V600E gene mutation |
| Tecfidera* | Adults with relapsing forms of multiple sclerosis |
| Tivicay | HIV-1 infection |
| Vizamyl | A radioactive diagnostic drug for use with positron emission tomography (PET) imaging of the brain in adults being evaluated for Alzheimer's disease (AD) and dementia |
| Xofigo* | Men with symptomatic late-stage (metastatic) castration-resistant prostate cancer that has spread to bones but not to other organs |

*Source*: Food and Drug Administration 2014, *Approved Drugs 2013*, viewed Apr 19, 2014, http://www.fda.gov/downloads/drugs/developmentapprovalprocess/druginnovation/ucm381803.pdf.

*First-in-class – drugs with a new and unique mechanism for treating a medical condition.

**TABLE 8.13 EMA New Medicines in 2013**

| New Medicines | Treatment For |
|---|---|
| Abilify Maintena | Schizophrenia |
| Aubagio | Multiple sclerosis |
| Bosulif | Chronic myelogenous leukemia |
| Brintellix | Major depressive episodes in adults |
| Cholib | Adjunctive therapy to diet and exercise, to reduce triglycerides and increase high-density-lipoprotein (HDL) cholesterol levels in adults with mixed dyslipidemia with high cardiovascular risk |
| Cholic Acid FGK | Inborn errors of primary bile acid synthesis |
| Cometriq | Medullary thyroid carcinoma |
| Deltyba | Pulmonary infections because of multidrug-resistant tuberculosis |
| Erivedge | Advanced basal-cell carcinoma |
| Evarrest | Hemostasis, and as an adjunct to hemostasis |
| Fluenz Tetra | Vaccine for the prevention of influenza in children and adolescents 24 months to less than 18 years of age |
| Giotrif | Lung cancer |
| Grastofil | A biosimilar medicine for the treatment of neutropenia |
| Hexacima | Primary and booster vaccination of infants and toddlers from 6 weeks to 24 months of age against diphtheria, tetanus, pertussis, hepatitis B, poliomyelitis, and invasive diseases caused by *Hemophilus influenzae type* B (Hib) |
| Hexyon | Primary and booster vaccination of infants and toddlers from 6 weeks to 24 months of age against diphtheria, tetanus, pertussis, hepatitis B, poliomyelitis, and invasive diseases caused by *Hemophilus influenzae type* B (Hib) |
| HyQvia | Replacement therapy for primary immunodeficiency syndromes and secondary hypogammaglobulinemia |
| Iclusio | Chronic myeloid leukemia or Philadelphia-chromosome-positive acute lymphoblastic leukemia |
| Imvanex | Vaccine against smallpox |
| Incresync | Type 2 diabetes |
| Inflectra | Rheumatoid arthritis, adult Crohn's disease, pediatric Crohn's disease, ulcerative colitis, pediatric ulcerative colitis, ankylosing spondylitis, psoriatic arthritis, and psoriasis |
| Invokana | Type 2 diabetes mellitus. |
| Izba | Ocular hypertension and open-angle glaucoma |
| Jetrea | Symtomatic vitreomacular adhesion |
| Kadcyla | Metastatic breast cancer |
| Lemtrada | Multiple sclerosis |
| Lidocaine/Prilocaine Plethora | Primary premature ejaculation in adult men |
| Lojuxta | Homozygous familial hypercholesterolemia, in addition to a low fat diet and other lipid-lowering medicinal products |
| Lonquex | Reduction in the duration of neutropenia and the incidence of febrile neutropenia in adult patients treated with cytotoxic chemotherapy for malignancy |

**TABLE 8.13 (*Continued*)**

| New Medicines | Treatment For |
|---|---|
| Maci | An implant used to repair cartilage defects at the ends of the bones of the knee joint |
| Memantine Accord | A generic medicine, for the treatment of Alzheimer's disease |
| Mirvaso | Facial erythema of rosacea in adults |
| Neudexta | Pseudobulbar effect |
| Neuraceq | Detection of beta-amyloid in the brain in patients being evaluated for Alzheimer's disease and other causes of cognitive impairment |
| Nexium Control | Reflux symptoms in adults |
| NovoEight | Hemophilia A |
| Opsumit | Pulmonary arterial hypertension in adults |
| Para-aminosalicylic acid Lucane | Multidrug-resistant tuberculosis |
| Pomalidomide Celgene | Multiple myeloma |
| Procysbi | Management of cystinosis |
| Provenge | Metastatic castrate-resistant prostate cancer |
| Raxone | Leber's hereditary optic neuropathy |
| Relvar Ellipta | Asthma and chronic obstructive pulmonary disorder (COPD) |
| Remsima | Rheumatoid arthritis, adult Crohn's disease, pediatric Crohn's disease, ulcerative colitis, pediatric ulcerative colitis, ankylosing spondylitis, psoriatic arthritis, and psoriasis |
| Sirturo | A combination therapy for pulmonary multidrug-resistant tuberculosis in adults |
| Somatropin Biopartners | Replacement therapy for endogenous growth hormone deficiency |
| Sovaldi | Chronic (long-term) hepatitis C in adults |
| Spedra | Erectile dysfunction |
| Stivarga | Metastatic colorectal cancer |
| Stribild | Human-immunodeficiency-virus-1 (HIV-1) |
| Tafinlar | Unresectable or metastatic melanoma with a BRAF V600 mutation |
| Tecfidera | Multiple sclerosis |
| Tivicay | Adults and adolescents over 12 years of age infected with human immunodeficiency virus (HIV) |
| Tybost | HIV-1 infection in combination with the protease inhibitors atazanavir and darunavir |
| Ultibro Breezhaler | COPD |
| Vipdomet | Type 2 diabetes |
| Vipidia | Type 2 diabetes |
| Vitekta | HIV-1 infection in adults who are infected with HIV-1 without known mutations associated with resistance to elvitegravir |
| Voncento | Prevention and treatment of bleeding in von Willebrand disease and hemophilia A (congenital FVIII deficiency) |
| Xigduo | Type 2 diabetes |
| Xofigo | Castration-resistant prostate cancer |
| Xoterna Breezhaler | COPD |
| Xtandi | Prostate cancer |

*Source*: European Medicines Agency 2014, *CHMP Meeting Highlights*, viewed Apr 19, 2014, http://www.ema.europa.eu/ema/index.jsp?curl=pages/about_us/document_listing/document_listing_000378.jsp&mid=WC0b01ac0580028d2a. Reproduced with permission of the European Medicines Agency.

(iii) National Authorization Procedure: submission is to one member state in its own language.

(iv) Abridged National Authorization Procedure: applicable for generics and submission in one member states; mutual recognition process required for extension to other states.

3. Essentially similar procedures with some specific country-based requirements for Japan, China, India, Australia, and Canada.
4. The CTD is a set of harmonized documents on Quality, Safety, and Efficacy to be submitted to the regulatory authorities for marketing approval to license a drug for sale.

## 8.12 REVIEW QUESTIONS

1. Compare and contrast NDA and BLA. Why are there different applications for small and large molecule drugs?
2. Explain the meaning of an orphan drug. Why is it necessary to have a separate approval route for orphan drugs?
3. What is meant by bioequivalence studies and why they are needed for generics applications?
4. Describe the Centralized Procedure in Europe for drug approval. Compare and contrast the Centralized Procedure with NDA/BLA.
5. Describe the Mutual Recognition Procedure and the resolution of disputes between member states.
6. Explain the clinical trial procedures in Australia.
7. How does the CTD help to expedite approval process for marketing application?

## 8.13 BRIEF ANSWERS AND EXPLANATIONS

1. Refer to Section 8.2.3. NDA is the application for small molecule drugs and BLA for large molecule drugs. They are legislated under different Acts. More information with respect to cell line/bank, test methods, and production processes are required for the BLA.
2. Refer to Section 8.2.4. The orphan drug approval mechanism is implemented to provide incentives to pharmaceutical companies to research and develop drugs for diseases with small patient groups where otherwise commercial returns are considered to be lower than other diseases with large patient pools.
3. Bioequivalence studies are designed to evaluate the PD and PK of drugs against reference off-patent drugs. They have to demonstrate that the generics behave similarly to the original drugs in terms of active component, formulation, mechanism of actions, bioavailability, and ADME. Since the generics are based on off-patent drugs, regulatory authorities have waived the need for preclinical and clinical trials. However, bioequivalence studies are conducted to establish that there are no unintended reactions from the generics.

4. Refer to Section 8.3.2. Note the requirement for the QP and Expert Reports in Europe. There are no separate applications in the Centralized Procedure for small and large molecule drugs.
5. Refer to Section 8.3.2.
6. The CTA in Australia follows the CTN and CTX schemes. Refer to Section 8.7.
7. The CTD streamlines the technical data on quality, safety, and efficacy, which are required by the regulatory authorities in their reviews of the marketing application. Sponsors are aware of the expectations in the evidence that need to be provided. Only Module 1 if for specific country or regional requirements. Other Modules, 2–5, can be submitted with little variations to different authorities.

## 8.14 FURTHER READING

Australia's Therapeutic Goods Administration website, http://www.tga.gov.au/

Central Drugs Standard Control Organization, India website, http://www.cdsco.nic.in/forms/contentpage1.aspx?lid=1424

China Food and Drug Administration website, http://eng.sfda.gov.cn/WS03/CL0755/

European Agency for the Evaluation of Medicinal Products website, http://www.emea.eu.int/

Food and Drug Administration website, http://www.fda.gov/

Food and Drug Administration, Center for Biologics Evaluation and Research website, http://www.fda.gov/cber/

Food and Drug Administration, Center for Drugs Evaluation and Research website, http://www.fda.gov/cder/

Health Canada website, http://www.hc-sc.gc.ca/index-eng.php

International Conference on Harmonization website, http://www.ifpma.org/ich1.html

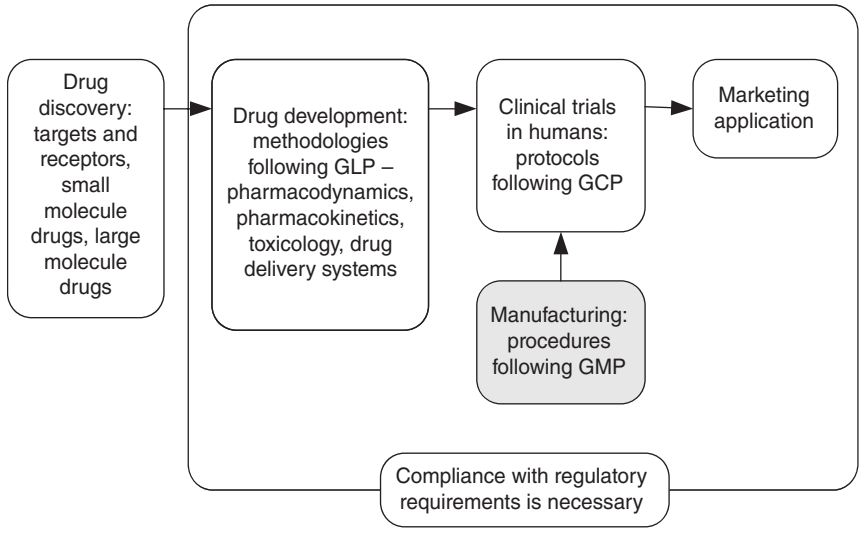
Ministry of Health, Labor and Welfare, Japan website, http://www.mhlw.go.jp/english/index.html

Pharmaceuticals and Medical Devices, Japan website, http://www.pmda.go.jp/english

Therapeutic Goods Administration, Australia website, http://www.tga.gov.au

# CHAPTER 9

# GOOD MANUFACTURING PRACTICE: REGULATORY REQUIREMENTS




## 9.1 INTRODUCTION

Successful clinical trials for a drug candidate lead to the filing of marketing authorization application to the regulatory authorities. Once the marketing authorization is

*Drugs: From Discovery to Approval*, Third Edition. Rick Ng.

approved, a pharmaceutical firm is ready to manufacture the drug for commercial sale to the target patient group. The manufacture of the drug must, however, be in accordance with Good Manufacturing Practice (GMP).

GMP is a quality concept and consists of a set of policies and procedures for manufacturers of drug products. These policies and procedures describe the facilities, equipment, methods, controls, and personnel training needed for producing drugs with the intended quality. The guiding principle for GMP is that quality cannot be tested into a product, but must be designed and built into each batch of the drug product throughout all aspects of its manufacturing processes. The term Quality by Design (QbD) is used (refer to Sections 9.8 and 9.9). Manufacturers are required to abide by GMP regulatory guidelines to ensure drugs are pure, consistent, safe, and effective. Regulatory guidelines are dynamic; they are revised and updated from time to time to implement new technologies, data, or information. Therefore, manufacturers have to keep abreast with regulatory developments by following current Good Manufacturing Practice (cGMP).

On a global level, GMP regulations are very similar for most developed countries. There are, however, differences in emphasis and implementation in specific areas. We explain the concept of GMP regulations and guidelines from the United States, Europe, International Conference on Harmonization (ICH), and Pharmaceutical Inspection Cooperation Scheme (PIC/S) in this chapter. In Chapter 10, we discuss the manufacturing processes for small molecule synthetic and large molecule protein-based drugs in accordance with the requisite quality processes under GMP.

## 9.2 UNITED STATES

GMP regulations came into effect in the United States in 1963. They have since undergone several major revisions. The implementation of GMP is the result of a number of tragedies in the United States and around the world. Legislations were introduced to ensure that drugs are safe for the patients and effective for treatments. Some of these unfortunate tragedies are presented in Exhibit 9.1.

The Food and Drug Administration (FDA) is charged with the responsibility of ensuring drug manufacturers comply with GMP regulations in the United States. GMP is defined by FDA as "a federal regulation setting minimum quality requirements that drug, biologics, and device manufacturers must meet. It describes in general terms known and accepted quality assurance principles for producing these products. Its

**Exhibit 9.1 Some Drug Tragedies**

In 1902, several children died after being administered contaminated diphtheria antitoxin.
In 1937, 107 people died when the drug sulfanilamide was wrongly formulated.
In 1955, 10 children died after being given improperly inactivated polio vaccine.
In 1960s, untold physical and emotional damage was caused by thalidomide (refer to Exhibit 7.1).

components are scientific understanding, documentation, analysis and measurements, and personnel matters. Its intended result is total quality assurance and product control."

The US FDA GMP is codified in the following regulations:

- *21 CFR Part 210*: *Current Good Manufacturing Practice in Manufacturing*, *Processing*, *Packing*, *or Holding of Drugs*; *General*
- *21 CFR Part 211*: *Current Good Manufacturing Practice for Finished Pharmaceuticals*
- *21 CFR Part 600*: *Biological Products*: *General*
- *21 CFR Part 610*: *General Biological Products Standards*.

Further details for each of these sets of regulations are presented in Exhibit 9.2 and 9.3. The areas where controls should be implemented are:

- Organization and Personnel
- Building and Facilities
- Equipment
- Control of Components and Drug Product Containers and Closures
- Production and Process Controls
- Packaging and Labeling Control
- Holding and Distribution
- Laboratory Control.

**Exhibit 9.2 FDA Current Good Manufacturing Practice (Parts 210 and 211)**

| | |
|---|---|
| *21 CFR Part 210*: *Current Good Manufacturing Practice in Manufacturing*, *Processing*, *Packing*, *or Holding of Drugs*; *General* | |
| 210.1 | Status of current good manufacturing practice regulations |
| 210.2 | Applicability of current good manufacturing practice regulations |
| 210.3 | Definitions |
| *21 CFR Part 211*: *Current Good Manufacturing Practice for Finished Pharmaceuticals* | |
| *Subpart A: General Provisions* | |
| 211.1 | Scope |
| 211.3 | Definitions |
| *Subpart B: Organization and Personnel* | |
| 211.22 | Responsibilities of quality control unit |
| 211.25 | Personnel qualifications |
| 211.28 | Personnel responsibilities |
| 211.34 | Consultants |

| | |
|---|---|
| *Subpart C: Building and Facilities* | |
| 211.42 | Design and construction features |
| 211.44 | Lighting |
| 211.45 | Ventilation, air filtration, air heating, and cooling |
| 211.48 | Plumbing |
| 211.50 | Sewerage and refuse |
| 211.52 | Washing and toilet facilities |
| 211.56 | Sanitation |
| 211.58 | Maintenance |
| *Subpart D: Equipment* | |
| 211.63 | Equipment design, size, and location |
| 211.65 | Equipment construction |
| 211.67 | Equipment cleaning and maintenance |
| 211.68 | Automatic, mechanical, and electronic equipment |
| 211.72 | Filters |
| *Subpart E: Control of Components and Drug Product Containers and Closures* | |
| 211.80 | General requirements |
| 211.82 | Receipt and storage of untested components, drug product containers, and closures |
| 211.84 | Testing and approval or rejection of components, drug product containers, and closures |
| 211.86 | Use of approved components, drug product containers, and closures |
| 211.87 | Retesting of approved components, drug product containers, and closures |
| 211.89 | Rejected components, drug product containers, and closures |
| 211.94 | Drug product containers and closures |
| *Subpart F: Production and Process Controls* | |
| 211.100 | Written procedures; deviations |
| 211.101 | Charge-in of components |
| 211.103 | Calculation of yield |
| 211.105 | Equipment identification |
| 211.110 | Sampling and testing of in-process materials and drug products |
| 211.111 | Time limitation on production |
| 211.113 | Control of microbiological contamination |
| 211.115 | Reprocessing |
| *Subpart G: Packaging and Labeling Control* | |
| 211.122 | Materials examination and usage criteria |
| 211.125 | Labeling issuance |
| 211.130 | Packaging and labeling operations |
| 211.132 | Tamper-resistant packaging requirement for over-the-counter human drug products |
| 211.134 | Drug product inspection |
| 211.137 | Expiration dating |

| | |
|---|---|
| Subpart H: Holding and Distribution | |
| 211.142 | Warehousing procedures |
| 211.150 | Distribution procedures |
| *Subpart I: Laboratory Controls* | |
| 211.160 | General requirements |
| 211.165 | Testing and release for distribution |
| 211.166 | Stability testing |
| 211.167 | Special testing requirements |
| 211.170 | Reserved samples |
| 211.173 | Laboratory animals |
| 211.176 | Penicillin contamination |
| *Subpart J: Records and Reports* | |
| 211.180 | General requirements |
| 211.182 | Equipment cleaning and use log |
| 211.184 | Component, drug product container, closure, and labeling records |
| 211.186 | Master production and control records |
| 211.188 | Batch production and control records |
| 211.192 | Production record review |
| 211.194 | Laboratory records |
| 211.196 | Distribution records |
| 211.198 | Complaint files |
| *Subpart K: Returned and Salvaged Drug Products* | |
| 211.204 | Returned drug products |
| 211.208 | Drug product salvaging |

*Source*: 1. Food and Drug Administration 2014, *Code of Federal Regulations Title 21, Part 210 Current Good Manufacturing Practice in Manufacturing, Processing, Packing, or Holding of Drugs, General*, viewed November 7, 2014, http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?cfrpart=210, 2. Food and Drug Administration 2014, *Code of Federal Regulations Title 21, Part 211 Current Good Manufacturing Practice for Finished Pharmaceuticals*, viewed November 7, 2014, http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?cfrpart=211

Selected items of these regulations are discussed in later sections. It should be noted that the applicable regulations for small molecule drugs are 21 CFR Parts 210 and 211, and for biopharmaceuticals the regulations are 21 CFR Parts 210, 211, 600, and 610. The reason is that biopharmaceuticals are less well defined chemically and they are sensitive to storage and manufacturing environment as well as the manufacturing processes. Biopharmaceuticals are normally prepared under aseptic (free from pathogenic organisms) conditions, as they are sensitive to degradation under normal terminal sterilization processes. Special techniques and analytical methods are required for the production and testing of biopharmaceuticals (refer to Chapter 10).

According to 21 CFR 210.1 (a), the regulations "contain the *minimum* current good manufacturing practice for methods to be used in, and the facilities or controls to be

**Exhibit 9.3 FDA Current Good Manufacturing Practice (Parts 600 and 610)**

| | |
|---|---|
| *21 CFR Part 600*: *Biological Products*: *General* | |
| *Subpart A: General Provisions* | |
| 600.2 | Mailing addresses |
| 600.3 | Definitions |
| *Subpart B: Establishment Standards* | |
| 600.10 | Personnel |
| 600.11 | Physical establishment, equipment, animals, and care |
| 600.12 | Records |
| 600.13 | Retention samples |
| 600.14 | Reporting of biological product deviations by licensed manufacturers |
| 600.15 | Temperature during shipment |
| *Subpart C: Establishment Inspection* | |
| 600.20 | Inspectors |
| 600.21 | Time of inspection |
| 600.22 | Duties of Inspector |
| *Subpart D: Reporting of Adverse Experiences* | |
| 600.80 | Postmarketing reporting of adverse experiences |
| 600.81 | Distribution reports |
| 600.90 | Waivers |
| *21 CFR Part 610*: *General Biological Products Standards* | |
| *Subpart A: Release Requirements* | |
| 610.1 | Test prior to the release required for each lot |
| 610.2 | Requests for samples and protocols; official release |
| *Subpart B: General Provisions* | |
| 610.9 | Equivalent methods and processes |
| 610.10 | Potency |
| 610.11 | General safety |
| 610.11a | Inactivated influenza vaccine, general safety test |
| 610.12 | Sterility |
| 610.13 | Purity |
| 610.14 | Identity |
| 610.15 | Constituent materials |
| 610.16 | Total solids in serums |
| 610.17 | Permissible combinations |
| 610.18 | Cultures |
| *Subpart C: Standard Preparations and Limits of Potency* | |
| 610.20 | Standard preparations |
| 610.21 | Limits of potency |

| | |
|---|---|
| *Subpart D: Mycoplasma* | |
| 610.30 | Test for mycoplasma |
| *Subpart E: Testing Requirements for Communicable Disease Agents* | |
| 610.40 | Test requirements |
| 610.41 | Donor deferral |
| 610.42 | Restrictions on use for further manufacture of medical devices |
| 610.44 | Use of reference panels by manufacturers of test kits |
| 610.46 | Human immunodeficiency virus (HIV) "lookback" requirements |
| 610.47 | Hepatitis C virus (HCV) "lookback" requirements |
| 610.48 | Hepatitis C virus (HCV) "lookback" requirements on the basis of review of historical records |
| *Subpart F: Dating Period Limitations* | |
| 610.50 | Date of manufacture |
| 610.53 | Dating periods for licensed biological products |
| *Subpart G: Labeling Standards* | |
| 610.60 | Container label |
| 610.61 | Package label |
| 610.62 | Proper name; package label; legible type |
| 610.63 | Divided manufacturing responsibility to be shown |
| 610.64 | Name and address of distributor |
| 610.65 | Product for export |
| 610.67 | Bar code label requirements |
| 610.68 | Exceptions or alternatives to labeling requirements for biological products held by the Strategic National Stockpile |

*Source*: 1. Food and Drug Administration 2014, *Code of Federal Regulations Title 21, Part 600 Biological Products: General*, viewed November 7, 2014, http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?cfrpart=600, 2. Food and Drug Administration 2014, *Code of Federal Regulations Title 21, Part 610 General Biological Products Standards*, viewed November 7, 2014, http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?cfrpart=610

used for, the manufacture, processing, packing, or holding of a drug to assure that such drug meets the requirement". Pharmaceutical companies should be implementing practices that would meet or exceed these minimum requirements for manufacturing quality products.

In addition to the regulations under 21 CFR, FDA publishes Guidance to Industry and documents called Points to Consider (PTCs) as guidelines and recommendations to industry to adopt as part of the GMP compliance program. Moreover, FDA recognizes ICH Quality Guidelines (refer to Section 9.4) and PIC/S GMP standards (refer to Section 9.5).

## 9.3 EUROPE

The principles and guidelines for GMP for human medicinal products and investigational medicinal products were laid down in the current EU Commission Directive 2003/94/EC on October 8, 2003. The basic requirements are similar to those of the United States as stated in *Article 4*: *Conformity with good manufacturing practice* – "the manufacturer shall ensure that manufacturing operations are carried out in accordance with good manufacturing practice and with the manufacturing authorization". The compliance system would necessitate controls of the following areas:

- Quality assurance system
- Personnel and training
- Premises and equipment
- Documentation
- Production
- Quality control
- Work contract out
- Complaint, product recall, and emergency unblinding (GMP is applicable to clinical trial materials and the requirements extend to revealing subjects assigned to placebo or active in emergency cases)
- Self-inspection
- Labeling.

The GMP requirements are presented in two guides as below:

- *EU GMP guide part I*: *Basic requirements for medicinal pro*ducts
- *EU GMP guide part II*: *Basic requirements for active substances used as starting materials*.

In addition to the two GMP guides, there are 19 different annexes for supplementary requirements on specific products and processes (refer to Section 9.5 for further details). Some examples of the annexes are:

- Manufacture of sterile medicinal products
- Manufacture of biological medicinal products for human use
- Manufacture of radiopharmaceuticals
- Manufacture of veterinary medicinal products other than immunologicals.

There are slight differences in the GMP guidelines between the United States and EU, for example:

- Inclusion of self-inspection by EMA but not FDA
- Continual training for personnel and periodic assessment by EMA but no periodic assessment explicitly required by FDA

- Cleanroom classifications of particulates at 0.5 μm by FDA and 0.5 and 5 μm by EMA
- Tamper-proof packaging requirement for OTC products by FDA, but not by EMA.

It should be noted that the GMP guidelines, whether the United States or EMA, are implemented with similar underlying principles to ensure manufacture of drug products with the intended quality as the desired outcome, even though there are minor differences. Regulatory authorities are mindful of the differences and strive for the need for harmonization, which is discussed the sections on International Conference on Harmonization (ICH) and Pharmaceutical Inspection Cooperation Scheme (PIC/S).

## 9.4 INTERNATIONAL CONFERENCE ON HARMONIZATION (ICH)

We discussed in Section 7.11 the tripartite harmonization of guidelines by the United States, Europe, and Japan with the formation of the International Conference on Harmonization (ICH). As noted there are four major categories of harmonized guidelines prepared by ICH: Quality, Safety, Efficacy, and Multidisciplinary. These guidelines have been gradually introduced in this book – Safety in Section 5.4, Efficacy in Section 6.4, and Multidisciplinary in Section 7.11. For Quality guidelines, these are presented in Exhibit 9.4.

**Exhibit 9.4 ICH Quality Guidelines**

| | |
|---|---|
| *Q1*: | *Stability* |
| Q1A | Stability testing of new drug substances and products |
| Q1B | Stability testing: photostability testing of new drug substances and products |
| Q1C | Stability testing for new dosage forms |
| Q1D | Bracketing and matrixing designs for stability testing of new drug substances and products |
| Q1E | Evaluation of stability data |
| Q1F | Stability data package for registration applications in climatic zones III and IV |
| *Q2*: | *Analytical Validation* |
| Q2 | Validation of analytical procedures: text and methodology |
| *Q3*: | *Impurities* |
| Q3A | Impurities in new drug substances |
| Q3B | Impurities in new drug products |
| Q3C | Impurities: guideline for residual solvents |
| Q4D | Impurities: guideline for elemental impurities |
| *Q4*: | *Pharmacopoeias* |
| Q4A | Pharmacopoeial harmonization |
| Q4B and Annexes | Evaluation and recommendation of pharmacopoeial tests for use in the ICH regions |

| | |
|---|---|
| *Q5*: | *Quality of Biotechnological Products* |
| Q5A | Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin |
| Q5B | Analysis of the expression construct in cells used for production of rDNA derived protein products |
| Q5C | Stability testing of biotechnological/biological products |
| Q5D | Derivation and characterization of cell substrates used for production of biotechnological/biological products |
| Q5E | Comparability of biotechnological/biological products subject to changes in their manufacturing process |
| *Q6*: | *Specifications* |
| Q6A | Specifications: test procedures and acceptance criteria for new drug substances and new drug products; chemical substances |
| Q6B | Specifications: test procedures and acceptance criteria for biotechnological/biological products |
| *Q7*: | *Good Manufacturing Practice* |
| Q7 | Good manufacturing practice guide for active pharmaceutical ingredients |
| *Q8*: | *Pharmaceutical Development* |
| Q8 | Pharmaceutical development |
| *Q9*: | *Quality Risk Management* |
| Q9 | Quality risk management |
| *Q10*: | *Pharmaceutical Quality System* |
| Q10 | Pharmaceutical quality system |
| *Q11*: | *Development and Manufacture of Drug Substances* |
| Q11 | Development and manufacture of drug substances |

*Source*: International Conference on Harmonisation 2014, *Quality Guidelines*, viewed November 7, 2014, http://www.ich.org/products/guidelines/quality/article/quality-guidelines.html. Reproduced with permission of ICH.

GMP is a part of the quality guidelines. In particular the ICH document – *GMP Guidance for Active Pharmaceutical Ingredients*, Q7, November 2000 (this document is almost identical to *PIC/S GMP Guide Part II*: *Basic Requirements for Active Pharmaceutical Ingredients*, refer to Section 9.5 and Exhibit 9.6) – sets out the requirements for active drug substance manufacture. It should be noted that when this guideline was conceived, the Steering Committee of ICH was aware of the global influence of this guideline and it invited experts from the World Health Organization (WHO), Australia, India, and China and industrial representatives from the generics industry, self-medication industry, and PIC/S (refer to Section 7.13) to participate in the preparation of this document. Hence, the Q7 document has been endorsed as a truly international document for GMP for active pharmaceutical substances. Another point to note is that at the time when the committee was preparing the Q7 document, ICH considered the existing GMP guidelines for drug product that have already been separately enacted in the United States, Europe, and Japan as adequate and did not require harmonization by ICH.

The United States, EU, and Japan have implemented this ICH Q7 GMP guideline, and in reality the EMA *EU GMP guide part II*: *Basic requirements for active substances used as starting materials* is equivalent to ICH Q7. Most of the requirements of ICH Q7 are derived from the 21 CFR regulations and EU GMP Directive. The important additional sections in ICH Q7 are Internal Audits (Self-inspection), Contract Manufacturers and Agents, Brokers, Traders, Distributors, Repackers, and Relabelers. The section on APIs for Use in Clinical Trials clarifies the regulatory authorities' expectations for drugs designated for clinical trials, as opposed to approved drugs manufactured on a routine production basis. With the increasing importance of biopharmaceuticals, a section on production via cell culture methods, harvesting, and viral removal has been added as Specific Guidance for APIs Manufactured by Cell Culture/Fermentation. ICH Q7 is now adopted by PIC/S (refer to Section 9.5).

Since late 2005, ICH has added four more quality guidelines to formalize the GMP requirements for drug substances and products to complement ICH Q7:

- Q8 – *Pharmaceutical Development*
- Q9 – *Quality Risk Management*
- Q10 – *Pharmaceutical Quality System*
- Q11 – *Development and Manufacture of Drug Substances*.

All these guidelines are intended to bolster the quality of drug substances and drug products to be manufactured and steer manufacturers in the direction to improve compliance, safety, and consistencies of the drugs and is based on a product life cycle management concept.

## 9.5 PHARMACEUTICAL INSPECTION COOPERATION SCHEME (PIC/S)

As introduced in Section 7.13 the Pharmaceutical Inspection Cooperation Scheme (PIC/S) is to foster "international development, implementation and maintenance of harmonized GMP standards and quality systems in the field of medicinal products". The GMP requirements under PIC/S are contained in the following documents:

- *PIC/S GMP Guide Part I*: *Basic Requirements for Medicinal Products*
- *PIC/S GMP Guide Part II*: *Basic Requirements for Active Pharmaceutical Ingredients*
- *PIC/S Guide* (*Annexes*).

For all intents and purposes these PIC/S GMP Guides are equivalent to the EU Guides and ICH Q7 (PIC/S GMP Guide Part I was developed in parallel with EU EMA guidelines and PIC/S GMP Guide Part II and the annexes were adopted from ICH Q7). With 44 participating authorities, inclusive of the United States, Europe, Japan, Africa (e.g. South Africa), America (e.g. Argentina, Canada), Asia (e.g. Malaysia, Singapore) and Australasia (e.g. Australia), and many other countries, the PIC/S Guides

and annexes are adopted as the harmonized documents for GMP compliance in the manufacture of drug substances and products.

Exhibit 9.5 and 9.6 tabulate the essential elements that constitute PIC/S GMP Guides Part I and Part II. A list of the Annexes is presented in Exhibit 9.7. These can be compared to Exhibit 9.2 and 9.3 to show the similarities in the intent to apply quality principles to GMP in the major countries.

## 9.6 SELECTED CORE ELEMENTS OF GMP

Selected core elements from PIC/S GMP Guides Part I and Part II are discussed in the following sections to highlight the extent of GMP requirements.

**Exhibit 9.5 PIC/S Guide to GMP for Medicinal Products Part I: Basic Requirements for Medicinal Products**

Chapter 1 – Quality management
- Principle
- Quality assurance
- Good manufacturing practice for medicinal products (GMP)
- Quality control
- Product quality review
- Quality risk management

Chapter 2 – Personnel
- Principles
- General
- Key personnel
- Training
- Personal hygiene

Chapter 3 – Premises and equipment
- Principle
- Premises
- Equipment

Chapter 4 – Documentation
- Principle
- Required GMP documentation
- Generation and control of documentation
- Good documentation practices
- Retention of documents

- Specifications
- Manufacturing formula and processing instructions
- Procedures and records

Chapter 5 – Production
- Principle
- General
- Prevention of cross-contamination in production
- Validation
- Starting materials
- Processing operations – intermediate and bulk products
- Packaging materials
- Packaging operations
- Finished products
- Rejected, recovered and returned materials

Chapter 6 – Quality control
- Principle
- General
- Good quality control laboratory practice
- Documentation
- Sampling
- Testing
- Ongoing stability program

Chapter 7 – Contract manufacture and analysis
- Principle
- General
- The contract giver
- The contract acceptor
- The contract

Chapter 8 – Complaints and product recall
- Principle
- Complaints
- Recalls

Chapter 9 – Self-inspection
- Principle

*Source:* Pharmaceutical Inspection Cooperation Scheme 2014, *PIC/S GMP Guide (Part I: Basic Requirements for Medicinal Products)*, viewed November 7, 2014, http://www.picscheme.org/publication.php?id=4

**Exhibit 9.6 PIC/S Guide to GMP for Medicinal Products Part II: Basic Requirements for Active Pharmaceutical Ingredients**

1. Introduction
   - Objective
   - Scope
2. Quality management
   - Principles
   - Quality risk management
   - Responsibilities of the quality unit
   - Responsibility for production activities
   - Internal audits (self-inspection)
   - Product quality review
3. Personnel
   - Personnel qualifications
   - Personnel hygiene
   - Consultants
4. Buildings and facilities
   - Design and construction
   - Utilities
   - Water
   - Containment
   - Lighting
   - Sewage and refuse
   - Sanitation and maintenance
5. Process equipment
   - Design and construction
   - Equipment maintenance and cleaning
   - Calibration
   - Computerized systems
6. Documentation and record
   - Documentation system and specifications
   - Equipment cleaning and use record
   - Records of raw materials, intermediates, API labeling and packaging materials
   - Master production instructions
   - Batch production records
   - Laboratory control records
   - Batch production record review

7. Materials management
    - General controls
    - Receipt and quarantine
    - Sampling and testing of incoming production materials
    - Storage
    - Reevaluation
8. Production and in-process controls
    - Production operations
    - Time limits
    - In-process sampling and controls
    - Blending batches of intermediates or APIs
    - Contamination control
9. Packaging and identification labeling of APIs and intermediates
    - General
    - Packaging materials
    - Label issuance and control
    - Packaging and labeling operations
10. Storage and distribution
    - Warehousing procedures
    - Distribution procedures
11. Laboratory controls
    - General controls
    - Testing of intermediates and APIs
    - Validation of analytical procedures
    - Certificates of analysis
    - Stability monitoring of APIs
    - Expiry and retest dating
    - Reserve/retention samples
12. Validation
    - Validation policy
    - Validation documentation
    - Qualification
    - Approaches to process validation
    - Process validation program
    - Periodic review of validated systems
    - Cleaning validation
    - Validation of analytical methods
13. Change control

14. Rejection and reuse of materials
    - Rejection
    - Reprocessing
    - Reworking
    - Recovery of materials and solvents
    - Returns
15. Complaints and recalls
16. Contract manufacturers (including laboratories)
17. Agents, brokers, traders, distributors, repackers, and relabelers
    - Applicability
    - Traceability of distributed APIs and intermediates
    - Quality management
    - Repackaging, relabeling, and holding of APIs and intermediates
    - Stability
    - Transfer of information
    - Handling of complaints and recalls
    - Handling of returns
18. Specific guidance for APIs manufactured by cell culture/fermentation
    - General
    - Cell Bank maintenance and record keeping
    - Cell CULTURE/fermentation
    - Harvesting, isolation, and purification
    - Viral removal/inactivation
19. APIs for use in clinical trials
    - General
    - Quality
    - Equipment and facilities
    - Control of raw materials
    - Production
    - Validation
    - Changes
    - Laboratory controls
    - Documentation
20. Glossary

*Source*: Pharmaceutical Inspection Cooperation Scheme 2014, *PIC/S Guide to GMP for Medicinal Products Part II: Basic Requirements for Active Pharmaceutical Ingredients*, viewed November 7, 2014, http://www.picscheme.org/publication.php?id=4

**Exhibit 9.7 PIC/S Guide to GMP for Medicinal Products: Annexes**

Annex 1. Manufacture of sterile medicinal products
Annex 2. Manufacture of biological medicinal substances and products
Annex 3. Manufacture of radiopharmaceuticals
Annex 4. Manufacture of veterinary medicinal products other than immunologicals
Annex 5. Manufacture of immunological veterinary medical products
Annex 6. Manufacture of medicinal gases
Annex 7. Manufacture of herbal medicinal products
Annex 8. Sampling of staring and packaging materials
Annex 9. Manufacture of liquids, creams, and ointments
Annex 10. Manufacture of pressurized metered dose aerosol preparations for inhalation
Annex 11. Computerized systems
Annex 12. Use of ionizing radiation in the manufacture of medicinal products
Annex 13. Manufacture of investigational products
Annex 14. Manufacture of medicinal products derived from human blood or plasma
Annex 15. Qualification and validation
Annex 16. [Qualified person in batch release] – specific to EU GMP, not adopted by PIC/S
Annex 17. Parametric release
Annex 18. [GMP Guide for active pharmaceutical ingredients] – now Part II of the PIC/S GMP Guide
Annex 19. Reference and retention samples
Annex 20. Quality risk management
Glossary

*Source*: Pharmaceutical Inspection Cooperation Scheme 2014, *PIC/S Guide to GMP for Medicinal Products: Annexes*, viewed November 7, 2014, http://www.picscheme.org/publication.php?id=4

### 9.6.1 Introduction: Scope

PIC/S GMP Guide Part I covers the manufacture of drug products, and Part II is applicable to the manufacture of active pharmaceutical ingredients (APIs) derived from a number of sources. Table 9.1 illustrates the scope of these API sources.

**TABLE 9.1 Application of PIC/S GMP Guide Part II to API Manufacturing**

| Type of Manufacturing | Application of This Guide to Steps (Shown in Gray) Used in This Type of Manufacturing | | | | |
|---|---|---|---|---|---|
| Chemical manufacturing | Production of the API starting material | Introduction of the API starting material into process | Production of intermediates | Isolation and purification | Physical processing and packaging |
| API derived from animal sources | Collection of organ, fluid, or tissue | Cutting, mixing, and/or initial processing | Introduction of the API starting material into process | Isolation and purification | Physical processing and packaging |
| API extracted from plant sources | Collection of plant | Cutting and initial extraction(s) | Introduction of the API starting material into process | Isolation and purification | Physical processing and packaging |
| Herbal extracts used as API | Collection of plants | Cutting and initial extraction | | Further extraction | Physical processing and packaging |
| API consisting of comminuted or powdered herbs | Collection of plants and/or cultivation and harvesting | Cutting/ comminuting | | | Physical processing and packaging |
| Biotechnology: fermentation/ cell culture | Establishment of master cell bank and working cell bank | Maintenance of working cell bank | Cell culture and/or fermentation | Isolation and purification | Physical processing and packaging |
| "Classical" fermentation to produce an API | Establishment of cell bank | Maintenance of the cell bank | Introduction of the cells into fermentation | Isolation and purification | Physical processing and packaging |

*Source*: International Conference on Harmonization 2002, *Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients, Q7*, viewed Novemeber 7, 2014, http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q7/Step4/Q7_Guideline.pdf. Reproduced with permission of ICH.

### 9.6.2 Quality Management

The first and foremost element for GMP is the quality system, which should be supported by senior management. This can be divided into Quality Assurance (QA) and Quality Control (QC). QA is a total system approach. It sets out the compliance policies and procedures for all facets of drug manufacturing. QC is the practical extension of QA. The role of QC is concerned with inspection and testing of environment, raw materials, in-process intermediates, and finished products.

All personnel involved in GMP production of drugs have to take ownership of quality. It is a requirement that processes and equipment for drug manufacturing must be approved and operated by trained, qualified personnel. Quality-related activities have to be recorded to enable traceability of data and information. Deviations and excursions of processes and results from specified conditions or criteria have to be reported, investigated, and resolved. Drug products have to be tested and meet specifications before being released by an authorized person, normally from the QA department. Responsibilities for the QA and QC departments and production activities need to be defined. Approved procedures are to be followed and processing conditions and data recorded.

Two important aspects are self-inspection (refer to Section 9.6.15) and product quality review (refer to Section 10.7). Self-inspections are implemented to regularly monitor the compliance activities in drug manufacture and to ensure rectification to these activities if deviations occur. Trending and statistical analysis of data provide early warning of impending problems. Product quality review checks the relevance and adequacy of the manufacturing activities. It provides input to update and improve the quality system.

Finally, the active role of responsible corporate management in Quality Management cannot be underestimated as this group contains decision makers and are ultimately held responsible for manufacturing. For example, the company should follow written procedures to keep management informed of regulatory inspections, serious GMP deficiencies, product defects, and related actions.

### 9.6.3 Personnel

Personnel engaged in GMP manufacturing of drug products are required to be formally trained in quality practices. They are only assigned to tasks for which they have been trained. This is to guarantee that drugs are manufactured by qualified personnel and is a key component of the concept that quality is built into each step of the manufacturing process.

Personnel are the main source of contaminants to drug products, and hence personnel cleanliness is an important factor (refer to Exhibit 9.8). Any personnel suffering from infectious diseases or having open wounds should be assigned to non-GMP production activities to reduce possibility of contamination.

### 9.6.4 Buildings and Facilities

Buildings must be designed with regard to the needs for manufacturing with minimum risk of contamination. There must be demarcation of areas for different activities. Such segregation reduces the possibility of contamination and material mix-ups.

For the manufacture of drug substances and products, certain processes have to be performed in clean areas. Specifications for environmental airborne particulates and viable microorganisms in cleanrooms are provided in EU Directive 2003/94/EC, Annex 1 and EN ISO 14644-1 (which replaced FS 209E in January 2002). Details for these specifications are summarized in Table 9.2.

The cleanliness is graded in accordance with the nature of operations: for example, Grade A for aseptic preparation and filling, Grade B as background environment for

**Exhibit 9.8 Human-Caused Particles**

About $10^7$ dead cells are shed from the body each day
About 2000 microorganisms per square centimeter of skin surface
Number of 0.3 μm particles shed from the body during specific activities:

| | |
|---|---|
| Motionless | 100,000 |
| Getting-up | 1,000,000 |
| Walking | 5,000,000 |

*Source*: Data from Hofmann, FK 2001, *GMP Compliance*, Centre for Continuous Education, Vista, CA.

**TABLE 9.2 Airborne Environmental Cleanliness Requirements**

| | Maximum Permissible Number of Particles or Microorganisms | | | |
|---|---|---|---|---|
| | Aseptic Core | Aseptic Process Area | Clean Preparation Area | Support Area |
| *EU 91/356/EEC Annex 1* | *Grade A* | *Grade B* | *Grade C* | *Grade D* |
| *At rest* | | | | |
| 0.5 μm particles/$m^3$ | 3,500 | 3,500 | 350,000 | 3,500,000 |
| 5 μm particles/$m^3$ | None | None | 2,000 | 20,000 |
| *In operation* | | | | |
| 0.5 μm particles/$m^3$ | 3,500 | 350,000 | 3,500,000 | Unclassified |
| 5 μm particles/$m^3$ | None | 2,000 | 20,000 | Unclassified |
| Viable organisms cfu/$m^3$ | <1 | <10 | <100 | <200 |
| *ISO 14644-1: 1999* | *ISO 5* | *ISO 7* | *ISO 8* | – |
| *In operation* | | | | |
| 0.5 μm particles/$m^3$ | 3,520 | 352,000 | 3,520,000 | Unclassified |

*Source*: European Commission 2008, *EudraLex Volume 4, Annex 1, Manufacture of Sterile Medicinal Products*, viewed May 19, 2014, http://ec.europa.eu/health/files/eudralex/vol-4/2008_11_25_gmp-an1_en.pdf. Reproduced with permission of the European Medicines Agency.

Grade A, Grade C for preparation of solutions to be filtered, and Grade D for the handling of components after washing. The "at rest" condition is defined as the condition where installation is complete with production equipment installed and operating, but with no operating personnel present. The "in operation" condition is when equipment is functioning and a specified number of personnel are present. The viable microorganisms are the permissible number of colony forming units (cfu) on a culture plate for a cubic meter of air sample. There are other limits for microorganisms present on surfaces

**TABLE 9.3 Recommended Limits for Microbial Contamination**

| Grade | Air Sample ($cfu/m^3$) | Settle Plate, Diameter 90 mm (cfu/4 h) | Contact Plate, Diameter. 55 mm (cfu/plate) | Personnel Glove Print, 5 Fingers (cfu/glove) |
|---|---|---|---|---|
| A | <1 | <1 | <1 | <1 |
| B | 10 | 5 | 5 | 5 |
| C | 100 | 50 | 25 | N/A |
| D | 200 | 100 | 50 | N/A |

Source: European Commission 2008, *EudraLex Volume 4, Annex 1, Manufacture of Sterile Medicinal Products*, viewed May 19, 2014, http://ec.europa.eu/health/files/eudralex/vol-4/2008_11_25_gmp-an1_en.pdf. Reproduced with permission of the European Medicines Agency

N/A = Not applicable.

and personnel that need to be monitored. Specifications for these microorganism limits for clearances in operation are recommended in EU Directive 2003/94/EC Annex 1 (Table 9.3**)**.

To control the cleanliness levels, the heating, ventilation, and air-conditioning system (HVAC) circulates air that is filtered through high efficiency particulate air (HEPA) filters, which remove up to 99.99% of particles 0.3 μm and larger. The number of air exchanges is also controlled, at a minimum of 20 air changes per hour, depending on room classifications.

Clean rooms are pressurized to prevent contaminants from entering. FDA specifies a minimum of 0.05 in. water (12.5 Pa) difference in pressure between clean rooms of different classifications, with the more critical, cleaner rooms having higher pressures. A schematic diagram showing the pressure gradient through air locks is shown in Figure 9.1. Materials can be transferred from one room to another via pass through boxes to avoid generation of contamination. Pass through boxes are to have interlocking system to prevent simultaneous opening of the doors on both sides. There should be clean rooms used for gowning, and personnel should apply hand sanitizer to prevent contamination.

Both the temperature and relative humidity are normally controlled by the HVAC to, for example, $21 \pm 2\,^{\circ}C$ and 30–50%, respectively, for operator comfort and to reduce growth of microorganisms at drier conditions. The facility is also designed to prevent product from escaping into the environment. Wastes (both solids and liquids) are decontaminated, and exhaust air is filtered before discharge. In some facilities, the direction of the flow of personnel, materials, products, and equipment is controlled to prevent cross-contamination.

For a facility manufacturing biopharmaceuticals, appropriate designs according to biosafety level (BSL1 to BSL4; refer to Exhibit 9.9 for biosafety definitions) have to be implemented.

Utilities such as gases and air piped to reaction vessels are filtered ($\leq$0.2 μm filters) to control the risk of microbial contamination. Water is considered a raw material in the manufacture of drug products. It requires a more detailed discussion and is presented in Section 9.7.1.

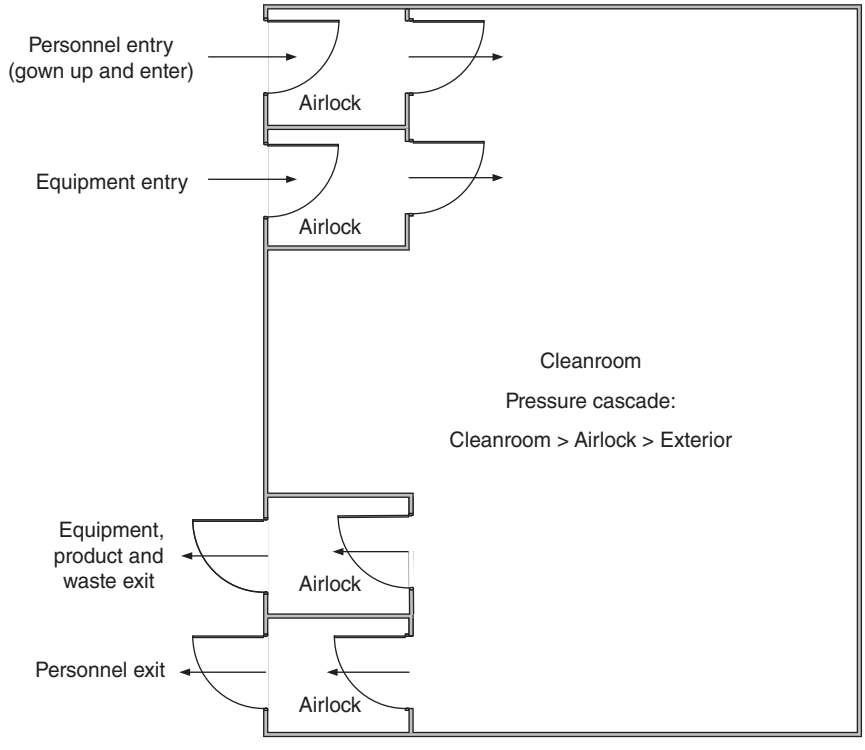



**Figure 9.1** Cleanroom pressure scheme. *Note*: Two entrances and exits for Grade B environment; single entrance and exit for Grades A and B environments.

### 9.6.5 Process Equipment

Appropriate equipment is used for manufacturing drugs. The equipment must be maintained and calibrated at defined periods to ensure it functions as intended. Critical equipment are to be validated (refer to Section 9.6.11). After each production batch, equipment is cleaned to prevent cross-contamination from residues. Ideally, this entails that equipment should be designed without difficult-to-clean areas. A discussion of cleaning is given in Section 9.7.2.

For computerized process equipment, the regulatory requirements are very specific, and these are detailed in Section 9.7.3.

### 9.6.6 Documentation and Records

Documentation comprises procedures, instructions, test methods, batch records, and so on that are prepared and controlled. Documentation is prepared, reviewed, and approved by qualified personnel. Approved copies of documents are distributed to relevant departments and superseded copies are retrieved and archived. The retention period for each type of document is specified. Documents are issued with document

**Exhibit 9.9 Biosafety Levels**

*BSL 1*: Biosafety Level 1 is suitable for work involving well-characterized microorganisms not known to consistently cause disease in healthy adults, and of minimal potential hazard to laboratory personnel and the environment. Safety equipment: none required. Microorganisms include *Bacillus subtilis*, *Naegleria gruberi*, and infectious canine hepatitis virus.

*BSL 2*: Biosafety Level 2 is suitable for work involving microorganisms of moderate potential hazard to personnel and the environment. Safety equipment: Class I or II biosafety cabinets or other physical containment devices; laboratory coats, gloves, face protection as needed. Microorganisms include hepatitis B virus, HIV, salmonellae, and mycoplasma.

*BSL 3*: Biosafety Level 3 is for work with indigenous or exotic microorganisms, which may cause serious or potentially lethal disease if inhaled. Safety equipment: Class I or II biosafety cabinets or other physical containment devices; protective laboratory clothing, gloves, respiratory protection as needed. Microorganisms include *Mycobacterium tuberculosis*, *B. anthracis,* and *Coxiella burnetii*.

*BSL 4*: Biosafety Level 4 is for work with dangerous and exotic microorganisms that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease. Safety equipment: Class III biosafety cabinet or Class I or II biosafety cabinets with full-body, air-supplied, positive pressure personnel suit. Microorganisms include Marburg virus, Ebola virus, Congo-Crimean hemorrhagic fever virus, and Nipah virus.

*Source*: Data from OSHA and American Biosafety Association Alliance, *Biosafety Levels (US)*, viewed May 20, 2014, http://www.absa.org/pdf/OSHABSLFactSheet.pdf.

and version numbers for ease of identification and reference. Master copies of documents are filed at secured locations with authorized access. Master copies stored in electronic media require validation in accordance with FDA regulation in 21 CFR Part 11 (refer to Section 9.7.3) to assess the security of access and data integrity. Operators are trained and retrained to only apply the latest approved documents.

Records include materials transfer records, batch records, materials/intermediates/finished product test records, shipping records, equipment calibration records, water test records, and environmental test records. They provide an audit trail for reviewing all the information related to the production of any batch of drug product. The data are required to be reviewed for product release.

### 9.6.7 Materials Management

Materials are managed to assure the following:

- Materials received match those that were ordered
- Identification labels are attached

- Where required, certificates of approval are provided
- Materials are quarantined and stored in specified conditions prior to QC inspection and test
- Segregation of approved materials from rejects
- Transfer of materials to relevant departments for use
- Receipt and quarantine of finished products is documented
- Storage of approved products pending shipment follows specific guidelines
- Shipment of products to designated receivers is documented
- All storage of intermediate and finished products must be validated.

It is necessary to ensure suppliers of materials have in place appropriate quality systems and that they are reliable. External audits may be required to inspect and confirm the supplier's facility and quality system.

### 9.6.8 Production and In-Process Controls

Materials, processes, and control parameters for drug production are stated in written documents. Production personnel follow procedures and record materials used, amounts weighed, and date of operation. Equipment, reaction vessels, and the production area are cleaned and their status recorded in logbooks. Throughout the production stages, equipment conditions (e.g., pH, pressure, stirring speed, and temperature) are also recorded. Adjustments to in-process control parameters, if permitted, are entered onto batch records.

Samples of intermediates and finished products taken for analysis are recorded, stating the time, date, and conditions for these samples. These samples must be accounted for in the product reconciliation sheet. Deviations/discrepancies in operating conditions and out of specifications (OOS) in test samples are reported and investigated (refer to Section 9.6.12). Figure 9.2 shows a mechanism for production and in-process controls.

Equipment, raw materials, intermediates, finished products, and packaging materials may require sterilization. A discussion of the sterilization process is presented in Section 9.7.6.

### 9.6.9 Packaging, Identification, and Labeling of APIs, Intermediates, and Finished Products

Proper identification of raw materials, intermediates, and finished products is necessary to prevent misuse and mix-ups and to allow traceability. Labels are controlled and accounted for to prevent mislabeling. Discrepancies in reconciliation of labels must be investigated. If required, packages may be sealed to provide an alert of mishandling or unauthorized tampering.

### 9.6.10 Laboratory Controls

The aim of laboratory controls is to ensure that only approved materials are used and only intermediates and drug products that meet specifications are released.

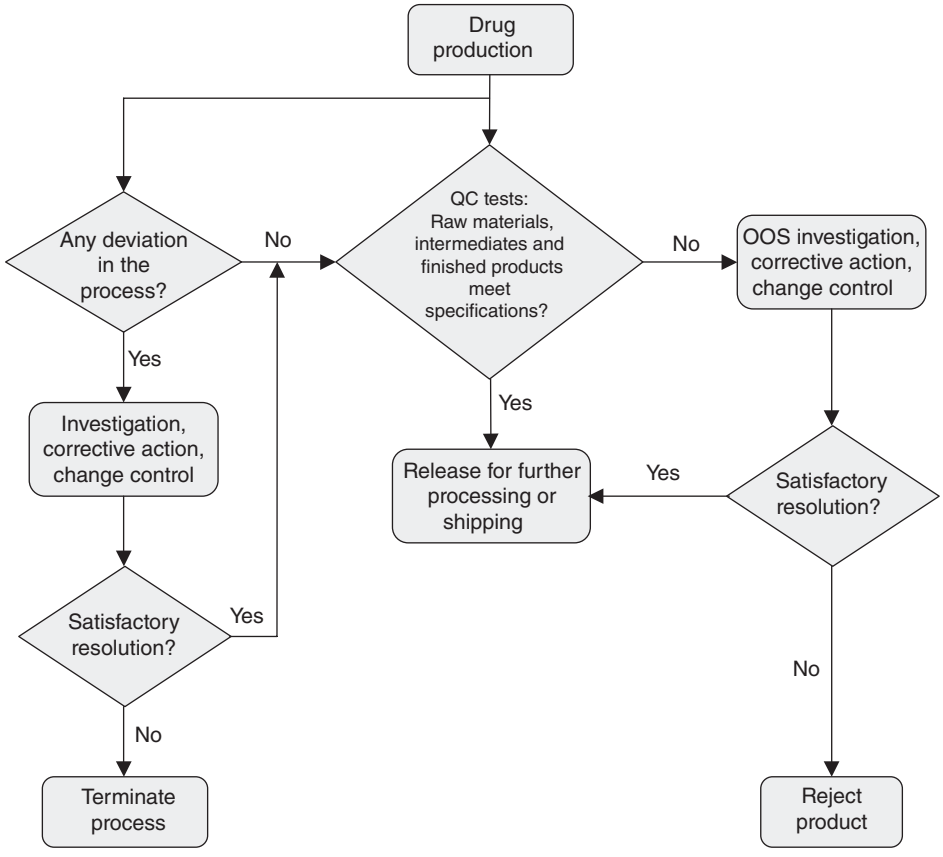



**Figure 9.2** Production and in-process control mechanism.

Laboratory controls commence with sampling and testing of incoming materials according to written procedures. Some aspects of the tests are identity, quantity, purity, activity, heterogeneity, stability, sterility, and safety. Intermediates and finished products are tested in accordance with preset specifications. Out of specification (OOS) results are investigated (refer to Section 9.6.12). OOS may be because of analyst or operator error, inappropriate method, production problems, or an inherent problem with the samples. It may also be necessary to trace the origins of materials: for example, in the case of raw materials for use in medium formulation for growing cells in protein drug production – if animal or plant components are used – the locations and herd or plantation where these components are obtained have to be recorded.

Test methods used in the laboratory are generally derived from pharmacopoeias such as the *US Pharmacopoeia*, *British Pharmacopoeia* or *European Pharmacopoeia*. For test methods that are not from recognized pharmacopoeias, validation of the analytical methods is required. The validation includes testing for accuracy, specificity, ruggedness, robustness, precision, detection limit, quantitation limit, and range. A discussion of analytical methods validation is presented in Section 9.7.5.

Samples are retained for possible future evaluation; normally, they are retained for 1 year after the expiry date of the batch or 3 years after distribution of the production batch, whichever is longer. Drug stability governs the effective date and storage conditions of the drug. Programs to evaluate stability of drug are an integral part of tests. Details for stability programs are discussed in Section 9.7.7.

### 9.6.11 Validation

In 2011, FDA updated its definition for Process Validation (*Guidance for Industry – Process Validation*: *General Principles and Practices*, January 2011) as:

> "The collection and evaluation of data, from the process design stage throughout production, which establishes scientific evidence that a process is capable of consistently delivering quality products."

The emphasis is the collection of data throughout the product life cycle of the drug and to ensure that quality is consistent. This concept of managing product life cycle quality is linked closely to the ICH documents Q8, Q9, Q10, and Q11 (refer to Section 9.9). There are three stages to process validation:

- Stage 1 – Process Design: The commercial manufacturing process is defined during this stage on the basis of knowledge gained through development and scale-up activities.
- Stage 2 – Process Qualification: During this stage, the process design is evaluated to determine if the process is capable of reproducible commercial manufacturing.
- Stage 3 – Continued Process Verification: Ongoing assurance is gained during routine production that the process remains in a state of control.

Table 9.4 shows the various activities classified by FDA as typical examples for each stage of the process validation. There is no explicit description in the guidance for the installation, operational, and performance qualification (IQ, OQ, PQ) of equipment, systems, and facilities. A pharmaceutical company, however, has to adopt a proactive policy of validation for its facilities, production processes, production equipment and support systems, analytical methods, and computerized systems. IQ, OQ, and PQ are prerequisites to a successful process validation program. A mindset of validated approach will help to assure drug product quality, optimize the processes, and reduce manufacturing cost.

The approach to validation commences with the Validation policy and then the development of Validation Master Plan (VMP), which details:

- Validation policy
- Organization of validation activities
- Personnel responsibilities
- Facilities, systems, equipment, and processes to be validated
- Documentation structure and formats

**TABLE 9.4 Stages of Process Validation**

| Stage | Intent | Typical Activities |
|---|---|---|
| Process Design | The commercial manufacturing process is defined during this stage on the basis of knowledge gained through development and scale-up activities | A combination of product and process design (Quality by Design) Product development activities Experiments to determine process parameters, variability, and necessary controls Risk assessments Other activities required to define the commercial process Design of Experiment testing |
| Process Qualification | During this stage, the process design is evaluated to determine if the process is capable of reproducible commercial manufacturing | Facility design Equipment and utilities qualification Process performance qualification Strong emphasis on the use of statistical analysis of process data to understand process consistency and performance |
| Continued Process Verification | Ongoing assurance is gained during routine production that the process remains in a state of control | Implement procedure for data collection from every batch Data trending and statistical analysis Product review Equipment and facility maintenance Calibration Management review and production staff feedback Improvement initiatives through process experience |

*Source*: Food and Drug Administration 2011, *Guidance for Industry – Process Validation: General Principles and Practices*, viewed November 7, 2014, http://www.fda.gov/downloads/drugs/guidancecomplianceregulatory information/guidances/ucm070336.pdf.

- Change control processes
- Planning and scheduling.

Following implementation of the VMP, the various progressive stages of validation activities are as defined below:

- *User Requirement Specification* (*URS*): Comprehensive description of the requirement and performance of the facilities, systems, equipment, or processes that have to be met to produce a drug product of the intended quality
- *Design Qualification* (*DQ*): Verification and documentation to demonstrate that the design of the facilities, systems, or equipment meets the requirements of the URS and GMP (refer to Exhibit 9.10 for the scope of DQ)
- *Installation Qualification* (*IQ*): Verification and documentation to demonstrate that the facilities, systems, or equipment as installed or modified, comply with the approved design and that all the manufacturer's recommendations have been duly considered (refer to Exhibit 9.10 for the scope of IQ)

- *Operational Qualification* (*OQ*): Verification and documentation to demonstrate that the facilities, systems, or equipment perform as intended throughout the anticipated operating ranges (refer to Exhibit 9.10 for the scope of OQ)
- *Performance Qualification* (*PQ*): Verification and documentation to demonstrate that the facilities, systems, or equipment and ancillary systems, when connected together, can perform effectively and reproducibly on the basis of the approved process method and specifications (refer to Exhibit 9.10 for the scope of PQ).

**Exhibit 9.10 Scopes of DQ, IQ, OQ, and PQ**

The following sets out the scopes generally expected to be performed for DQ, IQ, OQ, and PQ.

**DQ:**

- Verify
  - Design meets URS
  - Design complies with VMP
  - Software meets GAMP
  - Utilities are available and validated
  - Support documents are specific and available
  - System/equipment/instrument can be calibrated
  - System/equipment/instrument can be maintained
  - There is training for operating staff
  - System/equipment/instrument is safe to both product and personnel
  - System/equipment/instrument conforms to applicable standards.

**IQ:**

- Verify
  - Purchase documents (e.g. purchase order, invoice)
  - Model number and serial number
  - Utilities connected as specified
- Check
  - Manufacturer and supplier
  - Physical damage
- Confirm location and installation requirements per recommendation of manufacturers
- Installation shall be conducted per the instructions provided in the manual
- Ensure that all relevant documentation is received:
  - User manual
  - Maintenance manual

- Calibration certificate of sensors/measuring instrument
- Software manual
- Parts list
- Electrical drawings
- Mechanical drawings.

**OQ:**

- Verify
  - Alarm control and interlocks
  - All switches and push buttons function properly
- Perform calibration requirements identified in the manual or established by the validation team
- Operate the equipment at different parameter settings, for example, low, medium, and high speed/time/temperature per operations manual to verify the operating control
- Check software operations
- Establish procedures for operation, maintenance, and calibration
- Establish training program for relevant staff.

**PQ:**

- Check specific performance of system/equipment, for example,vial washing machine
- Perform visual inspection
- Check pH, conductivity, TOC of rinse water to WFI specifications
- Endotoxin
  - Unwashed vials: less than 10 EU/mL rinse water
  - Washed vials: less than 0.25 EU/mL rinse water
- Particulates
  - Positive and negative controls
- Spiked with about 500 particles of 40 μm glass beads
  - Rinse samples greater than 95% reduction
- Soiled samples
  - Use of positive and negative controls
  - Spiked with 10% sodium chloride or media/materials likely to encounter
  - After washing, rinse samples should have no traces of soiled materials.

DQ is performed by the supplier of the equipment or system at the supplier's factory as part of factory acceptance test (FAT). A Requirement Traceability Matrix is generated as part of the DQ. This ensures that all the requirements of the equipment are

recorded and tested in IQ, OQ, and PQ. IQ is based on the site acceptance test (SAT), OQ and PQ are performed on-site at the GMP facility. For a GMP manufacturing facility, the validation activities are facility design, HVAC system, environment control, laboratory and production equipment, water system, gases and utilities, cleaning, analytical methods, sterilization, and critical production processes. Validation protocols (IQ, OQ and PQ) are prepared for each item, listing all critical steps and acceptance criteria. Deviations are reviewed and resolved before the validation activity proceeds to the next stage.

After satisfactory execution of DQ, IQ, OQ, and PQ, process validation (PV) can be performed to verify the process can be consistently and accurately replicated and that the outcome is a drug product of the requisite quality. Further discussion on PV is presented in Section 9.7.4.

### 9.6.12 Corrective and Preventive Action (CAPA)

Deviations and OOS are discussed in Sections 9.6.8 and 9.6.10. Ideally, according to GMP regulations, all batches of pharmaceutical substance and product should be produced and tested exactly as per approved procedures and methods. In reality, the unexpected can always occur during the manufacturing and testing of drug substances and drug products. Approved written procedures must be in place to handle these deviations and OOS to ensure they are properly and carefully controlled to minimize the negative impact on the overall quality of the drug product.

Examples of deviations and discrepancies are:

- Yield percentage outside allowable limits
- Process equipment failures
- Facility/utility control failures
- Production process failures
- Label reconciliation variances.

Examples of OOS test results are:

- In-process test results
- Finished product test results
- Stability test results.

GMP implementation includes a system to handle:

- Corrective actions resulting from the investigation of OOS, deviations, product rejections, complaints, recalls, audits, regulatory inspections, and findings
- Preventive actions resulting from trending of process performance and product quality monitoring.

A systematic approach would need to be implemented to the investigation process with the objective to determine root cause such that corrective steps can be

implemented via the Change Control System. Corrective and preventive action (CAPA) methodology would result in product and process improvements and enhanced product and process understanding. Appropriate handling of deviation, discrepancy, and OOS would result in:

- A correction for the deviation, for example, rework, reprocess, resample, retest
- Corrective actions to rectify the root cause of the nonconformance, for example, retrain, process change.

There are a number of established methods for performing root cause analysis. These are failure mode and effects analysis (FMEA), Pareto principles, and Ishikawa diagram. Exhibit 9.11 presents an example of root cause analysis using the Ishikawa diagram.

**Exhibit 9.11 Root Cause Analysis**

Ishikawa (cause and effect/fishbone) diagram is a technique to determine the root cause of a problem. It displays all the possible causes of a target problem (cause) in a diagrammatic fashion and presents an overview of the problem systematically. Normally, there are six factors called 6Ms to consider:

- Manpower
- Methods
- Machines
- Materials
- Measurements
- Milieu (environment)

A series of questions are then asked on each "M" to probe the likely cause of the problem.

**Manpower:**

- Document interpreted properly?
- Information circulated to concerned personnel?
- Recipients understand the information?
- Operator properly trained?
- Too much judgment to perform activities?
- Guidelines for judgment available?
- Environment influences the activities?
- Distractions in the workplace?
- Fatigue a mitigating factor?
- Work efficiency acceptable?

- Operator responsible/accountable?
- Experience required for performing the activities?

**Methods:**

- Process capability established?
- Instructions clearly written?
- Instructions to latest version?
- Process under control?
- Mistake-proofing devices/techniques deployed?
- Tooling/machine properly designed and controlled?
- Process/design changed?
- Devices/tools/equipment properly labeled?
- Handling/packaging properly specified?

**Machines:**

- Correct tools used?
- Equipment affected by environment?
- Equipment calibrated, validated, and maintained?
- Software/hardware to latest version?
- Equipment properly programmed?
- Equipment used within its limits?
- Equipment controls within specifications?
- The machine right for the activity?

**Materials:**

- All needed information available and accurate?
- Information changed/updated?
- MSDS available?
- Materials properly tested?
- Materials substituted?
- Supplier's process defined and controlled?
- Materials right for the activity?
- Materials contaminated?
- Materials handled properly?

**Measurements:**

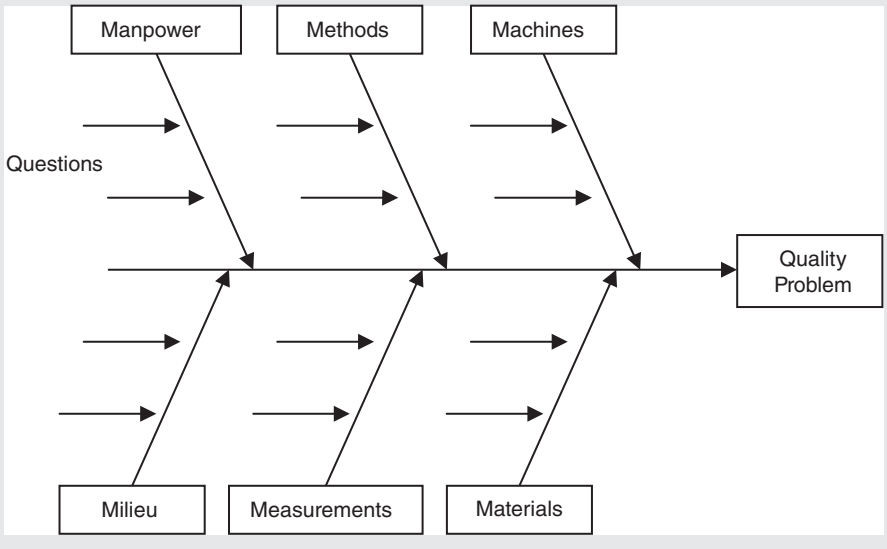
- Tools/gauge calibrated?
- Correct tools/gauge?

- Measurements vary between operators?
- Tools/gauge difficult to use?
- Tools/gauge with adequate resolution?
- Environment influences measurements?

**Milieu (environment):**

- Process affected by environment? temperature? relative humidity?
- Process affected by vibration, noise, lighting, UV, and so on?
- Process requires contamination control?
- Process affected by flow?
- Process affected by pressure?

Diagrammatically this is represented in the form of a fish bone with the 6Ms and the probing questions to isolate the underlying cause.




### 9.6.13 Change Controls

To reinforce the CAPA discussed in the previous section, a robust GMP system includes procedures to handle, review, and approve changes in raw materials, specifications, analytical methods, facilities, equipment, processes, computer software, and labeling and packaging. All the changes have to be documented with references for traceability.

Proposed changes have to be reviewed with reference to risk assessment and approved before being implemented. There may be justification to retest or revalidate

the affected system, equipment or process to ensure that quality of the drug product is not compromised. It is necessary to perform ongoing monitoring of changes for a time period and assess the long-term impact of the changes to ensure control system is put in place. In addition to corporate approval, approved products generally require regulatory approval by the overseeing regulatory authority (e.g. FDA) before implementation of major or critical changes.

### 9.6.14 Complaints and Recalls

GMP system includes the implementation of procedures to record and investigate oral and written complaints. The complaints records are periodically reviewed for trends and severity. Complaint records include:

- Name and address of complainant
- Name and phone number of person submitting complaint
- Nature of complaint
- Date complaint received
- Action initially taken
- Follow-up action
- Response provided to originator of complaint
- Final decision on the complaint.

For serious or life-threatening situation, regulatory authorities are to be informed and procedure for recall may need to be initiated. Recalled products should be identified and stored separately. Companies can set up "mock recalls" periodically to test the efficiency of their recall system.

### 9.6.15 Self-Inspection

The conduct of self-inspection is necessary to ensure adherence to GMP and detect and rectify any real or potential deviations. The frequency of self-inspections is not specifically defined in regulations but ideally should take place at least once per year. The scope of self-inspection should encompass:

- All major and important suppliers
- Personnel
- Premises, including personnel facilities
- Building and equipment maintenance
- Starting material and finished product storage
- Equipment
- Production and in-process controls
- Quality control
- Documentation

- Sanitation and hygiene
- Validation and revalidation program
- Calibration of instruments or measured systems
- Recall procedures
- Complaint management
- Label control
- Previous self-inspection results and any corrective actions taken.

## 9.7 SELECTED GMP SYSTEMS

In this section, we describe selected systems to illustrate the implementation of GMP concepts for these systems.

### 9.7.1 Water System

Two grades of water are used in drug manufacture: Purified Water (PW) and Water for Injection (WFI). In general, oral dosage drugs are prepared using PW, and parenteral injection drugs using WFI. Figure 9.3 illustrates a typical water system for generating pharmaceutical PW and WFI.

Incoming potable water (drinkable water) normally contains undissolved particulate matter and dissolved organic and inorganic compounds, as well as microorganisms. Several stages of treatment and purification are needed to produce PW and WFI.

Multimedia filters, which consist of a top layer of coarse and low-density anthracite, layers of silicas, and then dense finest media vitreous silicate, remove about 98% of particulates greater than 20 μm. These filters are regularly backwashed to avoid build-up of particulates. Finer filters (5–10 μm) are used to remove suspended matter and colloidal materials. To prevent scaling because of water hardness, sodium ions generated from brine are exchanged with calcium and magnesium ions in the water. Activated carbon or metabisulfite is used to remove chlorine.

In some cases, reverse osmosis is applied, and this removes almost all the particulates and organic materials, as well as microorganisms and endotoxins. Electrodeionization, which combines ion exchange membranes and resins, removes the last traces of dissolved ions from water under influence of a direct electric current. The last stage of the purification is ozone sterilization of water to inactivate residual microorganisms, as ozone is an efficient disinfectant (UV at 254 nm wavelength is then used to break up the spent ozone; ozone is a strong oxidant and can cause damage to mucus and respiratory tissues). The PW generated is then circulated to each point where it is used (point of use: POU).

To obtain WFI, the PW is distilled via several stills. Similarly, WFI is circulated to the POUs.

Several important points should be noted:

- PW and WFI are never stagnant; they are recirculated at 1–3 m/s to prevent growth of microorganisms

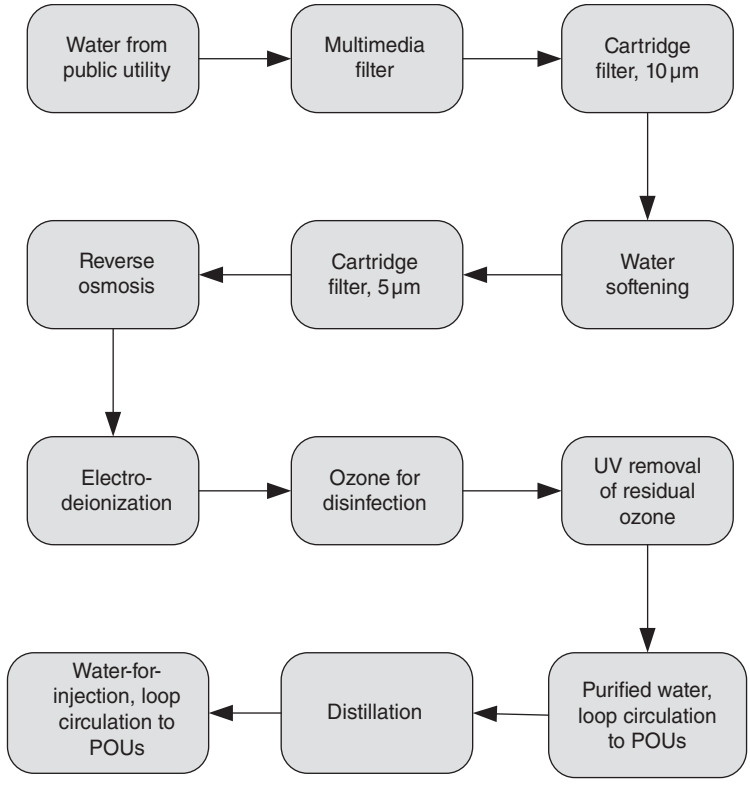



**Figure 9.3** Generation of purified water and water for injection.

- Pipeworks are designed to be self-draining with a slope of at least 1% over its length to prevent accumulation of water
- Spray balls are used to continuously wash the PW and WFI storage tanks
- Plastics such as polypropylene and acrylonitrile butadiene styrene are used for the construction of pretreatment tanks and pipeworks
- After purification, 316L grade stainless steel is used. This is resistant to corrosion and is electropolished or passivated to reduce roughness, which may act as sites for bacterial growth and future corrosion
- There should be no deadlegs, that is, areas where water may stagnate. The length of pipes without continuous flow of water should be less than six times the internal diameter of the pipe
- WFI is circulated hot at about 80 °C as hot water is self-sanitizing
- Cool loops can be sanitized using heat or ozone.

The quality of PW and WFI is constantly monitored. FDA has provided guidance for the validation of water systems. The validation program consists of three phases. Phases

**TABLE 9.5 The *US Pharmacopoeia* Specifications for Purified Water and Water for Injection**

| | Purified Water | Water for Injection |
|---|---|---|
| pH | 5.0–7.0 | 5.0–7.0 |
| Conductivity ($\mu$S/cm) | ≤1.1 at 20 °C | ≤1.1 at 20 °C |
| Total organic carbon (parts per billion) | <500 | <500 |
| Microbial | <100 cfu/mL | <10 cfu/100 mL |
| Endotoxin (EU/mL) | — | <0.25 |

cfu = Colony forming unit

1 and 2 are for 2–4 weeks each of continuous sampling and testing of water to establish the effectiveness of the pretreatment and purification and distillation processes. Phase 3 is routine monitoring of the water quality over the remainder of a 1-year period to gauge the influences of seasonal conditions on the water quality.

The specifications for PW and WFI according to the *US Pharmacopoeia* are given in Table 9.5.

### 9.7.2 Cleaning and Cleaning Validation

Cleaning of product contact surfaces such as reaction and storage vessels is an important aspect of pharmaceutical manufacturing. Residual materials are contaminants and may provide fertile grounds for microorganisms to grow. This is especially the case for biopharmaceutical production, because the soiled materials are normally protein-based, and, unlike synthetic drugs, biopharmaceutical drugs are not generally subjected to terminal sterilization.

There are several approaches to cleaning. The favored approach is clean-in-place (CIP), in which cleaning solutions are piped to the vessel under computer control. In cases where CIP is not suitable, clean-out-of-place (COP) is used. This approach is mostly for smaller items. COP may be carried out manually or with automated tanks. A third approach is manual cleaning, although this is prone to human error and is not generally adopted.

Different types of cleaning solutions are used. They are acids, bases, and detergents (Table 9.6).

The effectiveness of cleaning needs to be validated. The types of cleaning agents, concentrations, cleaning cycle, and temperature have to be determined. This is achieved by performing IQ, OQ, and PQ for each piece of equipment that has product contact surfaces. After cleaning, final rinse water samples using PW or WFI are collected. Direct surface sampling using swabs can be used as well. The samples are analyzed for pH, conductivity, microorganism levels, endotoxin, total organic carbon (TOC), residual materials, and other appropriate tests to determine levels of contaminants carried over from previous batches and residuals left by cleaning agents.

Other systems and areas that require cleaning are chromatographic columns and surfaces in the facilities, especially cleanrooms. A rigorous cleaning program has to be implemented to minimize potential product contamination. This includes a limit being

**TABLE 9.6 Types of Cleaning Agents**

| Cleaning Agent | Concentration |
|---|---|
| Acetic acid | 100–200 ppm |
| Peracetic acid | 100–200 ppm |
| Phosphoric acid | 1,000–2,500 ppm |
| Sodium hydroxide | 1,500–7,500 ppm |
| Sodium hypochlorite | 25–50 ppm |
| Solubilizing detergents | According to manufacturer's directions |

*Source*: Vos, JR and O'Brien, RW 1999, 'Cleaning and validation of cleaning in biopharmaceutical processing: A survey' in Avis, KE, Wagner, CM and Wu, VL (eds.), *Biotechnology: Quality Assurance and Validation*, Drug manufacturing Technology Series, Volume 4, Interpharm Press, Inc., Buffalo Grove, IL. Reproduced with permission of Taylor & Francis Group LLC.

set for the maximum carryover of contaminants and validated by the validation process (Exhibit 9.12).

There is a trend in the use of disposable, or single use systems to obviate the need for cleaning, both in terms of contamination control and cost-saving. Another aspect is operator safety, which can be enhanced through reduction in contact with potentially hazardous chemicals or microorganisms. Set-up costs and installation time for disposable systems are cheaper and faster, hence leading to the adoption of such technologies. Disposables may range from polymer-based syringes to storage containers and bioreactors. In these instances it is necessary to demonstrate that the systems used do not leach out materials when in contact with the solutions or solvents to be stored or used. Controlled experiments showing realistic case studies are required to convince regulatory authorities that the disposables present no harmful leachables affecting the safety and efficacy of the drugs.

### 9.7.3 Computer System Validation (CSV)

The pervasiveness of computerized systems within the pharmaceutical manufacturing facilities requires that these systems be validated to prevent potential problems from unauthorized access, computer software "bugs," and incompatible interfaces between software and hardware. FDA regulations under 21 CFR Part 11 (*Guidance for Industry*: *Part 11*, *Electronic Records*; *Electronic Signatures – Scope and Application*, August 2003) spell out the regulatory requirements for electronic signatures and electronic records to ensure that they are trustworthy and reliable. Currently, FDA enforces a narrow interpretation of Part 11, which applies when electronic records are used in place of paper records. The scope for Part 11 covers the following areas:

- Limiting system access to authorized individuals
- Use of operational system checks
- Use of authority checks
- Use of device checks

**Exhibit 9.12 Maximum Allowable Carryover**

Equipment is cleaned after a production batch. The maximum allowable carryover (MACO or MAC) of materials from one production batch (Product A) to the next batch (Product B) is given by the formula:

$$\text{MACO} = (\text{TD} \times \text{BS})/(\text{SF} \times \text{LDD})$$

where TD = standard therapeutic dose of Product A, BS = batch size of Product B to be manufactured in the same equipment, SF = safety factor (normally 1,000 is used), and LDD = largest daily dose of Product B to be manufactured in the same equipment

If the therapeutic dose of Product A is 100 mg, batch size for Product B is 10 kg, largest daily dose of Product B is 800 mg, and the safety factor is 1,000, the MACO is:

$$\begin{aligned}\text{MACO} &= (100 \text{ mg} \times 10,000,000 \text{ mg})/(1,000 \times 800 \text{ mg}) \\ &= 1,250 \text{ mg}\end{aligned}$$

For swab samples, the amount allowable is (MACO/Total Surface Area) × Swab Area.

For rinse samples, the amount allowable is (MACO/Total Volume) × Rinse Volume.

The recommended safety factors are: topicals (10–100), oral products (100–1,000), and parenterals (1,000–10,000).

Source: Data from Active Pharmaceutical Ingredients Committee 2000, *Guidance on aspects of cleaning validation in active pharmaceutical ingredient plants*, viewed May 20, 2014, http://apic.cefic.org/pub/pub-cleaning-validation.pdf.

- Determination that persons who develop, maintain, or use electronic systems have the education, training, and experience to perform their assigned tasks
- Establishment of and adherence to written policies that hold individuals accountable for actions initiated under their electronic signatures
- Appropriate controls over systems documentation
- Controls for open systems corresponding to controls for closed systems bulleted above
- Requirements related to electronic signatures.

Part 11 does not apply when computers are used for producing printouts and the regulated activities are based on a paper system. For systems that predate August 1997, FDA applies discretion in its enforcement, although these systems must comply with

predicate rules effective at the time. The details of Part 11 approach is presented in Exhibit 9.13.

**Exhibit 9.13 21 CFR Part 11 Electronic Records; Electronic Signatures – Scope and Application**

This regulation is far reaching and contains explicit requirements for computerized systems validation. It "applies to electronic records and electronic signatures that persons create, modify, maintain, archive or transmit … " As such, it requires persons to "employ procedures and controls designed to ensure the authenticity, integrity, and, when appropriate, the confidentiality of electronic records, and to ensure that the signer cannot readily repudiate the signed record as not genuine."

Examples of some selected requirements are:

*Section 11.10*(*a*): Validation of systems to ensure accuracy, reliability, consistent intended performance, and the ability to discern invalid or altered records.

*Section 11.10*(*b*): The ability to generate accurate and complete copies of records in both human readable and electronic form suitable for inspection, reviews, and copying by the agency (FDA).

*Section 11.10*(*d*): Limiting system access to authorized individuals.

*Section 11.10*(*e*): Use secured, computer generated, time-stamped, audit trails.

*Section 11.50*: Signed electronic records shall contain information associated with the signing that clearly indicates the printed name of the signer, the date and time of signing, and what the signature means.

*Overall Approach to Part 11 according to "Guidance for Industry Part 11, Electronic Records; Electronic Signatures – Scope and Application"*

The approach is based on three main elements:

- Part 11 will be interpreted narrowly
- For those records that remain subject to Part 11, enforcement discretion will be exercised with regard to Part 11 requirements for validation, audit trails, record retention, and record copying in the manner described in the guidance and with regard to all Part 11 requirements for systems that were operational before the effective date of Part 11 (also known as legacy systems)
- FDA will enforce all predicate rule requirements, including predicate rule record and recordkeeping requirements (Predicate rules are preexisting regulatory requirements such as GLP, GMP, and GCP guidelines).

FDA intends to enforce provisions related to the following controls and requirements:

- Limiting system access to authorized individuals
- Use of operational system checks

- Use of authority checks
- Use of device checks
- Determination that persons who develop, maintain, or use electronic systems have the education, training, and experience to perform their assigned tasks
- Establishment of and adherence to written policies that hold individuals accountable for actions initiated under their electronic signatures
- Appropriate controls over systems documentation
- Controls for open systems corresponding to controls for closed systems bulleted above
- Requirements related to electronic signatures.

*Source*: Data from Food and Drug Administration 2003, *Guidance for Industry: Part 11, Electronic Records; Electronic Signatures – Scope and Application*, viewed May 13, 2014, http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm072322.pdf.

Another industry guide for development and testing of computerized systems is the Good Automated Manufacturing Practice (GAMP) by the International Society for Pharmaceutical Engineering. This document sets out the various lifecycle stages for software systems design, testing, and validation (Figure 9.4).

User requirement specifications (URS) for the computerized system are provided by the pharmaceutical firm to the computer systems vendor. The vendor generates functional and design specifications as a basis for designing and coding software for the computerized system. The system is then built, together with all the interfaces to the

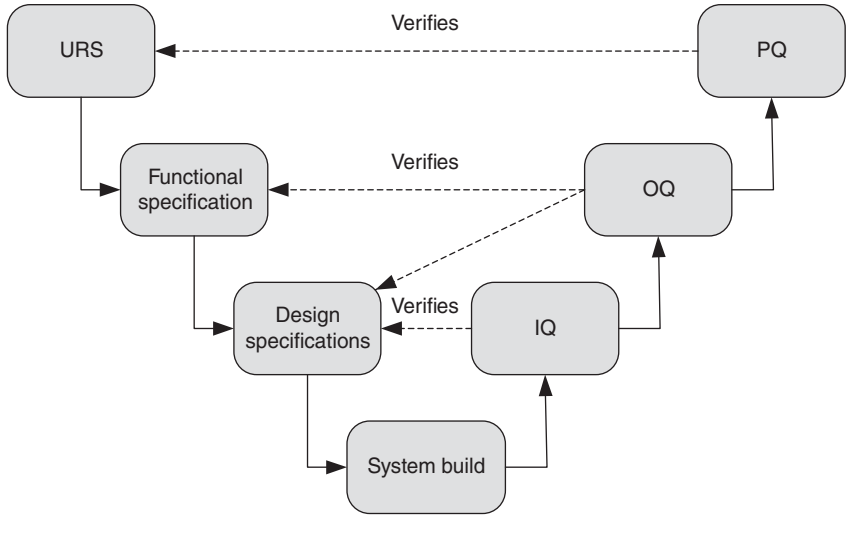



**Figure 9.4** Framework for specification and qualification of computerized systems.

hardware, and tested by the vendor. After installation of the computerized system, IQ, OQ, and PQ are performed at the pharmaceutical facility to verify that the system is able to meet the URS, design, and functional specifications.

Computer validation is to establish documented evidence to show that the computerized system will consistently function and meet its predetermined specification and quality attributes with a high degree of assurance. Some of the parameters tested in the validation process are:

IQ: Proper installation of computerized (hardware/software) system
- Correct software version
- All parts are present and connected correctly
- Software virus check
- Integrity of hard disk
- Availability of source code, manuals

OQ: Start-up routine
- Calibration routine
- Data transfer/backup
- Data integrity
- Power failure
- Auto lock off
- Human–machine interface
- Security access/audit trail
- System Stress test in event of power failure
- Alarm tests
- Operator data entry tests

PQ: System compatibility tests under operational conditions at defined limits.

In accordance with GAMP, there are different validation strategies depending on the categories of software, the degree of validation increases from general system to custom system (refer to Exhibit 9.14). Another important element of GAMP is traceability of URS functionality to lines of codes in the computer software. Establishment of this traceability would enable future modification and update of computer software simpler and more manageable.

### 9.7.4 Process Validation (PV)

Processes for the manufacture of drugs must be validated to ensure they consistently produce drugs of the desired specifications (refer to Section 9.6.11). Even in a seemingly straightforward production of a small molecule drug, there are generally many processing steps to achieve the active ingredient. The difficulty of producing proteins and antibodies in biological systems escalates even further, necessitating the control of multiparameters to manage the growth of cells and their expressions of the desired products.

**Exhibit 9.14 Categories of Software**

*Category 1*: *Operating systems*

These are established commercially available operating systems. They are not subject to specific validation; their features are functionally tested and challenged indirectly during testing of the application. Name and version number are documented and verified during IQ.

*Category 2*: *Firmware*

Instrumentation and controllers often incorporate firmware. The name, version, and any configuration and calibration for the firmware should be documented and verified during IQ, and functionality tested during OQ.

*Category 3*: *Standard software packages*

These are commercial, "off-the-shelf" software packages. The package is not configured, and process parameters may be input into the application. The name and version should be documented and verified during IQ. Compliance to URS should be tested during OQ. Supplier documentation should be assessed and used.

*Category 4*: *Configurable software packages*

These software packages can be configured according to user requirements. A supplier audit is usually required to confirm software has been developed according to documented quality system. Validation should ensure software meets URS requirements. Full life cycle validation is needed.

*Category 5*: *Custom* (*bespoke*) *software*

These software packages are developed to meet specific requirements of the user. A supplier audit is usually required to confirm the software has been developed according to a documented quality system. Validation should ensure the software meets URS requirements. Full life cycle validation is needed.

*Source*: Data from International Society for Pharmaceutical Engineering 2008, *GAMP 5: A Risk-Based Approach to Compliant GxP Computerized Systems*, ISPE, Tampa, FL.

Process validation (PV) entails firstly the definition of both the critical and noncritical parameters. Critical parameters are those that affect the safety, potency, efficacy, or quality of the drug. These parameters are derived from research and development scale-up, or experiences gained from manufacturing of other drug products. In the situation that these parameters are new and there is no prior information available, quality tools such as Design of Experiment (DOE, refer to Exhibit 9.15) should be devised and performed to obtain the data. Once they are defined, emphasis can be directed to designing a program to validate these parameters. Some established steps involve the evaluation of process consistency and yield over at least several batches, and comparing these with predetermined specifications (FDA emphasized evaluation over three batches previously; but the latest process validation guideline does not define the number of batches and instead focuses on life cycle approach). Input parameters that may

**Exhibit 9.15 Design of Experiment**

Design of Experiment (DOE) is a structured, methodical experiment to gather information concerning a process to optimize its performance or yield. DOE enables interactions of contributing factors to be collected and analyzed using statistical tools, such as ANOVA, and bypasses the conventional methods that vary each factor one at a time to determine its effects.

DOE designs can be divided into fully factorial and fractional factorial. Fully factorial combines levels of each factor with all levels for every other factor. Fractional factorial excludes some of these possible combinations by holding certain factor constant.

A DOE example of a process with three factors is presented. The experiment is performed with two levels, high and low, for each factor. This is a $2^3$ experiment, that is, eight possible combinations. Levels are represented by high (1) and low (−1) and the full factorial design matrix is as shown below:

| Exp No. | Factor 1 | Factor 2 | Factor 3 |
|---|---|---|---|
| 1 | −1 | −1 | −1 |
| 2 | −1 | −1 | 1 |
| 3 | −1 | 1 | −1 |
| 4 | −1 | 1 | 1 |
| 5 | 1 | −1 | −1 |
| 6 | 1 | −1 | 1 |
| 7 | 1 | 1 | −1 |
| 8 | 1 | 1 | 1 |

For example, in the granulation process for tablet manufacture, the factors to be studied may be binder, water, and granulation time. The DOE is conducted with binder level (Factor 1) high (2%) and low (1%), water level (Factor 2) high (45%) and low (25%), and granulation time (Factor 3) high (6 min) and low (3 (minutes). The yields from these experiments are obtained and analyzed using statistical ANOVA. From the results it is then possible to determine the major factors as well as interacting factors that can affect process performance and yield.

be considered as critical are temperature, flow rate, stirring speed, and they are varied and checked against output variables such as yield, purity, crystallization rate. Essentially, the process validation activity is to provide the basis for a robust manufacturing practice.

The prerequisites for performing the PV are:

- Planned process
- Critical parameters and their limits or ranges

- Validated equipment
- Validated computerized systems
- Approved materials
- Proposed in-process controls
- Specifications for intermediates and finished product
- Validated or compendial analytical methods
- Proposed standard operating procedures
- Proposed batch records
- Approved PV protocol.

The three stages discussed in Section 9.6.11 are applied to the PV. A life cycle approach in accordance with continued process verification is a practice that should be imbedded in GMP. Similarly, the EMA document, *Guideline on process validation for finished products – information and data to be provided in regulatory submissions*, February 2014, emphasizes continued process verification and design space verification (refer to Section 9.9).

### 9.7.5 Analytical Methods Validation

Generally, GMP manufacturers use compendial methods from the *US Pharmacopoeia*, *British Pharmacopoeia* or *European Pharmacopoeia* as much as possible for analyses and testing purposes, as these methods have been validated and accepted by regulatory authorities. However, manufacturers are expected to demonstrate that the compendial methods are suitable for the conditions under which the tests are performed.

There are occasions where new analytical methods have to be developed specifically for testing raw materials, intermediates, and finished products that are not covered by compendial methods. In these situations, the analytical methods are required to undergo a validation process to ensure they are suitable. One or more of the following parameters as defined in Exhibit 9.16 must be validated for newly developed analytical methods:

**Exhibit 9.16 Analytical Methods Validation**

*Specificity*: ability to assess unequivocally the analyte in the presence of components that may be expected to be present.

*Accuracy*: expresses the closeness of agreement between the value that is acceptable, either as a conventional time value or an acceptable reference value and the value found.

*Precision*: expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions.

*Repeatability*: expresses the precision under the same operating conditions over a short interval of time.

*Limit of detection*: the lowest amount of analyte that can be detected in a sample.

*Limit of quantitation*: the lowest amount of analyte that can be quantitatively determined in a sample with suitable precision and accuracy.

*Linearity*: ability to obtain test results that are proportional to the concentration of analyte in the sample.

*Range*: the interval between the upper and lower concentration (amounts) of analyte in the sample for which it has an acceptable degree of precision, accuracy, and linearity.

*Ruggedness*: interval between upper and lower concentration of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy, and linearity.

*Robustness*: a measurement of its capacity to remain unaffected by small, but deliberate, variations in method parameters; provides an indication of its reliability during normal use.

Source: Data from International Conference for Harmonization 2005, *Validation of Analytical Procedures: Text and Methodology*, Q2, viewed May 19, 2014, http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf.

The table below shows the recommended validation requirements for the analytical methods used to characterize the drug molecules:

*Source*: Adapted from Schniepp, S, Taylor, M, Loffredo, D and Vasinko, J 2007, *Method Validation: An Overview of Global Standards*, PDA Letter, XLIII, p.1, pp. 20–24 and p. 28

| Validation Parameter \ Analytical Method | Identification | Impurities (Quantitation) | Impurities (Limit) | Assay |
|---|---|---|---|---|
| Specificity | + | + | + | + |
| Accuracy | – | + | - | + |
| Precision: Repeatability | – | + | – | + |
| Intermediate precision | | | | |
| Reproducibility | | | | |
| Limit of detection | – | – | + | – |
| Limit of quantitation | – | + | – | – |
| Linearity | – | + | – | + |
| Range | – | + | – | + |
| Robustness | – | + | – | + |

Note: "+" means evaluation needed, "–"means evaluation not needed.

- Specificity
- Accuracy
- Precision
- Repeatability
- Limit of detection
- Limit of quantitation
- Linearity
- Ruggedness
- Robustness.

A rationale should be generated to explain and support the reasoning for validating the selected parameters. The use of reference standards during validation helps to reinforce the reliability of the analytical method developed. The conditions of how a test is performed may have a strong influence on the results. These conditions have to be recorded and followed.

### 9.7.6 Sterilization Processes

Parenteral drug products are required to be sterile. There are principally five different ways to sterilize a product. These are steam, dry heat, radiation, gas, and filtration. Selection of which method to use is based on the product that requires sterilization. For example, protein-based drugs are heat-sensitive, so the normal means for sterilizing these products is filtration. The rationale for sterilization validation is to show the reduction in microbial load or destruction of biological indicators.

Steam under pressure at 15 psig (103.4 kPa), 121 °C, is a very effective sterilant. Bacterial spores that are resistant to dry heat are killed by steam sterilization. The mechanism is thought to be that the steam causes denaturation of proteins and amino acids within the bacterial cells. An autoclave is a steam sterilization equipment. It is validated taking into account the loading pattern of items in the autoclave chamber, heat distribution and penetration, and the sterilization cycle used. Often, biological indicators such as *Bacillus stearothermophilus* (also known as *Geobacillus stearothermophilus*) and *Clostridium sporogenes* are used to challenge the effectiveness of sterilization.

Dry heat is used to sterilize and depyrogenate components and drug products. The definition of dry heat sterilization is 170 °C for at least two hours and depyrogenation cycle at 250 °C for more than 30 min. Typical equipment includes tunnel sterilizers (force convection, infrared, flame) and microwave sterilizers. An important aspect is the need to ensure air supply is filtered through HEPA filters. Biological indicators such as *Bacillus subtilis* can be used to gauge the performance of sterilization.

Radiation generates high-energy photons, which penetrate microorganisms and cause death through ionization. Commercial radiation sterilization employs gamma-ray radioisotopes such as cobalt-60 and cesium-137. The radiation dose is around $10^3$ to $4 \times 10^5$ Gy. Radiation may cause degradation in drug products and its effects have to be considered.

Ethylene oxide and hydrogen peroxide are the typical gases for gas sterilization. Their advantage is that they can be used at much lower temperature than steam sterilization: 27–60 °C for ethylene oxide and 25–40 °C for hydrogen peroxide. Another advantage is that they do not cause damage to the product or the packaging.

For protein-based drugs, filtration via a 0.2 μm filter is an effective way to achieve sterilization. Factors that determine the filtration efficiency are integrity of filter, pressure, temperature, flow rate, contact time of material with filter, pH, and viscosity. Validation of filters should include chemical compatibility of filter with the product and possibility of contaminant from the filters leaching into the product.

The effectiveness of sterilization can be established by culturing samples of the filtrate in a growth medium. Fluid thioglycolate medium and soybean-casein digest medium are normally used. Incubation is 7–14 days at 30–35 °C for fluid thioglycolate medium and 7–14 days at 20–25 °C for soybean-casein digest medium. The absence of microorganism colonies at the end of the growth cycle is an indication of sterility.

Several mathematical functions are used as indicators of microbial destruction. These are *D*, *Z,* and *F* values:

- *D value*: The time in minutes required for a 90% reduction of a specific microbial population under specified lethal conditions (1 log reduction).
- *Z value*: The number of degrees of temperature change necessary to change the *D* value by a factor of 10.
- *F value*: It is a measure of sterilization effectiveness. It is the number of equivalent minutes of steam sterilization at temperature 121.1°C delivered to a product calculated using a *Z* value of 10 °C.

In mathematical terms:

$$D = t/(\log\ N_o - \log\ N_t)$$

where

$t$ = Time at base temperature, for example, 121.1 °C
$N_o$ = Number of initial microorganism population
$N_t$ = Number of microorganism after time $t$

$$Z = T_1 - T_2/(\log\ D_2 - \log\ D_1)$$

where

$T$ = Temperature

Both the *D* and *Z* values are further illustrated in Figure 9.5. The *F* value can be derived as below:

$$\text{Sterilization lethality rate} = 10^{(T-Tb)/Z}$$

$$F_o \text{ or } F_H = \Delta t \times \Sigma \text{ lethality rate (at different temperatures)}$$

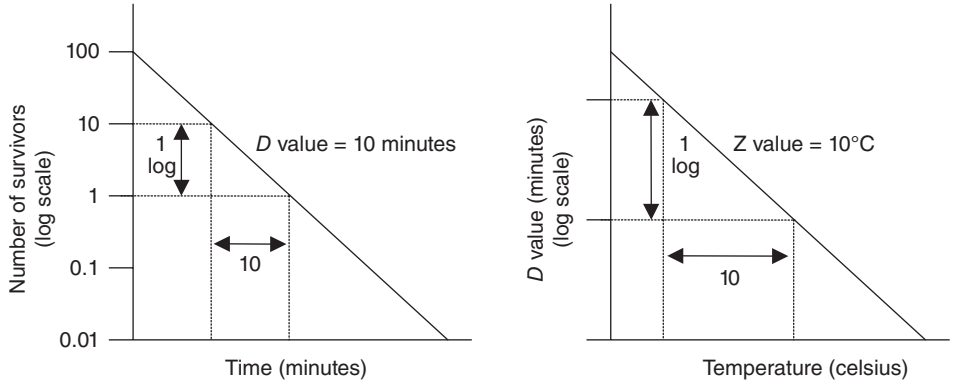



**Figure 9.5** Curves showing $D$ and $Z$ values.

where

$F_o$ = Moist heat (Steam sterilization)
$F_H$ = Dry heat
$\Delta t$ = Cycle time
$T$ = Actual sterilization cycle temperature
$T_b$ = Base temperature: 121.1 °C for $F_o$, 170 °C for $F_H$
$Z$ = Microbial death rate constant: 10 °C for $F_o$, 20 °C for $F_H$

### 9.7.7 Stability Evaluation

The quality of a drug changes over time under the influence of temperature, humidity, and light. It is a requirement that drug products have to be stable during transportation and storage over their projected shelf life. The ICH *Harmonized Tripartite Guideline*: *Stability Testing of New Drug Substances and Products*, Q1A(R2), February 2003, sets out the guidelines for testing the stabilities of new drug substances. It should be noted that the test conditions simulating Climate Zones III (hot and dry) and IV (hot and humid tropical) are considered as having the most adverse effects on drug products and as such are acceptable to other Climate Zones.

To evaluate the stability of a drug, stress testing is carried out to determine the effects of environmental conditions on the drug. For example, the effect of temperature is assessed over a range of temperatures in 10 °C intervals, humidity at conditions less than 75% relative humidity, oxidation and photolysis degradation processes, and hydrolysis of the drug at different pH levels. These evaluations are to assess any changes in the physical, chemical, biological, and microbiological properties of the drug in its container or packaging after it has been transported to various environmental conditions.

ICH has specified storage conditions to evaluate drug stability as part of the submission data for drug approval (Table 9.7).

**TABLE 9.7 Storage Conditions for Evaluating Drug Stability**

| Study | Storage Condition | Minimum Period Covered by Data at Submission |
|---|---|---|
| (*1*) *General case* | | |
| Long term | 25 °C ± 2 °C 60% RH ±5% RH | 12 months |
| Intermediate | 30 °C ±2 °C 65% RH ±5% RH | 6 months |
| Accelerated | 40 °C ± 2 °C 75% RH ±5% RH | 6 months |
| (*2*) *Drug substances intended for storage in a refrigerator* | | |
| Long term | 5 °C ±3 °C | 12 months |
| Accelerated | 25 °C ± 2 °C 60% RH ±5% RH | 6 months |
| (*3*) *Drug substances intended for storage in a freezer* | | |
| Long term | –20 °C ±5 °C | 12 months |

*Source*: International Conference on Harmonization 2003, *Stability Testing of New Drugs Substances and Products, Q1*, viewed May 19, 2014, http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q1A_R2/Step4/Q1A_R2__Guideline.pdf. Reproduced with permission of ICH.

RH = Relative humidity.

## 9.8 NEW cGMP INITIATIVES

In August 2002, FDA announced a new initiative to cGMP. The initiative is called *Pharmaceutical cGMPs for the 21st Century*: *A Risk-Based Approach*. It is intended to modernize FDA's regulation of pharmaceutical quality for veterinary and human drugs and selected human biological products such as vaccines. The goals were to:

- Encourage the early adoption of new technological advances by the pharmaceutical industry
- Facilitate industry application of modern quality management techniques, including implementation of quality systems approaches, to all aspects of pharmaceutical production and quality assurance
- Encourage implementation of risk-based approaches that focus on both industry and agency attention on critical areas
- Ensure that regulatory review, compliance, and inspection policies are based on state-of-the-art pharmaceutical science
- Enhance the consistency and coordination of FDA's drug quality regulatory programs, in part, by further integrating enhanced quality systems approaches into the Agency's business processes and regulatory policies concerning review and inspection activities.

This initiative has set forth a direction for FDA to restructure its oversight of pharmaceutical quality regulation. There were five guiding principles in the initiative:

- Risk-based orientation
- Science-based policies and standards

- Integrated quality systems orientation
- International cooperation
- Strong public health protection.

The risk-based approach merges science-based policies and standards with an integrated quality system. This is to ensure that FDA's resources are directed to address those areas that are considered to have higher risks; for example, companies with previous compliance problems, new companies with unknown history, and processes requiring aseptic procedures.

A result of the initiative is the publication of the document *Guidance for Industry – Quality Systems Approach to Pharmaceutical CGMP Regulations*, September 2006, which sets out "how implementing comprehensive quality systems can help manufacturers achieve compliance with 21 CFR Parts 210 and 211". Several key concepts that are critical to modern quality systems are:

- Quality – Every pharmaceutical product should have established identity, strength, purity, and other quality characteristics designed to ensure the required levels of safety and effectiveness
- Quality by Design (QbD) and Product Development – "QbD means designing and developing a product and associated manufacturing processes that will be used during product development to ensure that the product consistently attains a predefined quality at the end of the manufacturing process" (refer to Section 9.9: ICH Q8)
- Quality Risk Management – "Quality risk management helps guide the setting of specifications and process parameters for drug manufacturing, assess and mitigate the risk of changing a process or specification, and determine the extent of discrepancy investigations and corrective actions" (refer to Section 9.9: ICH Q9)
- Corrective and Preventive Action (CAPA) – This allows for remedial corrections to identify problems through systematic root cause analysis and implementation of preventive action (refer to Section 9.6.12)
- Change Control – This is to "manage change to prevent unintended consequences" (refer to Section 9.6.13)
- The Quality Unit – Establishment of a Quality Unit (QU) with "the authority to create, monitor, and implement a quality system" (refer to Section 9.6.2)
- Six-system Inspection Model – A systems-based approach to inspection by FDA. The six systems are: Quality System, Production System, Facilities and Equipment Systems, Laboratory Controls Systems, Materials Systems and Packaging, and Labeling System (refer to Section 10.3).

Other areas where the risk-based approach has been implemented are:

- 21 CFR Part 11, *Electronic Records*; *Electronic Signatures—Scope and Application*, August, 2003, which stipulates the use of risk-based approach to managing computer systems

- *Guidance for Industry – Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice*, September 2004, which details the requirements for aseptic processing for biological products
- *Guidance for Industry – Oversight of Clinical Investigations – A Risk-Based Approach to Monitoring*, August 2013, where risk-based approach is applied to clinical studies.

FDA also introduces the concept of Process Analytical Technology (PAT). PAT refers to systems that are used to analyze, monitor, and control manufacturing processes on a continuous basis. The quality attributes and specifications of raw materials, in-process intermediates, and processes are measured in real time and compared with predetermined parameters so that deviations can be rectified in a proactive nature to assure that the end products conform to the level of quality as expected. It is believed that a system based on PAT being implemented in a real time manner would improve manufacturing efficiency and simultaneously retain or improve the product quality through these interactive measurements and controls.

Some examples of PAT are chemical, physical, microbiological, mathematical, and risk analysis. FDA suggested the following tools, which may be used to manage PAT:

- Multivariate data acquisition and analysis tools
- Modern process analyzers or process analytical chemistry tools
- Process and endpoint monitoring and control tools
- Continuous improvement and knowledge management tools.

A diagrammatic representation of using PAT and Laboratory Information Management System (LIMS) controls for the manufacturing of pharmaceuticals/biopharmaceuticals is presented in Figure 9.6.

## 9.9 CASE STUDY #9.1

### 9.9.1 ICH Quality Documents

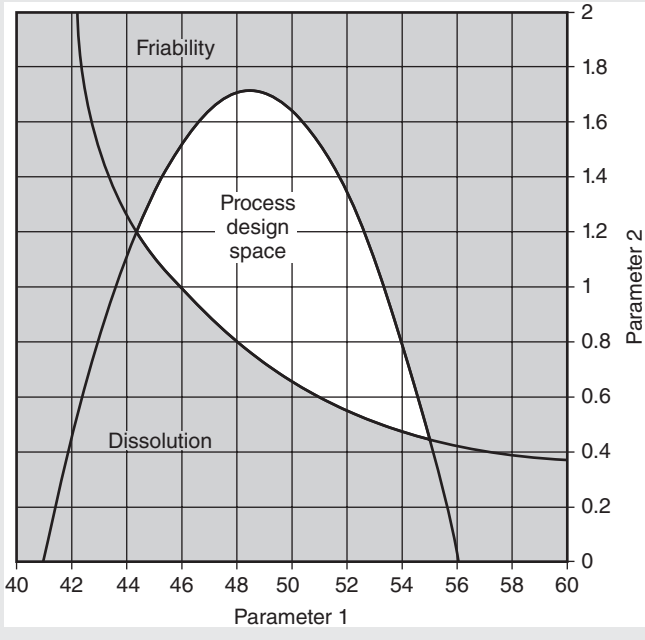
This case study presents the following ICH documents and reviews their impact to GMP:

- Q8 – *Pharmaceutical Development*
- Q9 – *Quality Risk Management*
- Q10 – *Pharmaceutical Quality System*
- Q11 – *Development and Manufacture of Drug Substances*.

*Q8* introduces the concept of design space (Exhibit 9.17) in the development of pharmaceuticals. Design space is defined as:

**Exhibit 9.17 Design Space**

A 2D design space is presented. There are two variables, Parameter 1 and Parameter 2, that can affect the dissolution time and friability of a tablet. The diagram below shows that only certain combinations of Parameter 1 and Parameter 2 would result in acceptable properties for dissolution and friability (the design space – white area). Experiments such as DOE (Exhibit 9.15) can be conducted to determine the design space.




*Source*: International Conference on Harmonization 2009, *Pharmaceutical Development, Q8*, viewed May 20, 2014, http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q8_R1/Step4/Q8_R2_Guideline.pdf. Reproduced with permission of ICH.

> The multidimensional combination and interaction of input variables (e.g., material attributes) and process parameters that have been demonstrated to provide assurance of quality.

Pharmaceutical companies are encouraged to gather information and knowledge from development studies and manufacturing experience to provide scientific understanding to support the establishment of design space. The specifications and controls for the variables in the design space would enable consistent delivery of quality drug

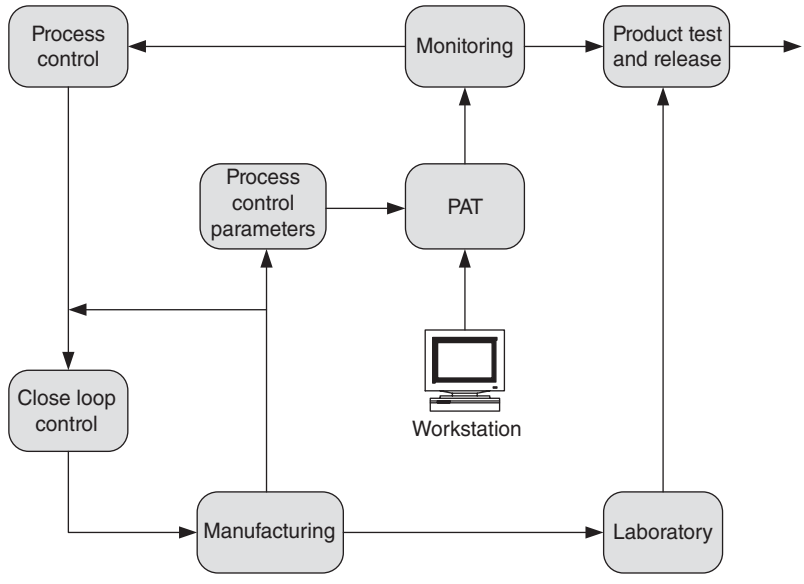



**Figure 9.6** Controls in manufacturing process. *Note*: Manufacturing includes controls on Building Management System (BMS) and Environment Management System (EMS).

product. In this way quality is built in by design (Quality by Design – QbD). Other elements in pharmaceutical development recommended for studies are:

- Quality Target Product Profile (QTPP) – characteristics such as dosage form, dosage strength, container closure system, dissolution, sterility, purity
- Critical Quality Attributes (CQAs) – the physical, chemical, biological, and microbiological properties of the drug substance and excipients, which can impact the drug product quality.

Further elaboration of these items is as below:

- Drug Substance – the physicochemical and biological properties of the drug substance that can influence the performance and manufacturability of the drug product; these properties include solubility, water content, particle size, crystal properties, biological activity, and permeability
- Excipients – the type of excipients, their characteristics, concentration, and compatibility can all influence the drug product performance
- Drug Product Formulation Development – the properties of the drug substance and its interactions with excipients, container closure system, and any relevant dosing device should be studied and the correlation between *in vitro*/*in vivo* studies should be established

- Overages – use of an overage of a drug substance to compensate for degradation during manufacture or a product's shelf life is discouraged, but if used, must be justified with consideration of safety and efficacy
- Physicochemical and Biological Properties – there should be appropriate studies conducted where these properties are relevant to the safety, performance, and manufacturability of the drug product.

*Q9* presents a systematic approach to quality risk management. It describes the principles and provides examples of tools for quality risk management, which can be applied to different aspects of pharmaceutical quality, from development to manufacturing and distribution throughout the life cycle of the drug substance, excipients, and drug product. The quality risk management process entails the identification, assessment, control, and communication of risks that can impact on the quality of the drug product. A model for the quality risk management process is given in Figure 9.7. Risk management tools are used to help support a science-based and practical decision process that is integrated into the quality system. Some of these risk management tools are:

- Basic risk management facilitation methods, for example flow charts, check sheets
- Failure Mode and Effects Analysis (FMEA) (refer to Exhibit 9.18)
- Failure Mode, Effects, and Criticality Analysis (FMECA)
- Fault Tree Analysis (FTA)
- Hazard Analysis and Critical Control Points (HACCP)
- Hazard Operability Analysis (HAZOP)
- Preliminary Hazard Analysis (PHA)
- Risk ranking and filtering
- Supporting statistical tools.

*Q10* describes specific quality system elements and management responsibilities to form a harmonized model for a pharmaceutical quality system throughout the life cycle of a drug product and links together with Q7 and the separate GMP requirements in different regions. Q10s objectives are to:

- Achieve Product Realization – to establish, implement, and maintain a system for the delivery of quality product
- Establish and Maintain a State of Control – to develop and use effective monitoring and control systems for process performance and product quality, and assure capability of processes (refer to Cp in Section 9.10)
- Facilitate Continual Improvement – to identify and implement appropriate process and product quality improvements
- Enablers: Knowledge Management and Quality Risk Management – use of process and product knowledge and quality risk management for continual improvement of process performance and product quality
- Design and Content Considerations – the design, organization, and documentation of a clear and well-structured pharmaceutical quality system is necessary

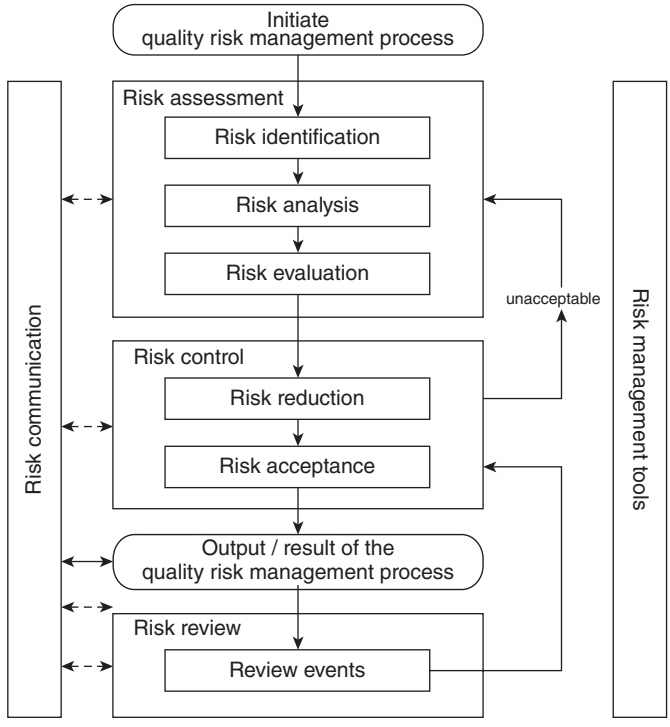



**Figure 9.7** Quality risk management process. (*Source*: International Conference on Harmonization 2005, *Quality Risk Management, Q9*, viewed May 19, 2014, http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q9/Step4/Q9_Guideline.pdf. Reproduced with permission of ICH.)

- Quality Manual – to establish a Quality Manual stating the quality policy, scope of the quality system, identification of quality system processes and linkages, and management responsibilities within the quality system.

*Q11* describes the approaches to developing and understanding the manufacturing process of drug substance. The critical quality attributes (CQAs) should be within certain limit, range, or distribution to ensure a desired drug product quality. The development of the manufacturing process should include identification of CQAs, definition of appropriate manufacturing process, and control strategy to ensure process performance and drug substance quality.

All these documents – Q8, Q9, Q10, and Q11 – integrate together a quality system on the basis of design space methodology using the concept of QbD, application of quality risk management process, defined management responsibilities, and CQAs of drug substance and excipients to enable a quality drug product to be manufactured consistently. By implementing these quality principles, the GMP system would comply with regulatory requirements. Such a GMP system is founded on science-based principles through systematic development and studies with risk-based orientation and would

**Exhibit 9.18 Failure Mode and Effects Analysis**

Failure Mode and Effects Analysis (FMEA) is a quantitative technique for evaluating a risk or failure. It identifies the types of risk/failure modes on the basis of past experience or common risk/failure mechanism logic. The risk/failure is analyzed by ranking the severity, probability, and detectability. A Risk Priority Number is calculated by multiplying the severity, probability, and detectability. An example of a FMEA worksheet is given below:

| Process Step | Potential Failure Mode | Potential Failure Effect | S E V | Potential Causes | P R O | Current Controls | D E T | P R N |
|---|---|---|---|---|---|---|---|---|
| What is the process step? | In what way could the process step go wrong? | What is the severity of the failure? | | What causes the failure? What probability does the failure occur? | | What are the existing controls? How difficult is it to detect the failure? | | |

where SEV = Severity, PRO = Probability, DET = Detectability, RPN = Risk Priority Number (SEV × PRO × DET)

Each of the factors is assigned a value on the basis of its effect:

- Severity
  - 10 – Exceed specification limit, reject because of potential to compromise patient safety
  - 7 – Exceed other nonspecification limits, investigate
  - 4 – Observed trend with no limit excursion, investigate
  - 1 – No limit excursion, no investigation
- Probability
  - 10 – Expected to occur greater than 50% of the time
  - 7 – Expected to occur greater than 10% of the time
  - 4 – Expected to occur greater than 1% of the time
  - 1 – Expected to occur less than 1% of the time
- Detectability
  - 10 – Cannot be detected
  - 7 – Not detectable until current process step is completed
  - 4 – Can be detected during current process step
  - 1 – Can be detected before the processing step

A calculated PRN of more than $4 \times 4 \times 4 = 64$ would require FMEA actions to eliminate the risk/failure mode by minimizing the severity, reduce probability, and/or improve detection.

meet the goals set forth in the FDA initiative: *Pharmaceutical cGMPs for the 21st Century*: *A Risk-Based Approach*.

*Source*: (1) International Conference on Harmonization 2009, *Pharmaceutical Development*, *Q8*(*R2*), viewed May 16, 2014, http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q8_R1/Step4/Q8_R2_Guideline.pdf, (2) International Conference on Harmonization 2005, *Quality Risk Management*, *Q9*, viewed May 16, 2014, http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q9/Step4/Q9_Guideline.pdf, (3) International Conference on Harmonization 2008, *Pharmaceutical Quality System*, *Q10*, viewed May 16, 2014, http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q10/Step4/Q10_Guideline.pdf, (4) International Conference on Harmonization 2012, *Development and Manufacture of Drug Substances (Chemical Entities and Biotechnological/Biological Entities)*, *Q11*, viewed May 16, 2014, http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q11/Q11_Step_4.pdf

## 9.10 CASE STUDY #9.2

### 9.10.1 Six Sigma and Lean Manufacturing

Implementing all the elements of quality element discussed in this chapter would ensure a GMP system that complies with regulatory requirement. However, efficiency and production yield can be improved further through the implementation of quality tools and techniques such as Six Sigma and Lean Manufacturing. This case study presents the concepts for Six Sigma and Lean Manufacturing, which can be adopted to improve productivity and reduce wastes in the manufacture of drug products.

#### *Six Sigma*

Six Sigma was developed by Motorola in 1986 and later adopted by General Electric in 1995. It was reputed that Six Sigma saved General Electric billions of dollars in production costs following improvements in product quality.

Six Sigma is a quality program that focuses on control of a process to $\pm 6\sigma$ (standard deviations) from its mean specification, that is, a process with an envelop of 12 standard deviations from lower specification level to higher specification level. This translates to an expected defects (rejects) of 3.4 items per million (a yield of 99.99966% it should be noted that these numbers are calculated using a correction factor of $1.5\sigma$ for long-term process drift) achieved in the semiconductor industry. In contrast, current pharmaceutical manufacturing practices are estimated to be operating at between 2 and $3\sigma$, with a yield of 69.2–93.3%. The Six Sigma concept is to identify critical quality attributes in a process and then control the process by reducing variations, improving process capabilities, increasing stability, and designing systems to sustain the improvements.

There are two categories of variations: common cause and special cause. Common cause variations are random variations in a process. It measures how well a process can be controlled. Special cause variations are nonrandom and have assignable causes. The approach is to use long-term strategy to manage common cause, but special cause variations would require immediate action to resolve.

Variations to a process can be calculated through the use of Process Capability Index (Cp). Cp is a measure of the ability of the process to produce outputs that meet the

specifications:

$$Cp = (USL–LSL)/6\sigma$$

where

USL = Upper specification limit
LSL = Lower specification limit
$\sigma$ = Standard deviation

when

$Cp < 1$ means the process is unacceptable
$Cp = 1$ means the process is acceptable
$Cp > 1$ means the process is desirable.

Six Sigma implementation commences with the formation of a team to investigate and control the variability in processes. The team would consist of:

- Champions – responsible for promoting the Six Sigma methodology throughout the company
- Master Black Belts – responsible for teaching executive managers and champions the basics of Six Sigma and help to select the right people for the Six Sigma Project; also mentors Black Belts and Green Belts
- Black Belts – responsible for leading Six Sigma project teams full time
- Green Belts – responsible for helping Black Belts in their functional area, working part time on project.

The Six Sigma method to control process variability is the Define-Measure-Analyze-Improve-Control (DMAIC) model:

- Define – to determine the objectives and scope of the project to control process variability
- Measure – to identify one or more products, map the process, assess process performance, estimate baseline capability, and quantify problems
- Analyze – to evaluate and reduce the variables and determine the root causes
- Improve – to discover variable relationships, establish operating tolerances, and validate measurements
- Control – to determine the ability to control the vital few factors and implement process control systems.

The outcome is a control plan which includes:

- Training plan – training process owner and operators; use of control charts; understanding and using implemented documentation; knowing the response plan and able to use it if necessary

- Documentation plan – new process step/s are integrated into normal process and systems, procedures, policies, are modified to sustain improvement
- Monitoring plan – team must document and monitor the process using metrics defined, evaluate solution, assess capability of process over time and establish control systems for long-term solution
- Response plan – establish checkpoints to signal out-of-control conditions and actions to undertake
- Institutionalization plan – align systems and structures, develop standards and procedures, and communicate to all stakeholders.

### *Lean Manufacturing*

Lean Manufacturing is a manufacturing quality practice that:

- Focuses on quantity control to reduce cost by eliminating waste
- Builds on strong foundation of process and product quality
- Integrates fully within organization
- Evolves continuously
- Perpetuates a strong culture that is managed consciously, continuously, and consistently.

Lean Manufacturing was developed by Taiichi Ohno of Toyota and on the basis of the earlier concepts of Henry Ford. Although Lean Manufacturing originated from the automotive industry, the underlying principles to improve efficiency and productivity are applicable to pharmaceutical manufacturing.

The focus of Lean Manufacturing is on reducing waste (*muda* in Japanese) and improving flow.

There are seven wastes:

- Transportation – This is the moving of products that are not required to perform the processing. Each time a product is moved it stands the risk of being damaged, lost, delayed, and so on as well as being a cost for no added value. Transportation does not make any transformation to the product that the consumer is willing to pay for.
- Inventory – Inventory encompasses all components, work in progress (WIP), and finished product not being processed. These represent a capital outlay that has not yet produced an income either by the producer or for the consumer. Any of these three items not being actively processed to add value is a waste.
- Motion - People or equipment moving or walking more than is required to perform the processing is categorized as motion. It also refers to the damage that the production process inflicts on the entity that creates the product, either over time (wear and tear for equipment and repetitive stress injuries for workers) or during discrete events (accidents that damage equipment and/or injuries to workers).

- Waiting – Waiting for the next production step. Whenever goods are not in transport or being processed, they are waiting. In traditional processes, a large part of a product's life is spent waiting to be worked on.
- Overproduction – Production ahead of demand. One common practice that leads to this waste is the production of large batches, as often consumer's needs change over the long timeframe that large batches require to complete. Overproduction is considered the worst waste because it hides and/or generates all the others. Overproduction leads to excess inventory, which then requires the expenditure of resources for storage space, and preservation, activities that do not benefit the customer.
- Overprocessing – Over-processing results from poor tool or product design and creates needless activity. It also includes more work being done on a product than what is required by the customer, and using tools that are more precise, complex, or expensive than absolutely required.
- Defects - The effort involved in inspecting and fixing defects. Extra costs are also incurred reworking the part/equipment, rescheduling production, and so on.

To improve production flow, the concept of Just-in-time (JIT) is introduced. JIT means delivery of the right quantity to the right place at the right time. This can be achieved through:

- Balanced Operations - Synchronization of production steps to avoid the waste of waiting; standardized operations to reduce variation in processes
- Pull - Manufacture with a limit on the maximum inventory; production is based on replenishment; minimize overproduction; use of a *Kanban* system – a scheduling system that helps determine what to produce, when to produce, and how much to *produce*
- Minimum Lot Size – Minimum lot size means reduce lead times; process can proceed faster leading to first piece through the process faster for quicker detection of defects and rectification of problems
- Flow – Parts and subassemblies do not stop except to be processed; minimum buffer stock between work stations
- Lead time reduction – This results in reduced inventories, reduced exposure to environmental factors, damage, obsolescence, and enable more precise planning
- Leveling – Leveling avoids unnecessary changes in production rates
- Cells – Work areas can be arranged so that processing steps are immediately adjacent to one another and reduces transportation and inventory wastes
- Single Minute Exchange of Dies (SMED) – SMED is designed to reduce changeover times by focusing on top bottleneck with longest time for changeover
- Stock – Only hold enough inventory to protect sales
- *Kanban* – A scheduling system, usually a card that contains information about part name, description, and quantity to signal replenishment in a "pull" system, where production is triggered by the removal of product and signals travel from product up the process chain to earlier processes

Lean Manufacturing uses techniques such as Spaghetti Diagram and Value Stream Mapping to improve flow. Both techniques are for analyzing and designing the flow of materials and information required to manufacture a product and include cycle times, inventories, changeover times, staffing, and modes of transportation.

The overall aim of Lean Manufacturing is to instill a culture of *Kaizen*, which is a Japanese word for continuous improvement. The cycle of *Kaizen* activity can be defined as:

- Standardize an operation and activities.
- Measure the standardized operation
- Gauge measurements against requirements
- Innovate to meet requirements and increase productivity
- Standardize the new, improved operations
- Continue the improvement cycle *ad infinitum*.

## 9.11 SUMMARY OF IMPORTANT POINTS

1. By regulations drugs are manufactured in accordance with Good Manufacturing Practice (GMP). The relevant codes in the United States for small and large molecule drugs are 21 CFR Parts 210 and 211; and 21 CFR Parts 600 and 610, respectively. For the EU, the requirements for GMP are stipulated in EU Directive 2003/94/EC.
2. With the formation of the International Conference on Harmonization (ICH) the GMP guidelines are presented in Q7. This document has been adopted by the United States, EU, and Japan.
3. The GMP guidelines adopted by the Pharmaceutical Inspection Cooperation Scheme (PIC/S) are:
   - *PIC/S GMP Guide Part I*: *Basic Requirements for Medicinal Products*
   - *PIC/S GMP Guide Part II*: *Basic Requirements for Active Pharmaceutical Ingredients*
   - *PIC/S Guide* (*Annexes*)

   These guidelines are equivalent to the EU and ICH guidelines and are used by the 44 participating countries around the globe.
4. The important elements for GMP are:
   - Quality system
   - Trained personnel
   - Suitable facilities
   - Appropriate equipment
   - Documentation and records
   - Materials management
   - Production and in-process controls
   - Identification and labeling
   - Laboratory controls

- Validation
- CAPA
- Change controls
- Complaints and recalls.

5. Some important GMP systems are:
   - Water system
   - Cleaning practices
   - Computer validation
   - Process validation
   - Test methods validation
   - Sterilization procedures
   - Stability evaluation.
6. The risk-based approach introduced by FDA is to focus pharmaceutical companies and regulatory authorities' resources into addressing potential risk areas on the basis of scientific approach. The aim is to ensure pharmaceutical companies adopt new scientific developments and equipment for manufacturing safe, effective, pure, and consistent products.
7. Six Sigma is a quality method to improve productivity and yield by controlling process variability. The Define-Measure-Analyse-Improve-Control (DMAIC) model is used.
8. Lean Maufacturing aims to improve production efficiency through the reduction of wastes and improvement in process flow.

## 9.12 REVIEW QUESTIONS

1. Using the PIC/S GMP Guides Parts I and II as reference, highlight the important elements of GMP.
2. Discuss the importance of Quality System, Trained Personnel, and Validation.
3. Explain the different grades of water in a pharmaceutical setting.
4. List and explain the important parameters for analytical test method validation.
5. How can contamination be controlled in a pharmaceutical plant?
6. Describe the life cycle approach to process validation.
7. Discuss the benefits or otherwise of Process Analytical Technology (PAT).
8. What are the benefits for the implementation of a risk-based approach in GMP manufacturing?

## 9.13 BRIEF ANSWERS AND EXPLANATIONS

1. Refer to Exhibit 9.5 and 9.6.
2. Quality system provides a global structure and coordination to implement manufacturing policies and procedures in methodical and controlled manner.

It also enables deviations and problems to be addressed in timely ways and recurrence reduced. Trained personnel are necessary to execute manufacturing and test activities by following established procedures and ensure compliance with GMP and that products are manufactured to specifications. Validation is conducted such that equipment, computers, processes, and analytical test methods work and function in the specified manner as intended.

3. Purified Water (PW) is used for cleaning and preparation of nonsterile drug compounds whereas Water for Injection (WFI) is used for final rinse and preparation of sterile materials. Refer to Section 9.7.1.
4. Refer to Exhibit 9.16.
5. Contamination is controlled at multilevels in a pharmaceutical plant. It starts with a clean, well-maintained facility. Raw materials are labeled, checked, and segregated at storage. Equipment are cleaned and tested for cleanliness prior to being used. Personnel are to follow procedures as written in SOPs. Samples and products are clearly labeled and stored. Flows of materials, equipment, personnel, tools, and wastes are controlled.
6. Refer to the three stages in Section 9.6.11 and Table 9.4.
7. PAT is used to analyze, monitor, and control manufacturing processes on a continuous real time basis. This process means predetermined characteristics are checked and deviations, if any, are corrected promptly such that product quality is maintained.
8. Refer to Section 9.8. Risk-Based Approach directs resources to address issues according to risk levels to assure safety and quality of the drug products being manufactured.

## 9.14 FURTHER READING

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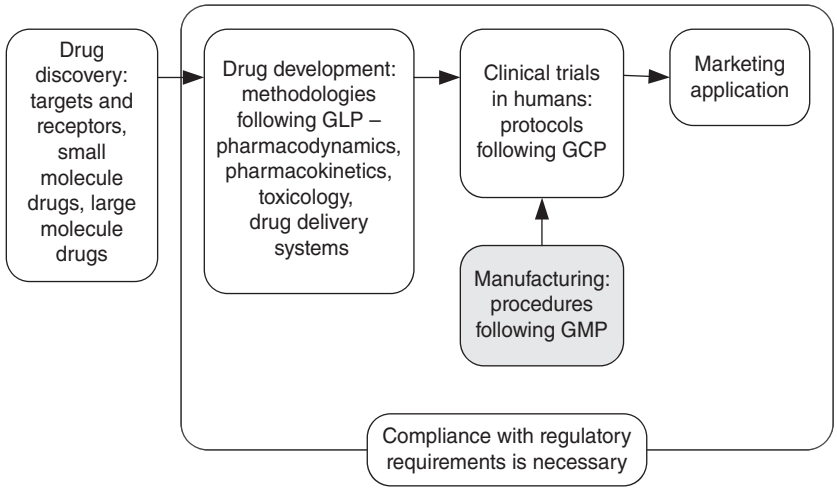
# CHAPTER 10

# GOOD MANUFACTURING PRACTICE: DRUG MANUFACTURING




## 10.1 INTRODUCTION

There are distinct differences in the manufacture of small and large molecule drugs; the former is mainly based on organic chemical synthesis, while the latter relies on biological systems or recombinant DNA (rDNA) technology. In this chapter, we describe the manufacturing processes for these drugs under GMP environments.

*Drugs: From Discovery to Approval*, Third Edition. Rick Ng.

Before the large scale manufacturing of drug under a GMP quality system is undertaken, there is a development process, which spans the discovery stage and the commercial production of the drug. From the discovery stage, lead compounds are identified. These lead compounds are tested in a number of stages, from laboratory *in vitro* assays to *in vivo* tests, pharmacology, toxicology, and finally into clinical trials as potential drug candidates. An increasing quantity of the drug material is needed as each stage progresses. The demand for material grows from milligrams to grams and kilograms. A development program is phased in to meet this demand by initially producing drugs on a laboratory scale. It then progresses to pilot plant scale to provide more drug material for clinical trials, and finally implements procedures, processes, equipment and setting up of a manufacturing plant for large-scale commercial production.

Broadly, the drug development program covers the following:

***General Items:***

*Raw Materials*: Raw materials have a significant impact on the manufacturing process. Issues such as availability and reliability of supply, reactivity, toxicity, handling, and storage have to be considered. Cost is another factor to take into account. Often, trade-offs between costs, manufacturing processes, and yields are considered.

*Safety*: Production of the requisite drug substance, called the active pharmaceutical ingredient (API) or bulk pharmaceutical chemical (BPC), may involve materials, solvents or intermediates that are volatile, toxic or even explosive. The development program has to determine the appropriate manufacturing processes to ensure that safety is not compromised and the API can be produced and purified to remove impurities and toxic residues. Formulations of finished product with excipients/additives need to ensure safety of the formulated product.

*Reproducibility of Manufacturing Processes*: The aim of GMP is to ensure the manufacture of safe, potent, pure, and effective drug in a consistent manner. The development program is used to evaluate procedures and processes that can be implemented in a large-scale manufacturing environment to ensure the drug product conforms to the intended safety, potency, purity, effectiveness and consistency on a routine basis.

*Environmental Factors*: In addition to conformance to GMP, the manufacturing plant has to comply with local environmental legislation. This may cover materials transportation, handling, storage, and disposal. The manufacturing plant is set up with systems for controlling gaseous emission, decontamination of solid waste and treatment of liquid discharge. All these factors are evaluated in the development program.

There are two major steps to manufacture a complete drug product. First is the preparation of the API. Then the next stage is to formulate the API with excipients and processed into a drug product of the specified finished dosage form (refer to Section 10.6) such as tablets and parenterals. These steps are different for the small and large molecule drugs.

**Exhibit 10.1 Fuzeon**

Fuzeon (enfuvirtide) is an anti-HIV drug. It interferes with the binding of HIV on the CD4 cell. It is a synthetic peptide with 36 amino acids, the N-terminus is acetylated while the C-terminal formed a carboxamide. The peptide binds to the gp41 subunit of the HIV envelop glycoprotein and prevents the HIV from fusing with CD4.

*Source*: Data from Food and Drug Administration 2011, *Fuzeon for Injection*, viewed May 25, 2014, http://www.accessdata.fda.gov/drugsatfda_docs/label/2011/021481s020lbl.pdf

### *Small Molecule Drugs:*

*Organic Chemistry Synthesis Route*: The production of API for small molecule drugs discussed in Chapter 3 requires ingenious and meticulous development of organic synthesis steps. Some drugs may require more than 50 steps to obtain the intended API. For example there are more than 100 production steps for the manufacture of Roche's AIDS drug enfuvirtide (Fuzeon), the first HIV fusion inhibitor, which was approved by FDA in March 2003 (Exhibit 10.1). In some cases, drug materials are directly isolated from natural products. In other cases natural product extraction constitutes the raw material or intermediate for production of the drug via semi-synthetic route (refer to Exhibit 3.4). Methods for chemical reactions, product purification, control parameters and analytical procedures are developed and they form the basis for implementing a GMP system to control the manufacturing processes. The Chemistry, Manufacturing and Control (CMC) information is required to be submitted to the regulatory agencies for marketing application approval.

### *Large Molecule Drugs:*

*Protein Synthesis Route*: Recent protein-based drugs are mainly produced using living systems of microbial, insect or mammalian cells. The rDNA technology development program commences with the selection of cell line and cloning methods for genes that express the intended protein molecule, the biologic API. Next is the experimentation to develop conducive environments for the cells to grow and divide to achieve high yields. This is followed by determination of effective purification procedures to extricate the protein API from cell debris and other components. Besides the rDNA production methods, there are other methods of obtaining the biologic API, including sourcing directly from animal or living things, for example, factor VIII for hemophilia A patients, which is obtained from blood plasma. Some traditional vaccines are obtained from egg-based systems (refer to Appendix 5). Another example is the anti-coagulant antithrombin, ATryn, which is obtained from the milk of goats that have been genetically modified (Exhibit 10.2). Irrespective of the source of the

**Exhibit 10.2 ATryn, Antithrombin (Recombinant)**

ATryn is a recombinant human antithrombin. It is a glycoprotein with 432 amino acids and a molecular weight of about 57.2 kDa. The antithrombin is produced by rDNA technology using genetically engineered goats. The antithrombin DNA coding sequence is inserted along with a mammary gland specific DNA sequence, which directs the expression of the antithrombin into the goat milk. The goats used are certified by USDA as scrapie-free and are controlled for specific pathogens.

ATryn is supplied in sterile, lyophilized powdered form.

*Source*: Data from Food and Drug Administration 2009, *ATryn, Antithrombin (Recombinant)*, viewed May 26, 2014, http://www.fda.gov/downloads/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/LicensedProductsBLAs/FractionatedPlasmaProducts/UCM134045.pdf

protein molecule, strict GMP compliance is necessary and CMC information for regulatory submission as for the small molecule drug is mandatory.

Drug development work also includes formulation, contamination control, stability studies and selection of drug delivery systems, as discussed in Section 5.6. Once the API has been prepared, the development progresses to the formulation stage where excipients are added:

- To modify processing properties for the manufacture of finished dosage forms (tablets, capsules, parenterals, etc.)
- As preservatives or buffers to ensure drug stability
- For efficient delivery of the drug to targets.

In API manufacture, whether via chemical synthesis, rDNA technology, or extraction from natural products, there are significant changes (both physical and chemical) from the starting materials to the API. In the formulation process, however, the quality and specifications of the API are retained. The addition of excipients to produce the drug product in a finished dosage form does not present physical or chemical changes to the API.

It should be noted that GMP regulations are necessary for the manufacture of approved drug products. Regulatory authorities such as FDA do not expect total GMP compliance for the manufacture of drugs designated for clinical trials. This is recognized, and ICH Q7 GMP Guidance Section 19, "APIs for Use in Clinical Trials", sets out the GMP expectations. It states "… controls used in the manufacture of APIs for use in clinical trials should be consistent with the stage of development of the drug product …" As a sponsor files for an Investigational New Drug (IND), the manufacturing information is presented in the CMC (Section 8.2.2). Initially, for Phases I and II clinical trials, regulatory authorities do not require total GMP compliance in the CMC. However, by Phase III, all quality systems, production processes and validation issues are expected to have been resolved to enable GMP manufacturing of drug products to be carried out routinely.

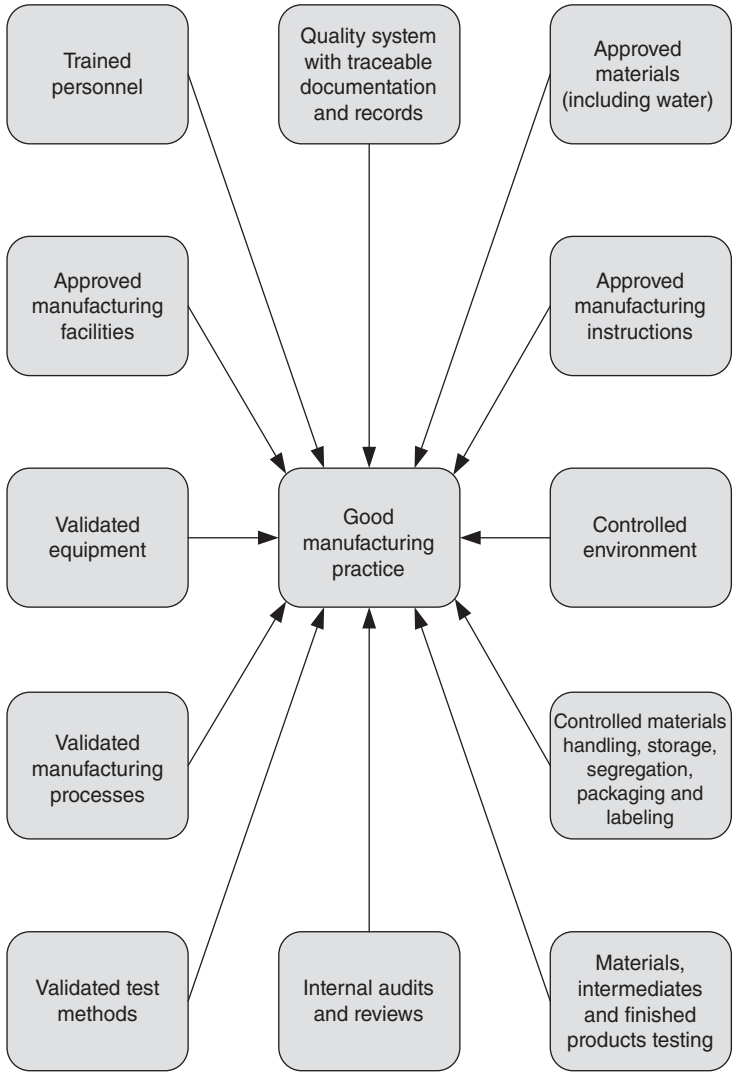



**Figure 10.1** Implementation of GMP in drug manufacture.

## 10.2 GMP MANUFACTURING

Manufacturing of drugs, whether the API or finished dosage form, must comply with GMP regulations as stated in Sections 9.2–9.5. Figure 10.1 shows the implementation of GMP concepts in drug manufacture.

The first requirement for GMP manufacture is the availability of trained personnel. Other requirements are:

- Raw materials that conform to specifications
- Water, in the form of Purified Water or Water-for-Injection, as required

- An environment with appropriate control for temperature, pressure, relative humidity. For aseptic production, cleanroom conditions monitored and controlled for particles and bioburden contamination are necessary
- Approved specifications for the intermediates and finished drug product
- Equipment that have been validated and maintained with current calibration
- Processes that have been developed and validated to ensure the production of pure and consistent product
- Operating procedures clearly written down, detailing each manufacturing step
- Approved batch records for registering all relevant information during the manufacturing process
- Quality records to document all tests pertaining to the raw materials, intermediates, and finished product
- Validated cleaning procedure to ensure reaction vessels and containers do not carry contaminants
- Validation of sterilization processes or aseptic processes, as applicable
- Satisfactory performance of the stability program.

All these items above must be in place before the manufacturing process begins. In the manufacturing process, deviations from specified conditions and processes may happen. For example, the pH for the reactions may be outside the range stated in the procedure or the reaction process may produce more heat leading to a greater temperature rise than is programmed. These deviations have to be resolved before the subsequent steps can proceed. Similarly, raw materials, intermediates and products that are outside specifications require out-of-specification (OOS) investigations. When resolved satisfactorily, the materials, intermediates, and products are released. Change controls and corrective actions are required when investigations show failure in controls that need rectification.

QC tests are carried out according to validated analytical methods or established methods from pharmacopeias: *US Pharmacopoeia* and *British Pharmacopoeia*. Exhibit 10.3 lists some of the QC analytical methods performed on drug intermediates and products.

## 10.3 GMP INSPECTION

Regulatory authorities inspect GMP facilities to ensure drugs are manufactured according to GMP requirements. The inspection programs used in most developed countries are similar. The following is an example of the FDA inspection program: *The Compliance Program Guidance Manual for FDA Staff*: *Drug Manufacturing Inspections Program 7356.002*, February 2002, states the strategy for inspection as:

- Evaluating through factory inspections, including the collection and analysis of associated samples, the conditions and practices under which drugs and drug products are manufactured, packed, tested and held, and

**Exhibit 10.3 Selected Analytical Methods**

*High Performance Liquid Chromatography* (*HPLC*): This is a separation method for characterizing or determining the purity of a drug material. The material is passed through a chromatographic column with solid matrix, which separates the material according to its physicochemical properties.

*Sodium Dodecyl Sulfate – Polyacrylamide Gel Electrophoresis* (*SDS-PAGE*): This method is used to separate proteins based on molecular weights. SDS is added to the proteins to produce a net negative charge. Under an electric field, the negatively charged proteins migrate to the anode. Smaller molecules migrate longer distances and are separated from larger molecules. Bromophenol blue is used as a color marker to show the progress of electrophoresis. Dithiothreitol (DTT) or mercaptoethanol is added to sample to disentangle disulfide linkages of proteins in a "reduced" condition.

*Isoelectric Focusing*: This is an electrophoretic method in which the proteins are separated based on their charge characteristics. This is accomplished by the proteins moving through a medium with a pH gradient. The protein stops at the point where the pH equals the protein's isoelectric point (pH where the protein has no net charge).

*Capillary Electrophoresis* (*CE*): The CE instrument consists of a source/sample vial, a destination vial and a small capillary filled with electrolyte joining the two vials. A voltage is applied and separates the sample according to size, affinity and charge, which is detected by UV absorbance.

*Quantitative Polymerase Chain Reaction* (*QPCR*): A method to determine the amount of DNA or RNA in a sample. Using the QPCR method, DNA can be amplified many times, allowing minute quantities to be assessed.

*Spectroscopy*: Drug compounds absorb visible, infrared and UV radiation at frequencies that are characteristic of the compounds. Quantitative measurements can be calculated from the absorbance readings at specific frequencies or wavelengths.

*Circular Dichroism*: This method is used to determine the enantiomers in racemic mixtures. The isomers rotate polarized light in different directions depending on their chiral characteristics.

*Atomic Spectroscopy*: This method is used to determine the concentration of an element in a drug substance. The intensity of the emission lines of the element measured at specific wavelengths shows its concentration.

*Mass Spectroscopy*: This is based on measurement of the ratio of mass to number of positive or negative charges of the substance to be analyzed. The pattern generated is characteristic of the drug substance. One method is the use of Matrix-Assisted Laser Desorption Ionization – Time of Flight (MALTI-TOF) mass spectroscopy. This gives partial sequences of peptide fragments. From these, the protein identity can be revealed through a database search.

*Limulus Amebocyte Lysate Test* (*LAL*): This test is used to detect the presence of endotoxins in the drug substance. It relies on the coagulation reaction between the endotoxin and the blood of a horseshoe crab.

*NMR* and *ELISA* methods are discussed in Chapters 3 and 4.

- Monitoring the quality of drugs and drug products through surveillance activities such as sampling and analyzing products in distribution.

FDA carries out inspections once every 2 years. FDA has adopted a systems approach to GMP inspection. A GMP facility is divided into six systems:

- *Quality System*: This consists of procedures and specifications to assure the overall compliance for the facility. Quality control, change control, batch release, internal audits, quality records are part of the Quality System.
- *Facilities and Equipment System*: This includes (i) Buildings and facilities along with maintenance; (ii) Equipment IQ, OQ, calibration, maintenance, cleaning, and validation of cleaning processes; (iii) Utilities such as HVAC, compressed gases, steam, and water systems.
- *Materials System*: This is concerned with segregation and storage of raw materials, components and finished products, inventory control and distribution of finished products.
- *Production System*: This includes manufacturing processes, sampling and testing, batch records and process validation.
- *Packaging and Labeling System*: This includes control and issuance of labels, packaging operations and validation of these operations.
- *Laboratory Control System*: This includes laboratory test methods, stability program and analytical method validation.

FDA carries out two types of inspections: surveillance inspections and compliance inspections. Surveillance inspections are the biennial inspections. Compliance inspections are to follow-up on non-compliance and previous corrective actions. Compliance inspections also include "For cause Inspections", which are inspections to audit a specific problem that has come to FDA's attention, for example, product recall and industry or public complaints.

There are two options of inspections: (i) the Full Inspection Option, and (ii) the Abbreviated Inspection Option:

- The Full Inspection Option is a surveillance or compliance inspection that is thorough and gives FDA a deep understanding of the cGMP program in a manufacturing facility. This type of inspection is conducted when FDA has little knowledge about the facility, such as a new facility or where the facility has a history of non-compliance or when FDA has doubt about the facility's quality system. The Full Inspection Option audits at least four systems in the facility, one of which must be the quality system.
- The Abbreviated Inspection Option is a surveillance or compliance inspection. It is a shortened inspection. This is performed when the facility has a satisfactory cGMP compliance record and there are no product problems or there has been little change since the last inspection. At least two systems are audited, including the quality system.

Figure 10.2 shows in simplified form the flow of an FDA inspection and the actions taken. A list of the pertinent FDA guides to inspections of drug manufacturers to determine their compliance to cGMP is given in Exhibit 10.4. Inspections of biopharmaceutical manufacturing facilities are carried out by Team Biologics, which is a partnership between the Office of Regulatory Affairs and the Center for Biologics Evaluation and Research (CBER) of FDA. Team Biologics consists of personnel with skills and experience in the biopharmaceutical manufacturing to ensure critical areas are inspected.

If the inspector believes the cGMP has been violated, Form FDA-483 is used to record the observations. Samples may be taken by the FDA inspector for analysis. In this case, Form FDA-484 is issued to the manufacturer for the receipt of samples. A

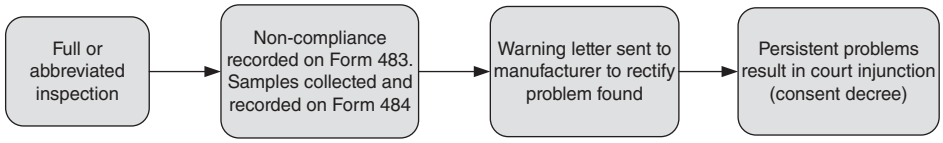



**Figure 10.2** FDA inspection and action process.

**Exhibit 10.4 FDA Guides to Inspections**

- Guide to Inspections of Dosage Form Drug Manufacturer's – CGMPR'S
- Guide to Inspections of Bulk Pharmaceutical Chemicals
- Guide to Inspections of High Purity Water Systems
- Guide to Inspections of Pharmaceutical Quality Control Laboratories
- Guide to Inspections of Microbiological Pharmaceutical Quality Control Laboratories
- Guide to Inspections of Lyophilization of Parenterals
- Guide to Inspections of Validation of Cleaning Processes
- Guide to Inspections of Computerized Systems in Drug Processing
- Guideline on General Principles of Process Validation
- Compliance Program Guidance Manual – Active Pharmaceutical Ingredient (API) Process Inspection
- Biotechnology Inspection Guide

*Source*: Data from 1. Food and Drug Administration 1993, *Dosage Form Drug Manufacturers cGMPs*, viewed May 26, 2014, http://www.fda.gov/iceci/inspections/inspectionguides/ucm074927.htm, 2. Food and Drug Administration 2006, *Active Pharmaceutical Ingredient (API) Process Inspection*, viewed May 26, 2014, http://www.fda.gov/downloads/aboutfda/centersoffices/officeofmedicalproductsandtobacco/cder/ucm095569.pdf, 3. Food and Drug Administration 2014, *Inspections 2014, Compliance, Enforcement, and Criminal Investigations*, viewed May 26, 2014, http://www.fda.gov/ICECI/Inspections/InspectionGuides/default.htm

normal practice for the manufacturer is to take more samples for internal analysis and compare with the FDA data when required.

After inspection, the inspector prepares a detailed Establishment Inspection Report (EIR). This is the FDA's primary record for the inspection. Time is given to the manufacturer to respond to the deficiencies found and recorded in Form FDA-483. Failure to comply with satisfactory resolution to the deficiencies found will result in FDA sending out a Warning Letter notifying the manufacturer to comply. If the manufacturer is unable to resolve the deficiency after the deadline set by FDA, then FDA may proceed to prosecute the manufacturer with an injunction. The injunction is a court order called Consent Decree, and the manufacturer may be required to cease operations until the problem is rectified (refer to Exhibit 10.5).

In Europe, inspections are conducted by Member States on behalf of the European Union. For drugs approved under the centralized procedure, inspections are coordinated by the European Medicines Agency (EMA; refer to Sections 7.3 and 8.3). For countries that are members of the Pharmaceutical Inspection Cooperation Scheme (PIC/S; Section 7.13), there is mutual recognition of inspections performed by members.

Some typical problems found in GMP inspections are:

- Out-of-Specifications: Insufficient investigations to determine root cause of problems and issues are not closed in a timely manner
- Product Sterility: The tests performed are superficial and not validated
- Environment Monitoring: Personnel are not monitored, inadequate monitoring, microorganisms are not monitored, there is no identification of contaminants, alert limits for contaminants are set too high
- Raw Materials, Components and Finished Product: No or insufficient audit procedure, test methods lack validation
- Training: There is no training plan, training is not documented, investigation of problems in manufacturing does not lead to retraining of staff involved
- Materials: There is no segregation of materials
- Calibration: Lack of scheduling and lapsed calibration validity
- Documentation: Manufacturing steps are not signed off, changes are not explained, obsolete copies of document are used
- Process: Personnel not following set procedures, procedures are not validated
- Internal Audit and Review: Infrequent internal audit performed, superficial audit insufficient to reveal problems, no follow-up on issues observed at internal audits
- Management: Lack or insufficient commitment on GMP issues.

Examples of some of the typical violations leading to drug product recall according to FDA are:

- cGMP deviations
- Sub-potent products
- Product failed *US Pharmacopoeia* dissolution test requirements

- Product failed endotoxin/pyrogen tests
- Presence of contaminants in product
- Label mix-up on the product
- Stability data do not support expiration date
- Product lacks stability
- Product failed content uniformity
- Product failed pH test requirements.

**Exhibit 10.5 GMP Consent Decree**

**Schering-Plough**

In May 2002 Schering-Plough was fined US$ 500 million for GMP violations by FDA under the consent decree scheme. The issue centered on the GMP violations of the manufacturing facilities at New Jersey and Puerto Rico. A total of 13 inspections were carried out by FDA from 1998 to 2002. The non-compliances were related to the facilities, quality assurance, manufacturing, equipment, laboratories and labeling.

In addition to the US$ 500 million that Schering-Plough had to pay, it also had to settle about US$ 500,000 for inspection costs, recalling of several products, suspension or discontinuation of certain products and revamping its quality system to ensure future compliance.

*Source*: Food and Drug Administration 2002, *The Food & Drug Letter*, Issue No. 653, June.

**GSK**

On April 28, 2005, FDA announced that GSK had signed a Consent Decree with FDA to correct manufacturing deficiencies at its Cidra, Puerto Rico facility.

The Consent Decree was initiated based on FDA's continued concerns that GSK's violation of manufacturing standards may have resulted in the production of drug products that could potentially pose risks to consumers.

The Decree required GSK to post a penal bond of US$ 650 million contingent upon GSK either successfully reconditioning drugs seized in March 2005, or destroying them and paying costs to the government. Under the terms of this Decree the company agreed to take measures to ensure that its Cidra facility and the two drugs, PaxilCR and Avandamet, fully comply with cGMP requirements and to ensure that ongoing shipments have the quality attributes they are required to possess. The Decree also requires that all corrections and the firm's compliance with cGMP requirements be certified by a third-party expert. Additionally, FDA will continue to monitor these activities through its inspections.

*Source*: Data from Food and Drug Administration 2005, *GlaxoSmithKline – Seizures and Subsequent Consent Decree*, viewed May 26, 2014, http://www.fda.gov/downloads/iceci/enforcementactions/enforcementstory/enforcementstoryarchive/ucm091066.pdf

In early 2007, EMA reported the analysis of GMP deficiencies in its document: *Good Manufacturing Practice*: *An Analysis of Regulatory Inspection Findings in the Centralised Procedure*. It detailed the deficiencies reported in 435 inspections of manufacturers of medicinal products and starting materials in EU and other countries in the period 1995 to 2005. Altogether there were 9,465 deficiencies, of which 193 were critical (2%), 989 major (10%) and 8,283 (88%) others. The top 20 deficiencies are presented in Table 10.1 below:

In November 2011 PIC/S conducted a survey on GMP deficiencies with its members. The top three deficiencies were: (i) Documentation – manufacturing, (ii) Design and maintenance of premises, and (iii) Documentation – quality systems (elements/procedures).

With FDA's initiative – *Pharmaceutical Current Good Manufacturing Practices* (*cGMPs*) *for the 21st Century*: *A Risk Based Approach*, and the implementation of ICH Q9 on *Quality Risk Management*, these initiative and guideline will have a major effect on the conduct of GMP inspections (refer to Sections 9.8 and 9.9). Inspections

**TABLE 10.1 Deficiencies of Manufacturers of Medicinal Products and Starting Materials in EU and Third Countries During 1995 to 2005**

Ranking of the Top 20 Major GMP Deficiencies for 1995/2005

| No. | Category of GMP Deficiency | Number |
|---|---|---|
| 1 | Contamination, microbiological – potential for | 112 |
| 2 | Documentation – quality system elements/procedures | 102 |
| 3 | Regulatory issues: Unauthorized activities | 66 |
| 4 | Design and maintenance of premises | 59 |
| 5 | Regulatory issues: Non-compliance with marketing authorization | 55 |
| 6 | Sterility Assurance | 53 |
| 7 | Documentation–manufacturing | 50 |
| 8 | Documentation–specification and testing | 46 |
| 9 | Equipment validation | 43 |
| 10 | Design and maintenance of equipment | 36 |
| 11 | Personnel issues: duties of key personnel | 35 |
| 12 | Supplier and contractor audit and technical agreements | 34 |
| 13 | Contamination, chemical/physical–potential for | 33 |
| 14 | Process validation | 33 |
| 15 | Environmental monitoring | 25 |
| 16 | Personnel issues: hygiene/clothing | 25 |
| 17 | Investigation of anomalies | 22 |
| 18 | In-process controls–control and monitoring of production operations | 18 |
| 19 | Line clearance, segregation and potential for mix-up | 18 |
| 20 | Personnel issues: training | 17 |

*Source*: European Medicines Agency 2007, *Good Manufacturing Practice: An Analysis of Regulatory Inspection Findings in the Centralised Procedure*, viewed May 25, 2014, http://www.ema.europa.eu/docs/en_GB/document_library/Other/2009/10/WC500005009.pdf. Reproduced with permission of the European Medicines Agency.

will be prioritized and more detailed for those GMP facilities categorized as potentially higher-risk drug manufacturing sites:

- Sites making sterile drugs
- Sites making prescription drugs
- Sites of new registrants not previously inspected by FDA.

## 10.4 MANUFACTURE OF SMALL MOLECULE APIs (CHEMICAL SYNTHESIS METHODS)

### 10.4.1 Conventional Synthesis Techniques

The manufacturing process for a small molecule AP is shown in Figure 10.3. Typically, the chemical reactions are performed in large reaction vessels. For commercial production of an API, the reaction vessel can typically range from 1,000 to 20,000 L in volume (Figure 10.4).

The reaction vessel is normally made of glass-lined stainless steel with a jacket for heating and cooling and consists of:

- Charge-hole for addition of solid raw materials
- Metered-pump input for liquids
- Supply of gases as reactants or inert blanket
- Stirrer for mixing the raw materials
- Condenser unit for solvent reflux
- Vents with filters for gas emission or depressurization
- Transfer line for discharge/separation of reactants/products
- Probes for measuring the temperature, pH, and pressure
- Sampling ports for withdrawals of samples for analysis.

Production of the API begins with the selection of a synthetic route, as determined in the development program. Raw materials are added into a reaction vessel. These raw materials as reactants are heated or cooled in the reaction vessel (normal range is from −15 °C to 140 °C; purpose-built vessels are needed for extreme reactions that require lower or higher temperature controls or pressurization of reaction processes). The chemical synthesis reactions are monitored and controlled via sensor probes (pH, temperature, and pressure) with in-process feedback controls for adjustments and alarms when necessary. Samples are withdrawn at defined intervals for analysis to determine the reaction progress. Catalysts, including enzymes, may be added to speed up and direct the reaction along a certain pathway.

It is important to maintain a uniform reaction environment within the vessel chamber by using a stirrer to agitate and mix the reactants. Gaseous discharge is vented through filters to the outside environment. As the reactions may generate substantial heat (exothermic) and pressure, or may even be potentially explosive, special precautionary features are designed into the vessel.

At the end of the synthesis reactions, the product can be pumped to another vessel or container via transfer lines. If the chemical reactions proceed to completion with

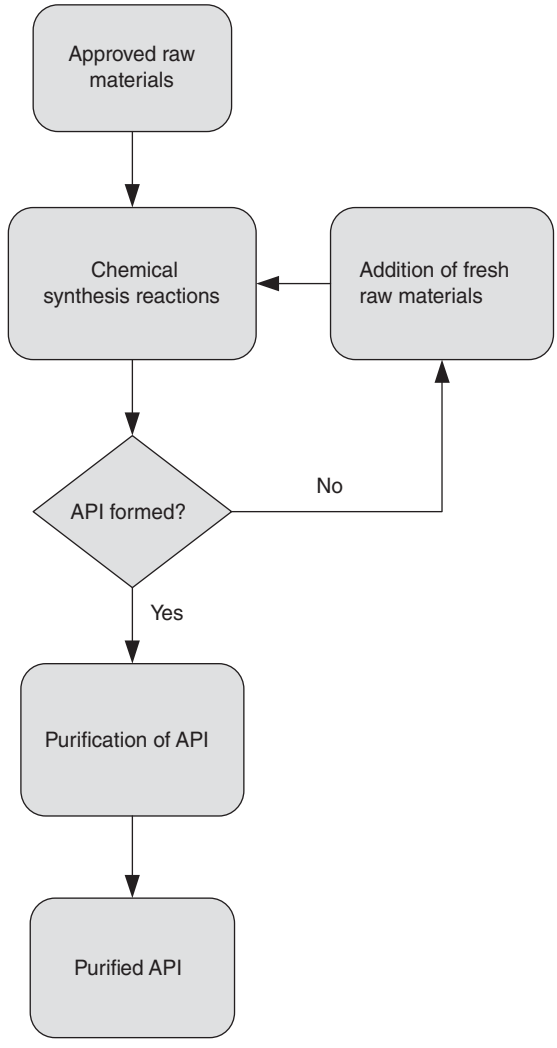



**Figure 10.3** Chemical synthesis of small molecule APIs.

negligible trace quantities of impurities, the next stage of production may commence in the same reaction vessel with addition of fresh raw materials. This process is called telescoping.

The finished product is centrifuged and purified via a number of processes, including filtration, fractional distillation, condensation, crystallization, and chromatographic separation techniques. The purified API is tested and then it is ready to be formulated into the finished dosage form, as discussed in Section 10.6. Exhibit 10.6 illustrates some of the typical reagents for API manufacture and Exhibit 10.7 presents selected chemical reactions as examples of the synthesis processes for API manufacture. Purification processes for APIs are described in Exhibit 10.8.

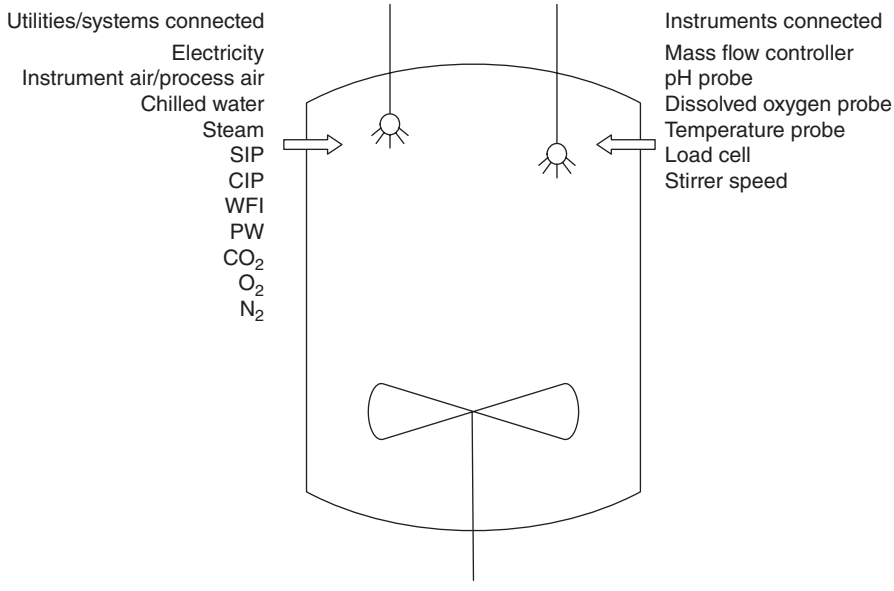



**Figure 10.4** Reaction vessel for the manufacture of an API – Schematic drawing.

**Exhibit 10.6 Typical Reagents for API Manufacture**

*Water*: Purified water, water-for-Injection

*Solvents*: Toluene, methanol, ethanol, ether, acetate, dimethyl sulfoxide, tetrahydrofuran, hexane, cyclohexane, dichloromethane, acetonitrile, acetone

*Oxidizing agents*: Hydrogen peroxide, chromic acid, potassium permanganate, manganese dioxide, ozone

*Reducing agents*: Hydrogen, lithium aluminum hydride, sodium borohydride, di-isobutyl aluminum hydride, iron metal

*Acids*: Sulfuric acid, hydrochloric acid, phosphoric acid, methanesulfonic acid, acetic acid, formic acid

*Bases*: Sodium hydroxide, ammonia, triethylamine, pyridine, butyl lithium, sodium hydride, α-methylbenzylamine

*Halogenation reagents*: Halogens, *N*-bromo- and *N*-chlorosuccinimide, thionyl chloride, phosphorus oxychloride

*Alkylating agents*: Dimethyl sulfate, methyl iodide, methyl tosylate

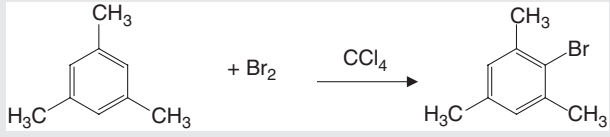
*Sulfur reagents*: Thiols and sulfides, hydrogen sulfide, sodium sulfide, sodium thiocyanate, thiourea, sodium metabisulfide

*Phosphorus reagents*: Phosphorus halides

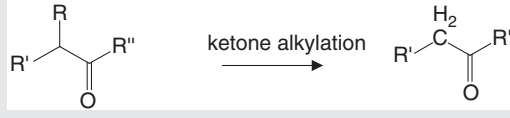
*Boron reagents*: Diborane, boron trifluoride, dialkyl borinates, aryl boronic acids.

**Exhibit 10.7 Selected Chemical Reactions as Examples for API Manufacture**

**Halogenation**

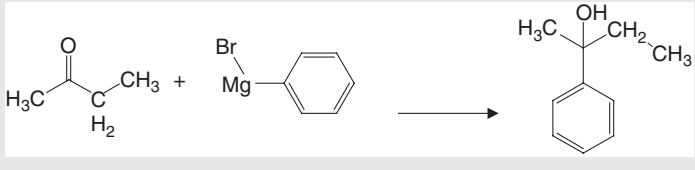



**Alkylation**




**Acylation**


**Grignard reaction**




*Source*: Adapted from Hornback, J.M 2005, Organic Chemistry, 2nd edn., Thomson Brooks/Cole, Belmont, CA.

Compliance to GMP for the production of API and finished dosage form is mandatory as discussed in Chapter 9 and Section 10.2. The quality system, quality control and validation of equipment and processes have to be developed and adhered to in the manufacturing process. Proper records and documentation are required to be kept in the forms of batch records, test records and manufacturing procedures. Reaction vessels and associated equipment must be calibrated, validated and cleaned to acceptable levels before being used; this is especially the case for multi-product plants where more than one API is manufactured.

**Exhibit 10.8 Purification of an API**

**Filtration/Fractional Distillation/Condensation**

Filters are used to remove solid particles from a solvent. The use of 0.2 μm filters can remove microbial contamination. Filtered solutions can be fractionally distilled and condensed to obtain the API.

**Crystallization**

Crystallization is used to separate the API from its solvent and impurities, or to separate racemic mixtures in solution. Crystallization occurs from a supersaturated solution. Important conditions are the temperature, concentration, stirring rate, and heating and cooling rate. Seeding with the desired API can assist in providing nucleation sites for the preferential crystallization of the API.

**Exhibit 10.9 Synthesis of Paclitaxel (Taxol)**

An introduction to Taxol (Bristol-Myers Squibb) is presented in Exhibit 3.4. The API is paclitaxel. The chemical name is 5β,20-Epoxy-1,2α,4,7β,10β,13α-hexahydroxytax-11-en-9-one 4,10-diacetate 2-benzoate 13 ester with (*2R*, *3S*)-*N*-benzoyl-3-phenylisoserine.

Early production of 1 kg of paclitaxel required extraction from about 13,000 kg of the Pacific yew tree bark. This process was refined, and paclitaxel is now produced by a semi-synthetic route. The starting material, 10-deacetyl baccatin III (10-DAB) is obtained from the needles of *Taxus baccata* (European yews) or *T. wallichiana* (Himalayan yews). The yield is around 1,000 kg of needles to produce 1 kg of 10-DAB.

*Source*: Data from Cabri, W and Di Fabio, R 2000, *From Bench to Market: The Evolution of Chemical Synthesis*, Oxford University Press, Oxford, UK.

As an example, we present in Exhibit 10.9 the synthesis of paclitaxel (Taxol, Bristol-Myers Squibb), an important anticancer drug for breast and ovarian cancer and Kaposi sarcoma. It illustrates the complexity in the synthesis of drug molecules.

### 10.4.2 Chiral Synthesis Techniques

Production of synthetic drug often gives rise to racemic mixtures of API enantiomers, that is, they are mirror images of each other (refer to Section 3.6). The first problem is that the production process generates equal amounts of the enantiomers and only one is active. In this case, only half the yield is effective for drug application. Another problem is that the ineffective isomer may create undesirable side effects when interacting with biological receptors that are chiral themselves. Examples of the differing effectiveness of chiral drugs are presented in Exhibit 10.10.

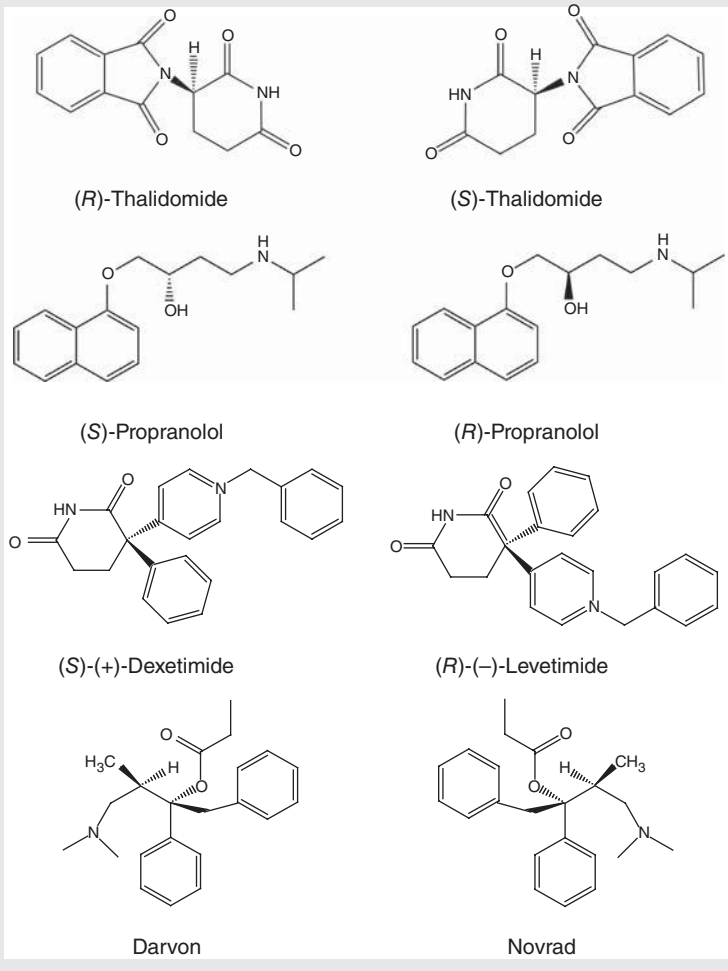
**Exhibit 10.10 Examples of Chiral Drugs**

*Thalidomide*: The (*R*)-enantiomer is a sedative and the (*S*)-enantiomer is teratogenic, that is, causes fetal deformity.

*Propranolol*: An antihypertensive drug: the (*S*)-enantiomer is 130-fold more potent than the (*R*)-enantiomer, a β-adrenoceptor antagonist.

*Dexetimide*: Dexetimide has 10,000-fold more affinity for the muscarinic acetylcholine receptor than its enantiomer, levetimide.

*Dextropropoxyphene*: The (*2R*,*3S*)-enantiomer marketed as Davron is an analgesic, whereas the (*2S*,*3R*)-enantiomer called Novrad is an antitussive.



Manufacturing of chiral drugs has become increasingly important; firstly to improve potency, secondly to improve yield, and thirdly to extend the patent life for approved drugs based on racemic mixtures. Stereoselective synthetic methods are used to produce chiral drugs. There are three basic methods being applied:

- Enzyme and non-enzyme catalysts
- Chiral building blocks
- Chiral auxiliary.

***Enzyme and Non-enzyme Catalysts:*** By nature, enzymes themselves are chiral and they catalyze a variety of chemical reactions with stereoselectivity. These reactions include oxidations, reductions, and hydrations. Examples of enzymes are oxidases, dehydrogenases, lipases, and proteases. Metoprolol, an adrenoceptor-blocking drug, is produced using an enzyme-catalyzed method.

Non-organic and organometallic catalysts are also used to channel the reactions towards the chiral synthesis pathway. The drug called levodopa, (*S*)-3,4-dihydroxy-alanine, is an effective drug against Parkinson's disease. It is stereoselectively manufactured using catalysts such as rhodium or ruthenium complexes.

***Chiral Building Blocks:*** Some drugs are made using chiral building blocks to generate the required chiral centre in the drug. The introduction of chiral centers ensures that the reaction proceeds in the desired direction. The preparation of enalapril, an ACE inhibitor, is an example of the use of chiral building blocks.

***Chiral Auxiliary:*** A chiral auxiliary is an intermediate formed by the attachment of a pure enantiomer to an achiral substrate. The attachment, called a chiral auxiliary, restricts the approach of reactants to react in specific ways to produce the chiral molecule. The antibacterial drug, aztreonam, is synthesized using the chiral auxiliary method.

## 10.5 MANUFACTURE OF LARGE MOLECULE APIs (RECOMBINANT DNA METHODS)

The manufacturing process for a typical large molecule protein-based API is shown in Figure 10.5. A description of the plasmid vector is given in Appendix 4. The production of these APIs uses "factories" that are living cells in the form of cell lines, which can grow indefinitely under appropriate conditions. As discussed in Section 4.3.3, monoclonal antibodies are conventionally produced using hybridoma cell lines, but now cell culture methods are the preferred technique. Other protein-based drugs are produced using a variety of cell lines: from bacterial or fungal to insect and mammalian cell lines.

Currently, about equal numbers of the approved protein drugs are derived from microbial and mammalian cells, although more drugs are expected to be produced from mammalian cell lines in the future. There are pros and cons for each type of cells as production "factories" for the protein drugs:

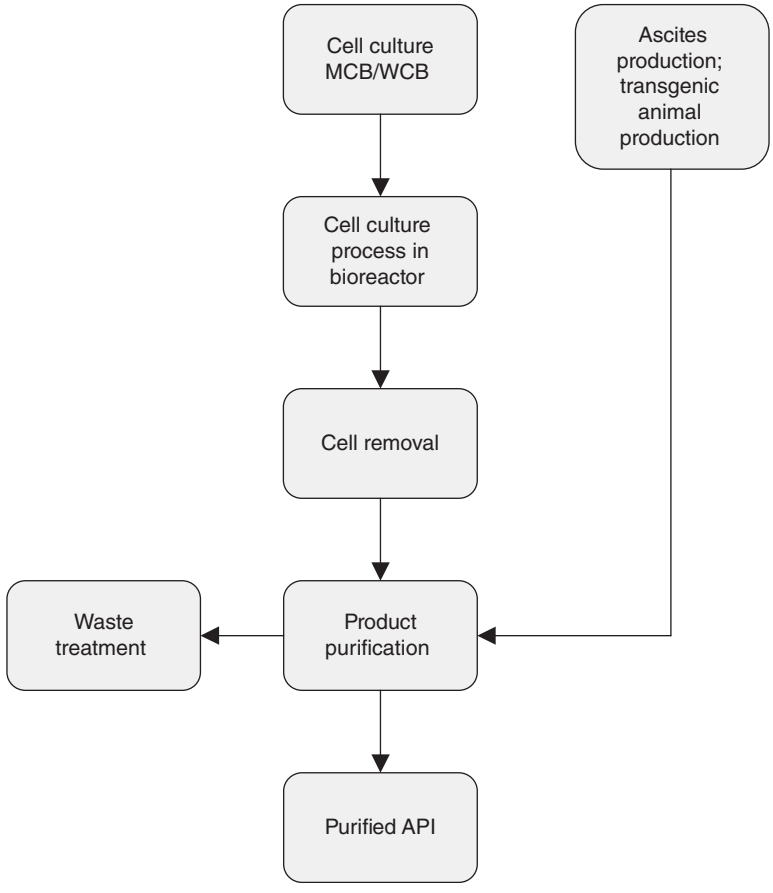



**Figure 10.5** Production of biopharmaceutical drug. Note: Ascites represents the old method of biopharmaceutical drug production.

### *Microbial Cells (for example Escherichia coli cells):*

*Advantages*: Cells grow rapidly on relatively inexpensive media; the fermentation technology for growing microbial cells is well established.

*Disadvantages*: The protein expressed is accumulated within the cell matrix (intracellular); protein does not undergo post-translational modifications (resulting in proteins which may be structurally different or less useful to humans); likely presence of lipopolysaccharides (pyrogens - microbial substances that cause fever) from host cells to contaminate product, and the need for more extensive chromatographic purification.

*Example*: Ranibizumab, which is the active ingredient in Lucentis (refer to Exhibit 4.13, treatment of macular degeneration) is produced using the *E. coli* 60E4 cells.

### *Mammalian Cells (for Example Chinese Hamster Ovary (CHO) and Baby Hamster Kidney (BHK) Cell):*

*Advantages*: Post-translational modification of protein product can be performed; extracellular expression of proteins, which requires less complex purification processes.

*Disadvantages*: Cells have complex nutritional requirements; higher production cost; cells grow more slowly and are susceptible to physical damage; requires specially designed bioreactors.

Example: Omalizumab, which is the active ingredient in Xolair (treatment of moderate to severe asthma) is produced using an adapted CHO cell line.

The recombinant technique involves transfecting cells with DNA that codes for the production of the intended protein. The process of transfection into a plasmid is shown in Exhibit 10.11 (also refer to Appendix 4). Once transfected, several clones are selected on their productivity of the target molecule. These clones are further cultivated separately and undergo serial dilution and clone selection again to ensure that only one clone is selected. Finally this clone is grown up to form a "pre bank" (typically around 6–10 vials) and one of these vials is used to form the master cell bank (MCB) to be used for production.

A MCB is set up which forms the first generation of these clones (usually at lease 400 vials are made, refer to Exhibit 10.12 about the guidelines for testing and characterizing cell lines and cell banks). They are stored in hundreds of 1–2 mL cryovials in liquid nitrogen freezers. The cells are kept in growth medium added with 5 to 10% dimethyl sulfoxide (DMSO) as a cryoprotectant and stored at −150 °C or below to preserve them indefinitely. From the MCB, a vial is taken and cells are grown to produce a working cell bank (WCB), and they too are maintained in liquid nitrogen freezers. Subsequently, a vial is taken from the WCB for each batch of production. This two-tier system of MCB and WCB can supply production needs indefinitely and also ensures the consistency and fidelity of protein production. For example, 200 vials each of the MCB and WCB for a production rate of 10 batches per year will last 4,000 years.

Cells from the WCB are cultured initially in flasks, which contain nutrient medium. The medium may contain the following:

- Amino acids
- Vitamins A, D, E and K
- Ionic salts ($Na^+$, $K^+$, $Mg^{2+}$, $Ca^{2+}$, $Cl^-$, $SO_4^{2-}$, $PO_4^{3-}$, $HCO^{3-}$)
- Glucose (as a source of energy)
- Organic supplements (proteins, peptides, nucleoside, citric acid, lipids, cholesterols)
- Hormones, growth factors, antibodies and antibiotics.

In certain cases, serum (fetal bovine serum (FBS)) is added to promote the growth of cells. However, the Bovine Spongiform Encephalopathy (BSE) problem has necessitated tight control on the quality of FBS (refer to Exhibit 10.13). This increases production and downstream processing costs. For new cell lines being developed, serum-free

**Exhibit 10.11 Recombinant DNA Techniques – Genetic Engineering**

The first step is the isolation of DNA genes that code for the production of the desired protein. The next stage is the insertion of these genes (foreign DNA) into a vector, or carrier. Common vectors used are the bacteriophage (a virus) and the bacterial plasmids, which are circular bacterial DNA.

Both the foreign DNA and plasmid are cleaved by enzymes called restriction endonucleases. They are mixed and then joined together using another enzyme called ligase.

The plasmid with the inserted DNA genes is transformed into bacterial or transfected into mammalian cells. Methods used include electroporation, microinsertion, or chemical mediated transfection. When these cells are cultured in medium with nutrients, they grow and divide. In the growth process, the foreign genes express proteins within the cell (intracellular) or outside the cells (extracellular). At the end of the growth cycle, the cells are killed and the proteins are extracted and purified as the protein drug material. The process is illustrated below, where the cells act as "factories" for producing the protein of interest.

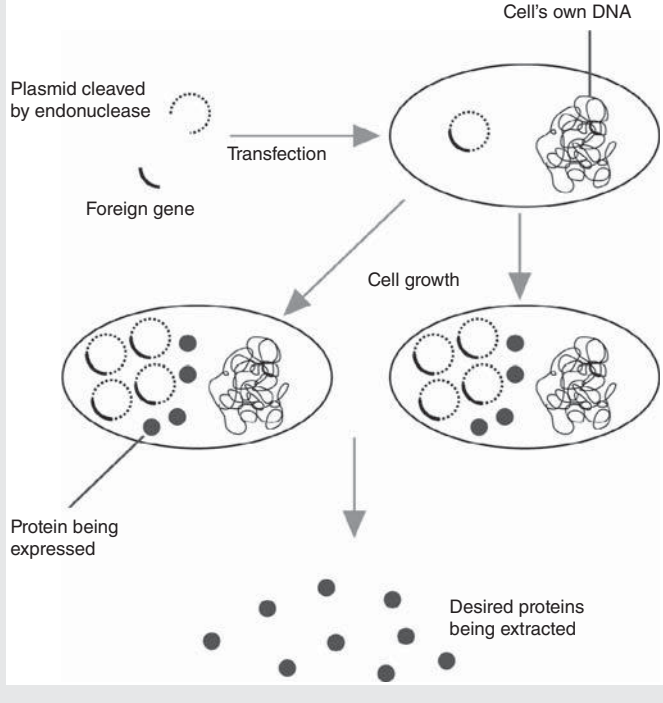


**Exhibit 10.12 Guidelines for Testing and Characterizing Cell Lines/Banks**

**Cell Line**

Need to know the origin, source and history of cells. The Host Cell Line need to be characterized fully and their history from inception to cell bank documented and tested.

Record of cultivation of cells, medium used, genetic manipulation, selection criteria, isolation methods, identification, characteristics, and tests for endogenous and adventitious agents.

*Human Cell Lines*: Characteristics of donor, tissue or organ of origin, ethnic and geographical origin, age, sex and general physiological conditions.

*Animal Cell Lines*: Species, strains, breeding conditions, tissue or organ of origin, geographical origin, age, sex and general physiological conditions.

*Microbial Cell Lines*: Species, strain, genotype, phenotype, pathogenicity, toxin production, and other biohazard information.

**Cell Bank**

Record of cell banking method and procedure.

*Characterization and Testing of Cell Banks*: Test for adventitious agents, endogenous agents and molecular contaminants (toxins, antibiotics) to confirm identity, purity, and suitability for manufacturing use.

*Evaluation of Stability*: Consistency of the coding sequence of the expression construct in generating the product.

*Karyology and Tumorigenicity*: May be required to test for safety of cell line. For those products with no cells, karyology and tumorgenicity not necessary but demonstration of residual host cell DNA is required. For those products with presence of live cells, karyology, and tumorgenicity are required.

*Source*: Data from International Conference on Harmonization 2004, *Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/Biological Products, Q5D*, viewed May 26, 2014, http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q5D/Step4/Q5D_Guideline.pdf

and protein-free media are used to circumvent the possibility of virus contamination from animal sources and the variation that may arise from use of serum from animal herd.

Most cells grow well under a fermentation process at a pH of around 7.0–7.4. However, as cells grow, $CO_2$ is produced. To maintain optimal growth, the media are often buffered with, for example, phosphate buffered saline or $NaHCO_3/Na_2CO_3$ as these interact with the $CO_2$ to control the pH. Cells go through different phases of growth

**Exhibit 10.13 Bovine Spongiform Encephalopathy**

Bovine spongiform encephalopathy (BSE or 'mad cow disease') is a progressive neurological degenerative disease in cattle. It is caused by a mutated protein called a prion. BSE was first reported in the United Kingdom in 1986. Variant Creutzfeldt-Jakob disease (vCJD) is a rare and fatal, neurodegenerative disease that occurs in humans. Evidence to date indicates it is possible for humans to acquire vCJD after consuming BSE-contaminated cattle products.

A number of measures have been taken to contain BSE. Thousands of cattle have been culled and there are controls prohibiting the feeding of mammalian proteins to ruminant animals (cows, sheep, and goats). There are also surveillance programs set up to monitor CJD in humans.

The FDA and European regulatory authorities have strongly recommended drug manufacturers not use materials derived from ruminant animals in countries where BSE has been reported. Manufacturers of protein drugs that require fetal bovine serum (FBS) for cell growth use FBS from countries such as the US, Australia and New Zealand—countries considered safe from BSE. As precautionary measures, newly developed cell lines for production of protein drugs are focusing on serum-free and protein-free growth medium.

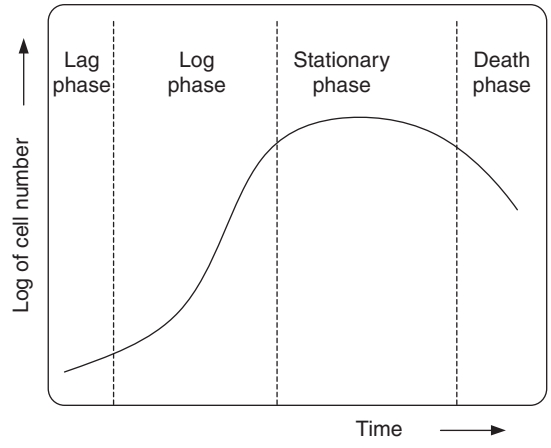



**Figure 10.6** Stages of cell growth.

(Figure 10.6). The cell viability, density and consumption of nutrients are constantly monitored (Figure 10.7).

When the cells grow to a certain density (number of cells per milliliter, around $1–10 \times 10^6$ cells/mL) and have an acceptable viability (normally >90% survival rate), they are transferred into larger vessels called bioreactors. There may be several steps

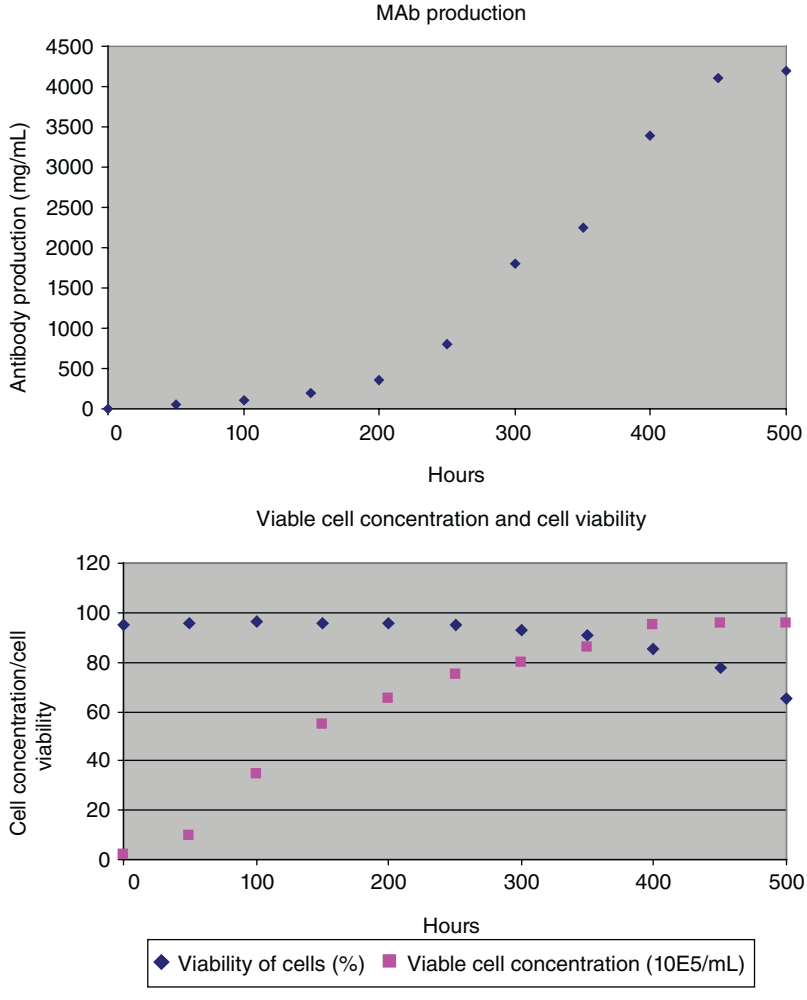



**Figure 10.7** Monitoring of cell growth over time. Note: Usually there are nutrient feeds that "kick start" the cell growth and the production of the MAb. This can be anything from a chemical inhibitor such as MTX or just as simple as changing the carbon substrate.

for growth in different size bioreactors (typically do not exceed a 1:5 ratio) before a final production bioreactor is used, which may be as large as 10,000–20,000 L.

Growth conditions are optimized in the development stage for specific cells. Some cells prefer to anchor onto solid substrates. In this case, microcarrier beads or hollow fibers are used to provide attachment for the cells. Some other cells grow best in suspension within the media. Yet, in other cases, continuous supply and harvesting of the cells are optimal; these are the perfusion techniques. In a perfusion system, typically the

**Exhibit 10.14 Cell Culture Methods**

**Suspension Process (Stirred/Sparged Tank)**

*Advantages*: Easy to operate in batch and fed-batch; easy to obtain cell sampling to determine cell concentration and viability; easy to scale up.

*Disadvantages*: Sensitive to shear force from stirrer; foaming when serum is added to media.

**Anchorage-Dependent Process (Microcarriers, Hollow Fiber)**

*Advantages*: Established technology; higher product titer than suspension process.

*Disadvantages*: Cells only grow when attached to solid substrates; cells require additional attachment factor; not easy to scale up; more cleaning validation issues and high disposal cost; more difficult to operate than Suspension process.

Apart from Bioreactors there are many processes still using static cell culture methods such as roller bottles. The advantage is less sophisticated controls, easily implemented and low capital expenditure.

cells remain in the bioreactor because a spin filter or a "Cell Settler" (a tube set at an angle so that at a define flow rate the cells remain in the tank) are used. The advantages and disadvantages of these methods are described in Exhibit 10.14. Cells are monitored for growth, viability, consumption of nutrient, discharge of metabolites, use of oxygen and carbon dioxide, and production of the target molecule.

As cells grow, proteins are secreted. At the end of the growth cycle, the proteins are harvested from clarified media. For cells (e.g., microbial cells) that produce intracellular proteins, the cell membranes are lysed to free the proteins. Yield of unpurified proteins may range from milligrams to 1–5 g/L. The proteins are then purified, normally via several stages through chromatographic columns (Exhibit 10.15), resulting in about 20–50% the amount from the cell culture process as purified product. The purified protein as an API is tested to ensure it meets specifications. Once it passes the requisite specifications, it is ready to be processed into the finished form (refer to Section 10.6).

Cells grow from a single vial (1–2 mL) to thousands of liters in bioreactors. Many generations of cell division and growth are involved. It is important that in this process, which may last from weeks to months, the cells do not mutate and they continue to faithfully express the intended protein. Cells at the completion of production are collected

**Exhibit 10.15 Purification using Chromatographic Techniques**

Chromatographic separation relies on the affinity of binding between different components of the API in liquid and the solid matrix column. The API is separated from the impurities by percolating the liquid through chromatographic columns filled with solid phase matrices. The matrices are made of different materials and separate the components on the basis of physicochemical properties such as charges, size and shape, hydrophobic and hydrophilic characteristics, complex formation with certain ions or metals, and interaction with dyes.

*Ion Exchange*: Separation is based on selective, reversible adsorption of charged molecules to an immobilized ion exchange group of opposite charge. An ion exchanger consists of an insoluble porous matrix to which charged groups have been covalently bound. Anion exchanger group: DEAE, Diethylaminoethyl; QAE, Quaternary aminoethyl; Q, Quaternary ammonium. Cation exchanger group: Carboxymethyl, sulfopropyl.

*Affinity*: Product to be purified binds to an affinity ligand that is coupled to a matrix. The ligand is specific for a particular type of protein/peptide molecule, or group of such molecules. The targeted product binds to the ligand under specific conditions of high or low ionic strengths and at a certain pH. After the unbound impurities are removed, the product can be eluted by using a gradient of increasing or decreasing ionic strength or by changing the pH.

*Hydrophobic*: Proteins and peptides differ from one another in their hydrophobic properties. Salt solutions are used to mediate the binding of molecules to a hydrophobic matrix substituted with a hydrophobic ligand.

*Reversed Phase*: This technique is based on hydrophobic regions on the surface of proteins and the hydrophobic groups covalently attached to the surface of the matrix. Organic solvents are required for elution. It is suitable for peptides and proteins up to $2.4 \times 10^4$ Da.

*Gel Filtration*: Separation is in accordance to size. Large molecules elute in void volumes and are eluted earlier. Small molecules penetrate pores of the matrix and elute later because of increase in path length.

It should be remembered that within the purification process that there must be two distinctly different active removal/inactivation steps for advantageous viruses. To qualify as an active virus removal or inactivation step the process step must achieve more than 4 log reduction of the model or target virus. Overall the process must produce over a 12 log reduction of the model or target virus.

**Exhibit 10.16 Etanercept (Enbrel)**

Etanercept (Enbrel, Immunex) is a dimerized protein molecule that consists of a portion of the tumor necrosis factor receptor coupled to the Fc portion of human IgG1 (see Section 4.3). It has 934 amino acids and a molecular weight of approximately 150 kDa.

Etanercept is produced by recombinant technology in a Chinese hamster ovary mammalian cell expression system. The cells from WCB are grown in proprietary media system and are cultured initially in flasks and then inoculated into the bioreactor vessel. The product is purified in a number of chromatographic steps, followed by viral inactivation and viral filtration steps.

The finished form is a sterile, lyophilized powder formulated with trimethamine, mannitol, and sucrose as excipients.

*Source*: Data from Food and Drug Administration 2012, *Enbrel (etanercept)*, viewed May 26, 2014, http://www.accessdata.fda.gov/drugsatfda_docs/label/2012/103795s5507lbl.pdf

as samples and grown further as an end of production cell bank (EPCB). Analysis conducted on the products from the EPCB is to verify that the protein is produced properly, even after many generations of cell reproductions.

Exhibit 10.16 presents the production of etanercept (Enbrel).

## 10.6 FINISHED DOSAGE FORMS

### 10.6.1 Examples of Different Dosage Forms

A finished dosage form is a drug formulated with an API and excipients in a form that is suitable for administering to patients. Some of the reasons for preparing finished dosage forms are:

- An exact quantity of the effective drug is incorporated into the formulation
- Drug products are easier to handle and this increases compliance in taking or administering the drug
- Preservatives and stabilizers can be added to the API to improve shelf life and result in less stringent storage conditions
- Taste, color and odor of the API can be masked by additions of excipients
- Delivery vehicles (Section 5.6) can be used to provide more specific targeting of the drug to receptors
- Extended drug effect can be maintained with controlled release formulations
- Different types of delivery mechanisms can be achieved for effective drug action, for example, intravenous injection, inhalation, sublingual application.

Table 10.2 lists the finished dosage forms for various routes of drug administration. The choice of which finished dosage form to administer to a patient depends on a

**TABLE 10.2 Finished Dosage Form Application**

| Route of Administration | Finished Dosage Form |
|---|---|
| Oral | Tablets, capsules, solutions, syrups, gels, powders |
| Sublingual | Tablets, lozenges |
| Parenteral | Solutions, suspensions |
| Topical | Ointments, creams, pastes, powders, lotions, solutions, aerosols |
| Inhalational | Aerosols, sprays |
| Rectal | Solutions, ointments, suppositories |
| Vaginal | Solutions, ointments, tablets, suppositories |
| Urethral | Solutions |

*Source*: Data from Ansel, LV Jr., Popovich, NG and Ansel HC 2010, *Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems*, 9th edn., Wolters Kluwer, Baltimore, MD.

number of factors. These factors include the nature of the disease, time required for onset of drug action, age of patient, site of intended receptor, and health status of patients. In general, where possible, drug manufacturers provide several dosage forms for an API to enable it to be applied in different ways for achieving reliable and effective therapy.

***Solids:*** A diagrammatic representation for the production of tablets and capsules is presented in Figure 10.8.

Solid dosage forms are the most common means for presenting the drug product for patient administration. Most APIs are in crystallized or powder forms. They are ground to predetermined sizes using mills or pulverizers. The APIs and excipients are then mixed using blenders or tumblers.

Tablets are manufactured through a compression process. Excipients such as binders, lubricants, colorants, flavorings, and disintegration modifiers are added. The production process has to ensure that tablets have the required mechanical strength and do not crumble. Tablets may be coated or uncoated. Uncoated tablets consist of granules of API and excipients compressed into tablets. Various substances are applied to coat tablets, from sugar to waxes, gums, plasticizers and flavorings. Effervescent tablets contain acids or carbonates, which, when mixed with water, release carbon dioxide to disperse the drug materials. Release modifiers are added to tablets to alter the time and duration of drug release. Enteric coatings are used to protect drugs from being dissolved in the acid environment of the stomach (refer to Section 5.3.2).

Capsules consist of shells for enclosing drug materials. Hard shells are made of gelatin, sugar, and water. Soft gelatin shells have additional glycerin or sorbitol to soften the wall. Powder or liquid can fill the capsule shells. Hard capsules consist of two prefabricated shell sections. The API and excipients in solid form or paste are placed in one section and it then is capped with the other section. Soft capsules are mainly filled with liquids and sealed in one operation. There are modified-release capsules for delayed-release or sustained-release application. Another type is the specially formulated shells that are resistant to acid in stomach, for drug release in the intestine.

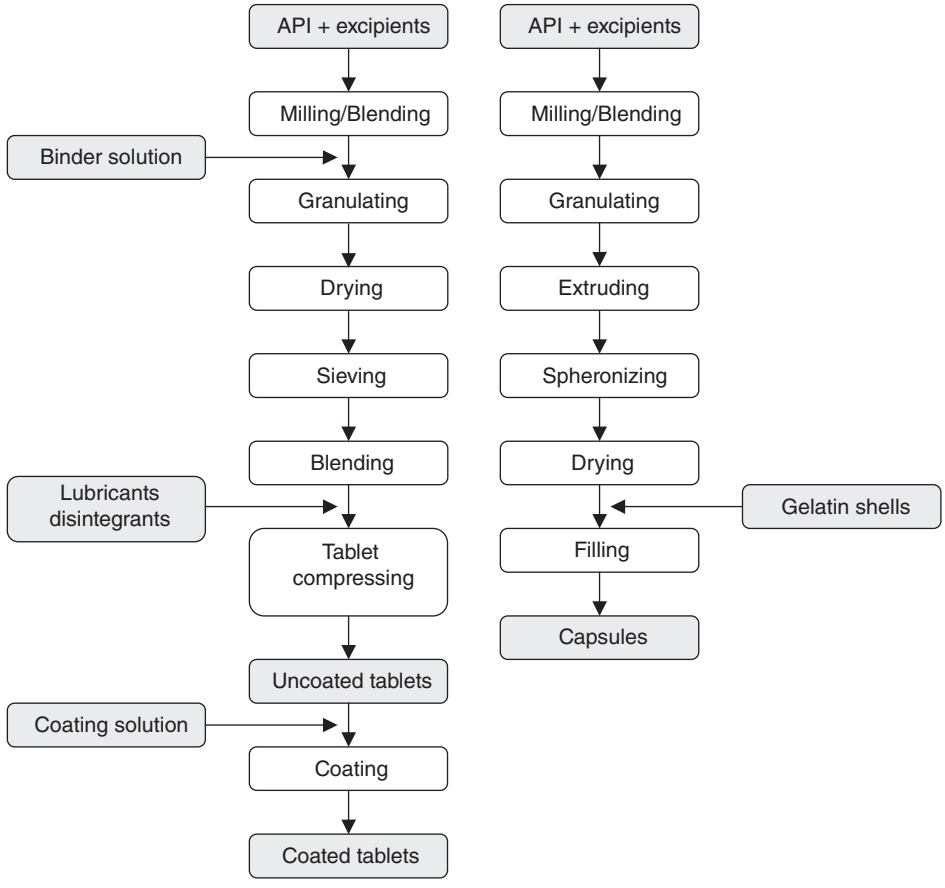



**Figure 10.8** Production of tablets and capsules.

An important specification for solid dosage form manufacture is the dissolution factor. The product is formulated and manufactured such that it will have the specified dissolution profile for maximum effectiveness.

***Liquids:*** The liquid dosage form comes in several categories: solution, emulsion, and suspension. They are prepared by dissolving the API in solvents such as water (purified water), alcohol, glycerin, or glycol. Flavorants, colorants, antioxidants, preservatives and agents for stabilizing, emulsifying, and thickening are often added to the solution to prepare the required liquid formulation. Important criteria in the manufacture of liquid dosage form are the uniformity of dispersion and the effectiveness of preservatives.

***Parenterals:*** The most important criterion for parenterals is that they have to be sterile for injection or infusion administration. Excipients are added to make parenterals isotonic with blood, improve solubility, and control pH of the solution. The solvent vehicles include water-for-injection, sterile sodium chloride, potassium chloride, or calcium

chloride solution, and nonaqueous solvents such as alcohol, glycol, and glycerin. Preservatives, antioxidants and stabilizers are normally added to enhance the properties of the drug product.

Manufacturing is performed in cleanroom conditions. Sterilization processes (refer to Section 9.7.6) in the forms of heat, steam, gas, or radiation are applied to ensure microorganisms are destroyed in the drug product. For protein-based drugs that can be damaged by the normal sterilization processes, the product is manufactured under aseptic conditions. Furthermore protein-based drugs can be lyophilized (freeze-dried) for stability and better shelf life. The lyophilized powder is reconstituted with sterile water just prior to injection. Both sterility and pyrogen tests are performed to ensure parenteral drug products are safe to be injected.

***Inhalants:*** Inhalants are pressurized dosage forms whereby powder or liquid drug substances are delivered in fine dispersions of aerosols or sprays by propellants. Typically, the particle size of the API is in the range of 2–20 μm for delivery to the respiratory system, and larger sizes for topical applications. Excipients such as preservatives, stabilizers and diluents are added. The manufacturing of inhalants involves filling the container with the API, propellant, liquid (if required), and excipients. The container is capped with a valve assembly and actuator for control of dosage. Production issues are the effectiveness of preservatives, container leakages, and control of dosage delivery.

***Ointments and Creams:*** Ointments are applied to the skin for topical treatment or to be absorbed into the blood system for delivery to target areas. They are semisolid preparations obtained by mixing the API with selected ointment bases depending on intended use. These bases include petrolatum, paraffin, mineral oil, lanolin, and glycols. Preservatives are often added to ensure the ointments will maintain the recommended shelf life.

Creams are less viscous than ointments. They are dispersions of the API in emulsions. Both oil-in-water and water-in-oil emulsions have their applications.

### 10.6.2 Packaging and Labeling

The finished dosage forms are packaged into blister packs, bottles, vials, syringes, aerosol containers, or tubes. Nowadays, packaging has tamper-proof designs to ensure the integrity of the packaging. Labeling of the packaging is in accordance with information submitted to regulatory authorities. Exhibit 10.17 describes the FDA regulations for packaging and labeling of intermediates, APIs and finished dosage forms.

### 10.6.3 Cold Chain

The need to transport temperature-sensitive raw materials and products, such as cell line, medium, large molecule drugs and vaccines means that some form of control during transportation is needed. For example working cell bank for the production of proteins may be transported in liquid nitrogen (−196 °C) and that of protein and vaccines in dry ice (−78 °C) in order to protect the integrity of the materials. Data loggers are used to record the temperature during the transit to provide evidence that

**Exhibit 10.17 Packaging and Labeling of APIs and Intermediates (ICH (1997) Guide for API: Good Manufacturing Practice). Also Including Finished Dosage Forms**

- Written procedures for receipt, identification, quarantine, sampling, examination, testing, release, and handling of packaging and labeling materials
- Records of shipment and packaging
- Containers suitable for intended use, not be reactive, additive or absorptive to intermediates or API and protect contents from deterioration and contamination
- Access to labels limited to authorized personnel
- Reconciliation of quantities of labels issued and used
- Procedures to ensure correct packaging and labels are used
- Labeling operations should prevent mix-ups
- Examination of containers and packages to ensure use of correct labels
- Transport materials with seals that will alert recipient possibility of alteration if seal has been breached.

the goods remained under the desired temperature and their functional integrity is not compromised.

## 10.7 PRODUCT QUALITY REVIEW

It is the responsibility of the pharmaceutical company to maintain a robust GMP system in manufacturing the drug product. Regular periodic reviews of all licensed drug products to verify consistency of existing processes, appropriateness of specifications for starting materials and finished products are to be conducted. These reviews would highlight trends and identify processes for improvement and rectification. The review should cover:

- Starting materials and primary product contact packaging materials
- Critical in-process controls and finished product results
- All failed batches and their investigations
- Significant deviations or non-conformances. Their investigations and CAPA taken
- All changes carried out to processes or analytical methods
- Manufacturing variations
- Stability monitoring programs and adverse trends
- Returns, complaints and recalls
- Adequacy of previous product process or equipment corrective actions
- Variations to marketing authorizations and post-marketing commitments
- Qualification status of critical equipment and utilities.

Process performance and product quality must be managed throughout the drug product life cycle by appropriate management review. This review includes the results of regulatory inspections and findings, audits and other assessments and commitments made to regulatory authorities.

## 10.8 MANUFACTURING VARIATIONS

Pharmaceutical companies have to ensure GMP is under control at all times for the manufacture of drug products, and processes, conditions and specifications are in accordance with the CMC information submitted to regulatory authorities (refer to Section 10.1). In reality as time goes by there are inevitable changes to processes, conditions and specifications. The drivers for these changes are innovations, continual improvements, the results of process performance and product quality monitoring, and corrective and preventive actions.

These post-market approval changes are considered as manufacturing variations. Manufacturing variations are notified or filed to the authorities immediately where prior approval is needed for major changes. For minor amendments of lesser impact these are communicated to the authorities on an annual basis. In order to evaluate, approve and implement these changes properly, companies must have an effective change management system. A change management system should be based upon sound principles, as presented in ICH Q10 guideline, *Pharmaceutical Quality System*, which describes a comprehensive model for an effective pharmaceutical quality system.

In US, manufacturing variations are classified as major, moderate, or minor depending upon their degree or potential to adversely affect the drug product's identity, strength, purity, or potency. The classifications of changes are determined on a risk-based approach:

- Major Change: A change that has a substantial potential to have an adverse effect on the safety or effectiveness of the product. Major changes require the submission of a Prior Approval Supplement (PAS) to FDA, for which FDA must approve before distributing the product made using the change.
- Moderate Change: A change that has a moderate potential to have an adverse effect on the safety or effectiveness of the product. Moderate changes require the submission of a *Changes Being Effected in 30 Days Supplement* to FDA at least 30 days before distributing the product made using the change.
- Minor Change: A change that has a minimal potential to have an adverse effect on the safety or effectiveness of the product.

In Europe the type of variations are divided as below:

- Type IA Variation: Any well-defined minor change, to be notified within 12 months following implementation
- Type IAin Variation: Minor change; to be notified immediately after the changes have been implemented

- Type IB Variation: Minor change; this procedure is also considered a notification, but a regulatory authority assessment is made within 30 days; Type IB variation needs to be approved prior to implementation
- Type II Variation: Any major change to the marketing authorization holder's proposed documentation; a Type II variation is a product change that does not meet Type IA and Type IB classifications or criteria, but is not so extensive as to require a line extension or new application procedure; Type II needs to be approved prior to implementation
- Line Extension Application: A major change that requires a full assessment in accordance with Article 17 of Directive 2001/83/EEC, and listed in Annex II of Regulations 1084/2003 and 1085/2003.

Some examples of the EU variations are given in Exhibit 10.18.

## 10.9 CASE STUDY #10.1

### 10.9.1 GMP Compliance Problems and Drug Product Supply Shortages

The ideal expectation from FDA and other regulatory authorities would be a pharmaceutical manufacturing industry that is in compliance with GMP and keeps producing high quality products without extensive regulatory oversight. In truth GMP non-compliance occurs and can lead to product recalls and eventually shortages of drug supply to needed patients. The consequences may result in a failure to treat specific medical conditions, or the use of less desirable, often more expensive, and unfamiliar alternative medicinal products, or there is an increased potential for errors and poorer patient outcomes.

Figure 10.9 shows the number of product recalls in US from 2007 to 2013. The major reasons for the recalls are:

- 2010 – Impurities/degradation products, GMP deviations, products marketed without an approved NDA/ANDA
- 2011 – GMP deviations, products marketed without an approved NDA/ANDA, impurities/degradation products
- 2012 – Impurities/degradation products, GMP deviations, lack of assurance of sterility
- 2013 (second Quarter) – Lack of assurance of sterility, impurities/degradation products, presence of particulate matter.

One drastic consequence of the recalls is that of drug shortages, which are illustrated in Figure 10.10. The data shows that the majority of the problems are due to issue with sterile injectables. The core reasons for drug shortages are:

- Quality issue (46%)
- Discontinuation (14%)

**Exhibit 10.18 Examples of EU Manufacturing Variations**

**Type IA Examples**

- Purely administrative change related to the identity and contact details of MAH, manufacturer, supplier (IAin)
- Change in invented name of product (IAin – Centralized, 1B – Nat)
- Change in name of active or excipient (IAin)
- Change in name or address of manufacturer, supplier of active (IA)
- Change in name/address of manufacturer/importer of finished product (IAin – responsible for batch release, IA – not responsible)
- Deletion of manufacturing site for active, intermediate, finished product, packaging (IA)
- Change in manufacturer of active (IA – if from same company, II – if another company).

Note: Type IA variations – minor variations with only a minimal impact or no impact at all, on the quality, safety or efficacy of the medicinal product, and do not require prior approval before implementation. These variations do not require immediate notification and reporting is on an annual basis. Type IAin variations – these minor variations require immediate notification.

**Type II Examples**

- Addition of a new therapeutic indication or to the modification of an existing one
- Significant modifications of the summary of product characteristics due in particular to new quality, nonclinical, clinical, or pharmacovigilance findings
- Changes outside the range of approved specifications, limits, or acceptance criteria
- Substantial changes to the manufacturing process, formulation, specifications, or impurity profile of the active substance or finished medicinal product
- Modifications in the manufacturing process or sites of the active substance for a biological medicinal product.

Note: Type II variation – a major variation which is not an extension and may have a significant impact on the Quality, Safety, or Efficacy of a medicinal product.

*Source*: Data from European Medicines Agency 2014, *European Medicines Agency post-authorisation procedural advice for users of the centralized procedure*, viewed May 25, 2014, http://www.ema.europa.eu/docs/en_GB/document_library/Regulatory_and_procedural_guideline/2009/10/WC500003981.pdf

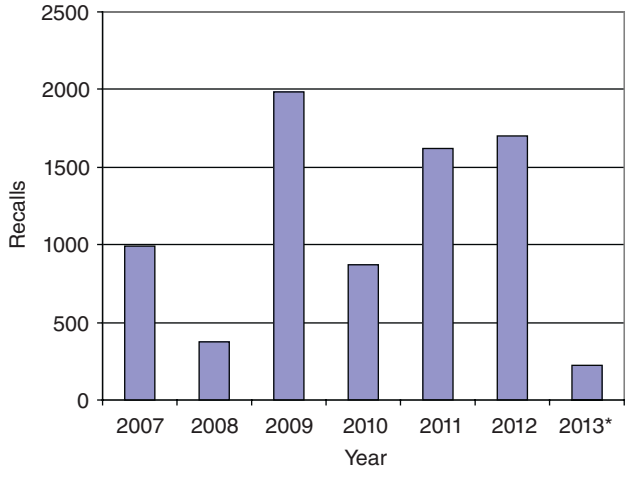



**Figure 10.9** Drug recalls in US (2007 to 2013). Note: 2013* – second quarter data only.

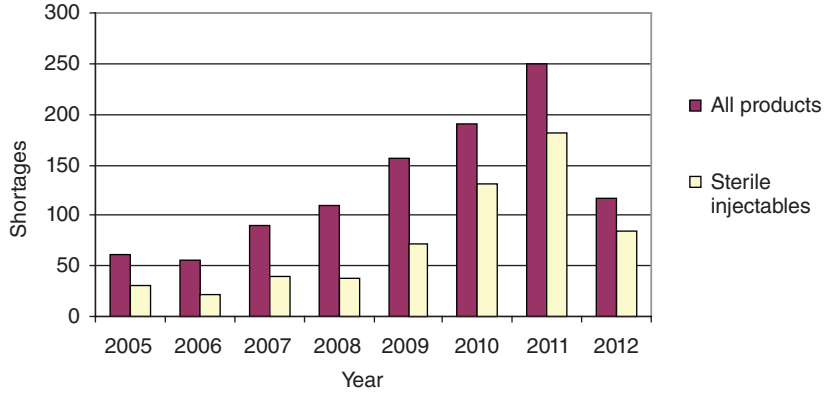



**Figure 10.10** Drug shortages in US (2005 to 2012).

- Raw materials (9%)
- Delays/capacity (6%)
- Loss of manufacturing site (3%)
- Increased demand (1%)
- Others (21%).

These data revealed that quality (GMP) issue causes significant drug shortages. This problem is similar in Europe. The regulator thus faces a dilemma as described by EMA below:

- In some cases defective medicines had to be left on the market to prevent shortages of life saving medicines as there is no available alternative and the risks linked to a

possible exposure with the defective product are considered less than those linked to the unavailability of the product
- In some cases of GMP non-compliance the ability of regulators to take action against a manufacturing site is restricted in order to avoid product shortages. Very difficult risk-benefit judgments had to be made between poor quality processes or product, or no product at all
- The switching of patients to alternatives may also be hindered by factors other than supply *per se* and beyond the control of the regulator.

Facing this dilemma FDA announced in October 2013 its action plan to remedy the drug shortages by:

- Releasing of a strategic plan to improve the agency's response to imminent or existing shortages, and for longer term approaches to address the underlying causes of drug shortages
- Issuing a proposed rule requiring all manufacturers of certain medically important prescription drugs to notify FDA of a permanent discontinuance or a temporary interruption of manufacturing likely to disrupt their supply.

Through this plan FDA aims to:

- Work with manufacturers to investigate the issue leading to the manufacturing disruption
- Identify other manufacturers that can make up all or part of the shortfall
- Expedite inspections and reviews of submissions from manufacturers of drugs that may prevent or mitigate a shortage
- Improve FDA's communications about shortages
- Clarify manufacturers' roles and responsibilities by encouraging them to engage in practices that will reduce the likelihood of a shortage
- Update FDA's internal procedures for responding to early notifications of potential shortages.

The plan is also intended to address the manufacturing and quality issues that are in most cases the root cause of the drug shortages, and they are:

- Broader use of manufacturing techniques to assist in the evaluation of manufacturing quality, as well as incentives for high-quality manufacturing
- Internal organization improvements within FDA to focus on quality, including a proposed Office of Pharmaceutical Quality within CDER
- Risk-based approaches to identify early warning signals for manufacturing and quality problems.

Similarly EU has an action plan to deal with shortages, which includes "requiring all marketing authorization applicants submit a risk-analysis of their manufacturing

process, identifying any weaknesses and, depending on the severity, provide a contingency plan and proposals to strengthen the identified weaknesses".

*Source*: 1. Food and Drug Administration 2013, FDA News Release: *FDA Takes Two Important Actions on Drug Shortages*, viewed May 24, 2014, http://www.fda.gov/newsevents/newsroom/pressannouncements/ucm373044.htm, 2. Stearn, DW 2013, *Compliance and Quality*, GPHA Technical Conference, Rockville, MD, viewed May 24, 2014, http://www.fda.gov/downloads/aboutfda/centersoffices/officeofmedicalproductsandtobacco/cder/ucm374190.pdf, 3. European Medicines Agency 2012, *Reflection Paper on Medicinal Product Supply Shortages Caused by Manufacturing/Good Manufacturing Practice Compliance problems*, viewed May 24, 2014, http://www.ema.europa.eu/docs/en_GB/document_library/Other/2012/11/WC500135113.pdf

## 10.10 CASE STUDY #10.2

### 10.10.1 Generics and Biosimilars

***Generics:*** From earlier chapters we know that generics are copies of "innovator" or "branded" drugs when their patents have expired. These generics are small molecule drugs and they are formulated with the same active ingredients, dosages and routes of administration as the innovator drugs. Typically they are sold at a fraction of the price charged by the pharmaceutical companies for their patented products. The price disparity of patented drugs and generics is due to the recovery of costs by the innovator pharmaceutical companies for expenses and risks taken in the discovery and development of the patented drugs, including preclinicals and clinical trials. By the time the patented drugs are approved by regulatory authorities, pharmaceutical companies typically have less than 10 years to recoup their investments. In contrast companies producing the generics do not have to invest in drug discovery nor the extensive preclinicals and clinical trials that are mandatory to bring a new drug to market. They do, however, need to demonstrate bioequivalence which costs miniscule amounts compared the expenses invested by the innovator pharmaceutical companies.

Generics came into effect through the Drug Price Competition and Patent Term Restoration Act of 1984 in the US, generally known as the Hatch-Waxman Act, which paved the way for the entry of generics as the US Congress sought to lower drug prices for consumers by encouraging generic competition. Since then many generics have appeared as soon as the patents of innovative drugs expired. In the US generics approval is via the ANDA process in accordance with the Federal Food, Drug and Cosmetic Act (FD&C Act), Section 505(j) (refer to Figure 8.5). An important criterion is that generics must show bioequivalence to the patented drug, that is, absorbed into the body in similar rate and extent.

In the EU, generics can be applied through an abridged procedure or centralized procedure according to Regulation 726/2004, Article 3(3). The applicant does not have to provide results of preclinical tests and clinical trials if there is evidence to demonstrate the product is a generic copy of an approved, reference drug. The generic is defined as a medicinal product that:

- Contains the same qualitative and quantitative composition in active substance as the reference medicinal product

- Possess the same pharmaceutical form as the reference medicinal product
- The bioequivalence of which has been demonstrated by appropriate bioavailability studies.

***Biosimilars:*** Biosimilars, or in some cases referred to as biogenerics and follow-on biologics, are by definition copies of off-patent biopharmaceuticals/biologics. The lack of consensus on the terms: biosimilars, biogenerics, and follow-on biologics, amply demonstrates the uncertainty in this area. The problem stems from the fact that biologics, by their very nature, are not as well defined as small molecule drugs and as such they are difficult to characterize precisely. They are sensitive to processing and storage conditions, for example, pH, pressure, temperature, mixing speed, in addition to the nutrients and growth factors added; all of which affect how the cells grow and express the proteins. Furthermore proteins are known to undergo post-translational modifications (glycosylation, sulfation, acetylation, phosphorylation etc.) that affect how the amino acids fold and interact in their 3D structures. Currently manufacturers of protein-based drugs have to demonstrate comparability of products if they make changes to their processes. This is to show that products remain as pure, safe, and effective irrespective of the changes and they are under control by the manufacturers.

Thus arguments abound as to what would constitute similarity in these protein drugs to claim them as biosimilars or biogenerics, considering that these proteins have thousands of atoms with molecular weight often in excess of 40 kDa with complex constructs, compared to small molecules of nominally less than 0.5 kDa with relatively simple structures.

This debate is particularly pertinent as some of these first generation biologics produced by rDNA technology have come off patent, as shown in the Table 10.3 below, and the generics industry aims to tap into this market, whilst at the same time regulatory authorities are under pressure to work towards making drugs more affordable to patients.

Although a number of assays and technologies are available to characterize and test protein molecules, such as peptide mapping, protein sequencing, carbohydrate analysis, electrophoresis, ELISA, mass spectroscopy and so on, they are not the definitive methods as those used for small molecule drugs. Hence the test for similarity is not as well-defined. However as technology progresses, this notion of similarity and that processes have great influence on structures is being challenged. Examples provided are the human growth hormones (hGH) from different sources which have all been approved and appeared to be similar in treatment efficacy: they are produced by Pharmacia and Ferring using standard *E. coli*, Eli Lilly and Novo Nordisk with special strain of *E. coli* and Serono through expression in mouse cell line.

Prior to 2010 there was no legislation for biosimilar approval in the United States. In 2006, FDA approved the first recombinant Omnitrope (somatropin), a human growth hormone for long-term treatment in pediatrics for growth failure and replacement therapy in adults with growth hormone deficiency. Omnitrope was approved as a biosimilar by comparing with Gonotropin, a product approved in 1995.

In 2010 a new act, the Patient Protection and Affordable Care Act, provided FDA with authority to approve biosimilars. The document, *Guidance for Industry - Scientific*

**TABLE 10.3 Biologics Products Facing Biosimilars Challenge**

| Product Class | Leading Brands | Company | EU Patent Expiry | US Patent Expiry |
|---|---|---|---|---|
| Erythropoietin alpha | Epogen/Procrit// Eprex/Erypo | Amgen/J&J | Expired | Expired |
| Erythropoietin beta | NeoRecormon | Roche/Wyeth/Chugai | Expired | Expired |
| Interferon-β1-a | Avonex, Rebif | Biogen Idec, Serono | Expired | Expired |
| Interferon-β1-b | Betaferon | Bayer (Schering) | Expired | Expired |
| Granulocyte-colony stimulating factor | Neupogen | Amgen | Expired | Expired |
| Interferon-α-2b | Intron A | Schering Plough | Expired | Expired |
| Interferon-α-2a | Roferon-A | Chiron | Expired | Expired |
| Soluble TNF-α receptor | Enbrel | Amgen/Wyeth | Expired | Expired |
| TNF-α antibody | Remicade | Centocor, Schering-Plough and Tanabe | Expired | Expired |
| CD20 antibody | MabThera/Rituxan | Genentech/Roche | Expired | 2015 |
| ErbB2 receptor antibody | Herceptin | Genentech/Roche | 2014 | 2014 |
| EGFR antibody | Erbitux | BMS | Expired | 2015 |
| VEGF antibody | Avastin | Roche | 2019 | 2017 |

*Source*: Ledford, H 2007, 'Biotechs go generic: The same but different,' *Nature*, 449, pp. 274-276. Reproduced with permission of Macmillan Publishers Ltd.

*Considerations in Demonstrating Biosimilarity to a Reference Product*, February 2012, presents FDA's approach to determine biosimilarity. To establish biosimilarity, there should be comparison "of the proposed product and the reference product with respect to structure, function, animal toxicity, human pharmacokinetics (PK) and pharmacodynamics (PD), clinical immunogenicity, and clinical safety and effectiveness". Even though the clinical study is not as extensive as that of the innovator reference product, FDA expects the applicant to demonstrate "there are no clinically meaningful differences between the biological product and the reference product in terms of the safety, purity, and potency of the product".

In EU the regulatory framework for biosimilar is detailed in: *Guideline on Similar Biological Medicinal Products*, October 2005. EMA acknowledged that biosimilars are unlike generics and that such biologics would require stringent testing before marketing authorization. Applicant would need to present "comparability studies to generate evidence substantiating the similar nature, in terms of quality, safety, and efficacy, of the new similar biological medicinal product and the chosen reference medicinal product

authorized in the Community". Due to the complex nature of vaccines and allergens, EMA would only consider this type of biosimilars on a case by case basis.

*Source*: 1. Food and Drug Administration 2012, *Guidance for Industry – Scientific Considerations in Demonstrating Biosimilarity to a Reference Product*, viewed May 24, 2014, http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM291128.pdf, 2. European Medicines Agency 2005, *Guideline on Similar Biological Medicinal Products*, viewed May 24, 2014, http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003517.pdf

## 10.11 SUMMARY OF IMPORTANT POINTS

1. Small molecule drugs are produced using organic synthesis processes whereas large molecule drugs are derived mainly from living cells, such as microbial and mammalian cells.
2. Regulatory authorities inspect GMP facilities to ensure compliance to GMP. FDA carries out surveillance and compliance inspections. A systems based approach is adopted: Quality, Facilities and Equipment, Materials, Production, Packaging and Labeling, and Laboratory Control. Deficiencies are reported in Form FDA-483, which may lead to warning letter and consent decree if unresolved.
3. Typically small molecule drugs are produced using reactions such as oxidation-reduction, acid-base, halogenation, alkylation and substitution. The resulting drug substance, called APIs, are recovered and purified from solvents. More recent methods aim to isolate chiral compounds to improve drug-target interactions.
4. Most large molecule drugs are produced in microbial, insect or mammalian cell culture systems using rDNA techniques. Foreign genes that express the desired drug molecules are inserted into plasmid vectors which are then introduced into the microbial or mammalian cells and grown in nutrient-rich media. The drug molecules are recovered through a series of chromatographic purification steps. As the end products are sensitive to environmental factors, they are not amenable to final heat sterilization. As such aseptic processes have to be practiced to prevent contamination.
5. Purified drug substances are mixed with excipients into finished dosage forms: solids, liquids, parenterals, inhalants, and ointments and creams, then packaged and labeled and shipped for distribution.
6. Non-compliance to GMP may lead to drug recalls and drug shortages. Patients could be adversely affected by drug shortages. FDA and EMA are introducing new action plans to deal with GMP non-compliance and drug shortages.
7. As novel drugs come off patents, generics are produced and increasingly compete with novel drugs. Biosimilars (refer to Section 10.10), however, due to the complexity of the drug molecules, are closely reviewed by regulatory authorities and only a handful have been approved.

## 10.12 REVIEW QUESTIONS

1. Differentiate the production processes for small and large molecule drugs.
2. Describe the system-based approach of FDA inspections, with reference to the surveillance and compliance inspection program.
3. Explain selected reaction synthesis and purification steps for the small molecule drugs.
4. Discuss the importance of testing and characterizing cell lines and cell banks.
5. Why is there a need to remove bovine serum from cell culture media?
6. Distinguish the characteristics of the different types of columns for protein purification.
7. Explain what is meant by biosimilars and the regulatory status with respect to approving these drugs.

## 10.13 BRIEF ANSWERS AND EXPLANATIONS

1. Refer to Sections 10.1, 10.4 and 10.5: organic synthesis route for small molecule drugs and living cells for large molecule drugs.
2. Refer to Section 10.3.
3. Refer to Section 10.4 and Exhibits 10.6 to 10.10.
4. As living cells are complex, it is necessary to establish they are of known history and free of components and viruses that may have deleterious effects on the drug molecules and humans when administered with such drug molecules.
5. Refer to Exhibit 10.13.
6. Refer to Exhibit 10.15.
7. Refer to Section 10.10. The regulatory authorities have introduced legislations for biosimilar approvals. As a minimum comparability studies and certain clinical data are necessary. For more complex molecules, the biosimliar application will be reviewed on a case by case basis.

## 10.14 FURTHER READING

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# CHAPTER 11

# FUTURE PERSPECTIVES

## 11.1 PAST ADVANCES AND FUTURE CHALLENGES

Drug discovery and development underwent astounding changes in the last two decades. These have been fuelled by advances in many areas, especially cell and molecular biology, recombinant DNA technology, genomics, proteomics, bio- and chemical informatics, as well as laboratory equipment and automation. In tandem with these advancements, there were changes in regulatory requirements, with the aim to approve drugs in an efficient manner for those in need of the medication. Great strides have also been made in the harmonization of regulations to adopt some form of international regulatory standards, in the hope of lowering regulatory costs and expediting approvals. New and efficient manufacturing technologies and processes have enabled purer and more effective drugs to be produced.

Against this backdrop of advances, the pharmaceutical industry also faces unprecedented challenges in many areas. The pipelines for new drugs are drying up, in spite of huge investments allocated for research and development. Drugs are not being discovered and developed fast enough to fill the pipeline. The prospects of generating more blockbuster drugs are not encouraging. Technologies, such as combinatorial chemistry and high throughput screening, have yet to live up to expectations of speeding up drug discovery. Clinical trials have become more complex, lengthy and expensive. Competitions from generics and biosimilars are gathering pace. In addition, there are ethical, social, political, and intellectual property issues that need due consideration. There are many more diverse questions, such as gene therapy, cloning, intellectual property,

*Drugs: From Discovery to Approval*, Third Edition. Rick Ng.

sustainable biodiversity, transgenic production systems, bioterrorism, cost of treatment, and quality of life, that challenge the industry to face squarely.

In this chapter, we discuss some of these issues and the likely course of events that may unfold in the decades ahead.

## 11.2 SMALL MOLECULE PHARMACEUTICAL DRUGS

There are two distinct routes to the discovery of small molecule drugs: (i) from natural sources and (ii) from rational design.

### 11.2.1 Drugs From Natural Products

Proponents of drugs from natural products argue that natural sources provide a vast diversity of chemical compounds. These compounds with myriad chemical compositions and structures serve as reservoirs for many pharmacologically active lead compounds to be discovered.

As described in Exhibit 3.2, there are now regulations enacted to protect the environment with respect to natural product collection. Bioprospecting from natural habitats has to take into account the 1993 *Convention on Biological Diversity*, the sovereign rights of nations over their biological resources have to be respected.

Hitherto, the normal source of collections has been from terrestrial habitats. However, marine bioprospecting represents a vast and relatively untapped area that is likely to be intensified. Exhibit 11.1 describes the diversity and life forms in marine habitat and examples of several drugs derived from this source.

It can be envisioned that laboratory equipment and assay systems will continue to play crucial roles in drug discovery from natural products. Although high throughput technologies have been able to screen hundreds of thousands of samples per day, there will continue to be a push for even higher density screening throughputs. Further miniaturization of liquid dispensing and more specific and sensitive assay systems will continue to be developed. Larger compound libraries and more comprehensive databases will provide another natural progression to widen the boundaries of chemical diversities, with the hope that drug candidates will be found within these boundaries.

### 11.2.2 Drugs From Rational Design

The use of technologies such as X-ray crystallography, NMR, bioinformatics, computational chemistry, and combinatorial chemistry have yet to realize their full potential to design safe and effective drugs with high success rates. The additional use of microarrays and proteomics to identify targets that cause diseases will help to better define the drug targets and identify possible binding sites. These technology platforms in combination provide powerful means to test potential drug candidates and their interactions with putative targets, with the ultimate aim of interrupting or diverting disease pathways.

An often-quoted limitation of rational drug design is the lack of biodiversity and chemical space (the various possible chemical compositions in nature, estimated to be as high as $10^{100}$ different compounds) in the libraries of compounds examined. The use

**Exhibit 11.1 Marine Bioprospecting**

There are 34 fundamental phyla of life: 17 occur on land and 32 in the sea (including some overlaps), more chemical diversity is found among marine life forms. Most of these are from invertebrate organisms—sponges, tunicates and mollusks. Some of the compounds from marine life forms are extremely potent, given that these organisms have to defend themselves from attacks in vast volumes of water that dilute the compound.

The range of climatic conditions, from tropical waters to cold arctic ocean, shallow continental shelves to great ocean depths with high pressure and low oxygen content, means that there is a potentially huge supply of life forms with extensive biodiversity.

A list of approved drugs derived from marine life forms is presented below:

| Chemical Compound | Brand Name | Marine Organism | Mechanism | Therapy Area |
|---|---|---|---|---|
| Brentuximab vedotin (SGN-25) | Adcetris | Mollusk | Chimeric monoclonal antibody that targets CD30 expressed on cancer cells | Hodgkin's lymphoma, anaplastic large cell lymphoma |
| Cytarabine (arabinofuranosyl cytidine) | Cytosar-U Depocyt | Sponge | Interferes with DNA synthesis | Acute myeloid leukemia, non-Hodgkin's lymphoma |
| Eribulin | Halaven | Sponge | Apoptosis of cancer cells | Metastatic breast cancer |
| Trabectedin (ecteinascidin 743) | Yondelis | Tunicate | DNA backbone cleavage and cell apoptosis | Soft tissue sarcoma, ovarian cancer |

*Source*: Adapted from 1. Willis, RC 2002, 'Nature's pharma sea', *Modern Drug Discovery*, 5, pp. 32–38, 2. Newman, DJ and Cragg, GM 2014, 'Marine-Sourced Anti-Cancer Pain Control Agents in Clinical and Late Preclinical Development', *Marine Drugs*, 12, pp. 255–278.

of advanced software, together with artificial intelligence for simulation, is an important tool to extend the chemical space and provide a greater diversity of chemical structures for use as scaffolds to test for potential drug candidates. There are likely to be more useful rules such as the Lipinski's rule (refer to Section 3.3.4) being implemented to test the scaffolds and functional groups to be attached. The development of intelligent, information-rich systems will be a key to the success of rational design technological platforms. Combinatorial chemistry with chiral selectivity will help to design and

generate potent drugs more expeditiously. Increasing contributions will be expected to come from siRNA and systems biology as these two fields of studies develop.

Imatinib mesylate (Gleevec, Novartis), zanamivir (Relenza, GlaxoSmithKline), and oseltamivir (Tamiflu, Roche) are examples of drugs (Exhibits 3.7 and 3.11) that show the successful contributions of rational drug design. A recent example is that of ibrutinib (Imbruvica, Pharmacyclics) which was discovered and developed using the rational drug design approach (Exhibit 11.2). It was approved by FDA via the Breakthrough Therapy Designation for the treatment of mantle cell lymphoma (MCL) and chronic lymphocytic leukemia (CLL).

## 11.3 LARGE MOLECULE BIOPHARMACEUTICAL DRUGS

The biopharmaceutical industry for producing large molecule protein-based drugs has grown exponentially in the past 30 years (refer to Exhibit 4.1). The knowledge base of life sciences doubles every 14 months. Molecular biology has enabled many types of proteins being expressed in different cell systems. Manufacturing processes for producing consistent, pure and potent biopharmaceuticals are now widely available.

To date, most approved protein-based drugs are for replacement therapies, therapeutics, and prophylactics. Products for replacement therapies are recombinant versions of natural proteins such as insulin and erythropoietin. Their characteristics and functions are relatively well defined and known. Biopharmaceuticals such as antibodies for therapeutics and vaccines for prophylactics are more complex and require more tests and characterizations. Controls for the reliability, contamination-free and fidelity of expression systems are key strategies to producing these antibodies and vaccines.

The high cost of biopharmaceuticals has been due in part to the stringent requirements of aseptic manufacturing processes, low productivity of cell lines, rigorous process control, and stability/sensitivity issues of the proteins. Some efforts are likely to be directed at developing more robust protein expression systems, high productivity cell lines, better control parameters for efficient, and reliable manufacturing processes, and more stable formulations. Production of biopharmaceuticals using transgenic plants and animals is another technological development and may also lower manufacturing costs (refer to Section 11.12).

Most forecasts predict that we are at the threshold of seeing many more effective and potent biopharmaceuticals for a variety of treatments. An example is the emergence of immunotherapeutics, which is to stimulate the body's own immune system to work better or smarter in the fight against diseases, particularly cancer (Exhibit 11.3).

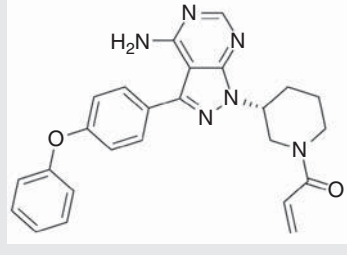
## 11.4 TRADITIONAL MEDICINE

In spite of the discovery and development of many new 'Western' drugs, there remains a demand for traditional medicine (TM). TM is also called Complementary Medicine (CM) or Alternative Medicine (AM). It is likely that TM demand will continue when there are still unmet needs to be filled. The quandary with Western drugs is that pharmaceutical firms have to ensure a reasonable return to shareholders' investment. With

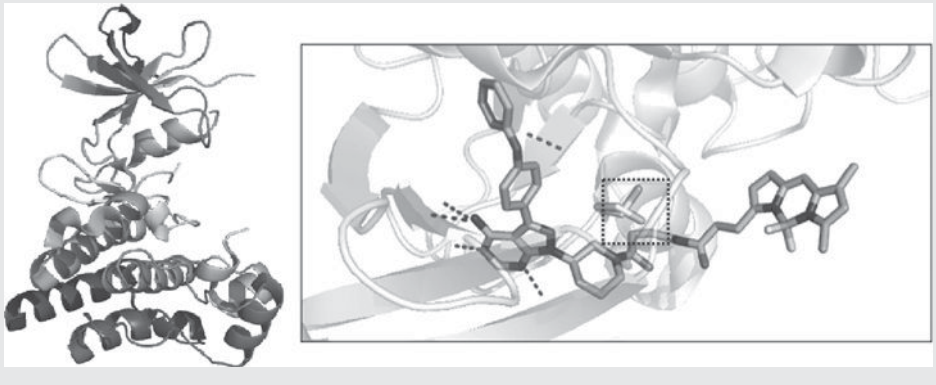
**Exhibit 11.2 Imbruvica**

Imbruvica (ibrutinib) is an inhibitor of the enzyme Bruton's tyrosine kinase (BTK). It is approved by FDA for the treatment of mantle cell lymphoma (MCL – a rare form of non-Hodgkin's lymphoma) in 2013 and for chronic lymphocytic leukemia (CLL – the most common type of leukemia in adults) in 2014.

Imbruvica is a white powder which has an empirical formula of $C_{25}H_{24}N_6O_2$ and a molecular weight 440.50. The chemical name for Imbruvica is 1-[(3R)-3-[4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl]-1-piperidinyl]-2-propen-1-one. The chemical structure is shown below:




Imbruvica forms a covalent bond with a cysteine residue in the BTK, as depicted in the figure below:



BTK molecule

Imbruvica bonded with the cysteine residue (dotted square). Hydrogen bonding with O and N atoms shown in dotted lines (left). A fluorescent dye is attached to Imbruvica (right) for enhanced imaging.

BTK is a signaling molecule of the B-cell antigen receptor (BCR) and cytokine receptor pathways which lead to development of malignancies. The interaction between Imbruvica and BTK stops the chain of signaling pathways between the cell surface receptor and the genes within the nucleus. This leads to the stoppage of proliferation of malignant B-cell.

The Imbruvica mechanism of action has opened up a new research area in the development of BTK-targeted anti-cancer drugs.

*Source*: Turetsky, A *et. al*. 2014, 'Single cell imaging of Bruton's Tyrosine Kinase using an irreversible inhibitor', *Scientific Reports*, Article Number 4782, doi: 10 1038/srep04782, viewed June 8, 2014, http://www.nature.com/srep/2014/140424/srep04782/full/srep04782.html. Data from 1. de Lartigue J 2014, *Success of First BTK Inhibitor Opens New Options in B-cell Malignancies*, OncLive, viewed June 8, 20145, http://www.onclive.com/publications/Oncology-live/2014/February-2014/Success-of-First-BTK-Inhibitor-Opens-New-Options-in-B-Cell-Malignancies; 2. Food and Drug Administration 2013, *Imbruvica*, viewed June 8, 2014, http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/205552lbl.pdf.

**Exhibit 11.3 Immunotherapeutics**

The American Cancer Society defines immunotherapy as:

> "Treatment that uses certain parts of a person's immune system to fight diseases such as cancer."

This can be achieved through -

- Stimulating the body's own immune system to work harder or smarter to attack cancer cells
- Providing immune system components, such as man-made immune system.

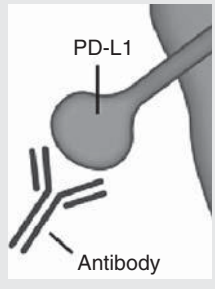
A number of immunotherapeutics have been approved recently to treat cancer. Amongst these are Provenge (sipuleucel-T) and Zytiga (abiraterone) for the treatment of prostate cancer, and Yervoy (ipilimumab) and Zelboraf (venurafenib) for treatment of metastatic melanoma.

Some details about these drugs are:

- *Provenge*: treatment is a three step process; (i) patient's white blood cells are extracted, (ii) the white blood cells are incubated with an immune signaling factor (granulocyte macrophage stimulating factor, Provenge), (iii) the incubated product is infused into the patient.
- *Zytiga*: a small molecule inhibitor of the enzyme CYP17 lyase. This enzyme is expressed in testicular, adrenal and prostatic tumor tissues and is required for androgen biosynthesis.
- *Yervoy*: a monoclonal antibody that stimulates the cytotoxic T lymphocytes to target and destroy cancer cells. It is approved for treatment of metastatic melanoma and currently undergoing clinical trials for treatment of non-small cell lung cancer, small cell lung cancer, bladder cancer and prostate cancer.

- *Zelboraf*: a small molecule B-Raf enzyme inhibitor: the first drug designed using fragment-based lead discovery platform.

Another development in immunotherapeutics is nivolumab. The T cells in the human body are required for immune response (refer to Exhibit 4.7). Many cancer cells make a molecule called "programmed cell death 1 ligand 1 (PD-L1)". The PD-L1 attaches to the PD-1 receptor on the surface of T cells and kills the T cells or renders them ineffective. Nivolumab targets the PD-L1, thus blocking it from binding to PD-1, and sparing the T cells to mount an immune response against the cancer cells.




*Source*: Data from 1. American Cancer Society 2014, *What is Immunotherapy*?, viewed June 9, 2014, http://www.cancer.org/treatment/treatmentsandsideeffects/treatmenttypes/immunotherapy/immunotherapy-what-is-immunotherapy; 2. Food and Drug Administration 2010, *Provenge*, viewed June 9, 2014, http://www.fda.gov/downloads/BiologicsBloodVaccines/CellularGeneTherapyProducts/ApprovedProducts/UCM210031.pdf; 3. Food and Drug Administration 2011, *Zytiga*, viewed June 9, 2014, http://www.accessdata.fda.gov/drugsatfda_docs/label/2012/202379s004lbl.pdf; 4. Food and Drug Administration 2011, *Yervoy*, viewed June 9, 2014, http://www.accessdata.fda.gov/drugsatfda_docs/label/2012/125377s033lbl.pdf; 5. Food and Drug Administration 2014, *Zelboraf*, viewed June 9, 2014, http://www.fda.gov/safety/medwatch/safetyinformation/ucm364374.htm; 6. Mkrtickyan, M *et al*. 2013, 'Anti-PD-1 antibody significantly increases therapeutic efficacy of Listeria monocytogenes (Lm)-LLO immunotherapy', *Journal of ImmunoTherapy of Cancer*, 1:15, doi:10.1186/2051-1426-1-15.

many drug discovery and development programs costing billions of dollars, most pharmaceutical firms concentrate on blockbuster drugs with large market potential. The way is thus left open for the treatment of many therapies to be filled by other means such as TM.

Another perceived view about Western drugs is the adverse effects. It is sometimes viewed that Western medicine is akin to a "sledgehammer" method rather than the more holistic approach using TM. Costs of Western medicine can be prohibitive, especially for patients in countries where there are no comprehensive healthcare systems. For example, treatment with pegylated interferon for hepatitis C or antibody for colon cancer can cost tens of thousands of dollars per year. Limitations of Western drugs in some cases, such as resistance to antibiotics, also highlight the problems and pave way for people to consider TM.

For TM to be accepted into mainstream medical treatment, a necessary scenario is the application of scientific methodologies and controls for TM development, evaluation and production. Many of the tools for high throughput screening (HTS) and assay systems can be used to test the efficacy of TM, similar to the irrational approach of screening natural products. Pharmacology studies have to be conducted in accordance with Good Laboratory Practice (GLP).

The conditions for growing and harvesting of traditional herbs and plants are sources of variability and contamination. Good Agricultural Practice (GAP) is required to minimize contamination, such as elimination of heavy metals, use of approved fertilizers, and also to improve agricultural methods for ensuring consistent production levels of active ingredients. Factors such as climatic variations and processing conditions may also affect the quality of TM. Better characterization methods, as well as Good Manufacturing Practice (GMP), are needed for scientifically-based development and production of reliable, consistent, and efficacious TM.

TM of proven quality, efficacy and safety can help to maintain good health. Recognizing the contributions from TM, the World health Organization (WHO) has developed the *WHO Traditional Medicine Strategy 2014–2023* that aims to help member states develop regulations for harnessing effective TM and applying safety practices to improve patient health and wellbeing. A summary is presented in Exhibit 11.4.

Some regulatory authorities have foreseen the future impact of TM and set up appropriate guidelines. The EU has legislations for traditional herbal products. Another example is the Therapeutic Goods Administration of Australia, which has set up a CMs section that controls the regulatory practices for TM.

**Exhibit 11.4 The WHO Traditional Medicine Strategy, 2014–2023**

Two key steps are proposed for TM strategy:

- Member states should define and better understand TM within their own national situation
- Member states should develop policies, regulations and guidelines for those TM that meet the health needs of their people.

To achieve these key steps, WHO recommended to the member states to:

- Build knowledge base for active management of TM through appropriate national policies
- Strengthen quality assurance, safety, proper use and effectiveness of TM by regulating products, practices and practitioners
- Promote universal health coverage by integrating TM services into health service delivery and self-health care.

*Source*: Data from World Health Organization 2013, *WHO Traditional Medicine Strategy 2014–2023*, viewed May 31, 2014, http://apps.who.int/iris/bitstream/10665/92455/1/9789241506090_eng.pdf?ua=1

## 11.5 PERSONALIZED MEDICINE

The challenge is that, one day, drugs will be tailor-made for individuals and adapted to each person's own genetic makeup. In this way, the drugs will be used optimally and adverse events minimized, if not eliminated. Environment, diet, age, lifestyle, and state of health can all influence a person's response to drugs, but an understanding of a person's genetic makeup is thought to be the key to creating personalized drugs.

An individual's blood samples can be collected and analyzed. Through the study of single nucleotide polymorphisms and pharmacogenomics, the genes that cause diseases can be pinpointed. The results will show the individual's disease condition or predisposition to some ailments. In this way, treatment or preventive measures can be prescribed. Exhibit 11.5 shows an example of how differences in genetic make-up affect the effectiveness of a drug on individuals.

**Exhibit 11.5 Genetic Variations**

Single-nucleotide polymorphisms (SNPs) are DNA sequence variations among individuals. SNPs are the most common form of human variation, occurring approximately every kilobase. On average there are about 5–10 million SNPs between two individuals across the entire genome. SNPs are associated with various medical conditions. Studying the differences in SNP patterns among various human populations may help to predict disease risk and response to therapies. Hence it is hoped that knowledge of SNPs will improve medical treatment and care.

*Source*: Barnes, MR and Breen, G (eds.) 2010, *Genetic Variation*: *Methods and Protocols*, Springer, New York, NY.

In one study, researchers from Vanderbilt University in Nashville, US, compared responses to a $\beta$-blocker called atenolol among 34 patients. All patients had genetic variations affecting one of the building blocks of the receptor that binds to $\beta$-blocker drugs, which affected the way the receptor responded to the binding of the drug.

Thirteen had one type of genetic variation, Gly389, and 21 had another variation called Arg389.

Patients with the Arg389 variant achieved a significantly lower resting blood pressure and heart rate with the drug than did Gly389 patients, suggesting that the drug was more effective for them. However, this finding held true only at rest, and not during exercise.

*Source*: Data from Sofowora, GG *et al*. 2003, 'A common $\beta$1-adrenergic receptor polymorphism (Arg389Gly) affects blood pressure response to $\beta$-blockade', *Clinical Pharmacology & Therapeutics*, 73, pp. 366–371

It is envisaged that cheaper and more affordable drugs may also result as drugs are designed for specific groups of people. Clinical trials may be shortened considerably and save millions of dollars. Large patient population groups to trial a drug may no longer be required. These drugs can also be introduced faster into the market to treat people in need of such tailor-made medications.

The approval of trastuzumab (Herceptin) in 1998 was the first therapy using genetic biomarker for the treatment of women with HER-2 positive metastatic breast cancers. A list of selected drugs and their respective biomarkers is presented in Table 11.1. A recent personalized drug is Kalydeco, which was approved in 2012 by FDA for cystic fibrosis patients with the G551D genetic mutation.

## 11.6 GENE THERAPY

Currently, there is still a gap for the potential of gene therapy to be fulfilled. Gene therapy clinical trials have been conducted for diseases such as severe combined immunodeficiency disease (SCID, "bubble baby" syndrome), sickle cell anemia, cystic fibrosis, familial hypercholesterolemia, and Gaucher's disease.

The aim of gene therapy is to supply healthy genes to replace those that are missing or flawed. One key to the success of gene therapy is the vectors that are used to transport the genes (refer to Section 4.6). Another critical success factor is the understanding of the effects and functions of genes. Of the estimated 20,500 human genes, we know the functions of relatively few. Although some diseases such as sickle cell anemia and cystic fibrosis are caused by single genes, there are other diseases that may be the result of multiple gene disorders, and the relationships of these genes have to be studied.

There are two types of gene therapy: somatic cell and germ cell gene therapy. Somatic cells are non-reproductive cells and, as such, somatic cell gene therapy affects the individual only. The change in gene is not passed on to the next generation. Germ cell gene therapy involves changes to the reproductive cells, the sperm and egg, with the result that the new genes are passed on to future generations. Although most researchers support research on somatic cell gene therapy, there are differences in opinion concerning germ cell gene therapy. For example, the ethical questions are:

- If gene therapy can remedy missing or faulty genes, why can it not be applied to germ cell gene therapy to stop the fault from passing onto future generations, such as in the case of Huntington's disease?
- Gene therapy is costly. Who decides which patient receives the therapy? Who pays for the treatment?

Refer to Exhibits 4.16, 6.17 and 11.15 for more information on gene therapy.

## 11.7 CLONING AND STEM CELLS

Cloning can be divided into therapeutic cloning and reproductive cloning. Therapeutic cloning is synonymous with stem cell research. Under proper control and environment,

**TABLE 11.1 Selected Drugs and Their Genetic Biomarkers**

| Drug | Original Approval Date | Therapeutic Area | Biomarker |
|---|---|---|---|
| Arsenic Trioxide | 2000 | Oncology | PML/RAR$\alpha$ |
| Tretinoin | 1995 | Oncology | PML/RAR$\alpha$ |
| Brentuximab Vedotin | 2011 | Oncology | CD30 |
| Capecitabine | 1998 | Oncology | DPD |
| Flurouracil | 1998 | Oncology | DPD |
| Cetuximab | 2004 | Oncology | EGFR; KRAS |
| Panitumumab | 2006 | Oncology | EGFR; KRAS |
| Crizotinib | 2011 | Oncology | ALK |
| Denileukin Diftitox | 1999 | Oncology | CD25/IL2 |
| Exemestane | 1999 | Oncology | ER/PR |
| Fulvestrant | 2002 | Oncology | ER/PR |
| Letrozole | 1997 | Oncology | ER/PR |
| Imatinib | 2003 | Oncology | C-Kit, PDGFR, FIP1L1 |
| Lapatinib | 2007 | Oncology | HER2 |
| Pertuzumab | 2012 | Oncology | HER2 |
| Trastuzumab | 1998 | Oncology | HER2 |
| Everolimus | 2009 | Oncology | HER2 |
| Nilotinib | 2007 | Oncology | Ph Chromosome |
| Dasatanib | 2006 | Oncology | Ph Chromosome |
| Imatanib | 2003 | Oncology | Ph Chromosome |
| Rasburicase | 2002 | Oncology | G6PD |
| Tositumomab | 2003 | Oncology | CD20 antigen |
| Vemurafenib | 2011 | Oncology | BRAF |
| Citalopram | 1998 | Psychiatry | CYP2C19 |
| Valproic Acid | 1978 | Psychiatry | UCD |
| Pimozide | 1984 | Psychiatry, Neurology | CYP2D6 |
| Aripiprazole | 2002 | Psychiatry, Neurology | CYP2D6 |
| Iloperidone | 2009 | Psychiatry, Neurology | CYP2D6 |
| Tetrabenazine | 2008 | Psychiatry, Neurology | CYP2D6 |
| Thioridazine | 1962 | Psychiatry, neurology | CYP2D6 |
| Ivacaftor | 2012 | Pulmonary | CFTR |
| Celecoxib | 1998 | Analgesics | CYP2C9 |
| Maraviroc | 2007 | Antivirals | CCR5 |
| Lenalidomide | 2005 | Hematology | Chromosome 5q deletion |

*Source*: Food and Drug Administration 2013, *Paving the Way for Personalized Medicine*, FDA's Role in a New Era of Medical Product Development, viewed May 31, 2014, http://www.fda.gov/downloads/scienceresearch/specialtopics/personalizedmedicine/ucm372421.pdf.

embryonic stem cells can potentially be directed to grow and develop into different tissues that are invaluable for repairing damaged or diseased tissues and organs. Reproductive cloning is the replication of another living being with genes from only one individual. An example is the cloning of Dolly the sheep in 1996 (Exhibit 11.6).

As discussed in Section 4.7, stem cells have the potential to treat medical conditions beyond the scope that can be offered by drugs alone. However, there are many

**Exhibit 11.6 Dolly the Sheep (1996–2003)**

In February 1997, the Scottish scientist, Ian Wilmut, and colleagues at the Roslin Institute announced the birth of a cloned sheep called Dolly in July 1996. They had removed the nucleus from the egg cell of a sheep and replaced it with the nucleus from an adult sheep. Dolly was born from a surrogate mother sheep and is an exact clone of the adult sheep, unlike offspring from the reproductive process, in which the offspring inherits the genes from both parents.

Dolly suffered from premature arthritis in 2002 and had to be put down in February 2003 at the age of 6½, because of progressive lung disease common in older sheep. It is not known whether Dolly's premature death is related to cloning; her life was about half the normal sheep lifespan of 12 years.

scientific and ethical hurdles to overcome. On the scientific front, stem cell research activities will intensify over the next decade. These challenges can be broadly divided into (i) determining how to develop stem cells into specific tissues, and (ii) implanting these stem cells or tissues into the body without rejection by the recipient's immune system. On the ethical front, it is expected that there will be more debates on the moral issues of stem cell research. Most scientists consent to therapeutic cloning (stem cell research) but not reproductive cloning. The ethical issue of stem cell research concerns harvesting cells from embryos that are a few days old. This action destroys the embryos. Some questions are:

- What is the legal and religious status of the embryos?
- Who has the right to give informed consent?
- Under what conditions can this consent be given?

In November 2003, the members of the Europe Parliament voted to approve embryonic stem cell research, using techniques similar to that adopted for cloning Dolly the sheep, although severe restrictions were put in place. For US scientists, however, the US legislation meant that they were only allowed to perform research using 12 existing sources of the embryonic stem cells, and were not allowed to create any new sources.

Currently there are three types of stem cells:

- *Adult Stem Cells*: Present in unspecialized cells and umbilical cord blood; able to differentiate into some or all other cell types
- *Human Pluripotent Stem Cells*: Developed from eggs that have been fertilized *in vitro*; able to develop into all cell types
- *Induced Pluripotent Stem Cells*: Use of laboratory techniques to reprogram adult cells, such as skin cells to develop into other cell types.

The first stem cell related product approved by FDA is Hemacord, a hematopoietic progenitor cells from cord blood for transplantation into patients with disorders affecting the hematopoietic system (Exhibit 11.7).

**Exhibit 11.7 Hemacord**

Hemacord contains hematopoietic progenitor cells, monocytes, lymphocytes, and granulocytes. It is obtained from human cord blood that is recovered from umbilical cord and placenta. Hemacord is used on patients with hematopoietic disorders (disorders in blood cells formation).

Each unit of Hemacord contains greater than $5.0 \times 10^8$ nucleated cells with a minimum of $1.25 \times 10^6$ viable CD34+ cells and are suspended in 10% dimethyl sulfoxide (DMSO) and 1% Dextran 40, at the time of cryopreservation. The minimum dosage is $2.5 \times 10^7$ nucleated cells/kg at cryopreservation. Patients are matched to at least 4 of 6 specific antigens. Administration is via intravenous infusion.

The infused cells from Hemacord migrate to the bone marrow, where they grow, divide, and mature. These cells then circulate in the bloodstream to other parts of the body and help to restore blood counts and functions.

*Source*: Data from Food and Drug Administration 2011, *Hemacord*, viewed June 9, 2014, http://www.fda.gov/downloads/BiologicsBloodVaccines/CellularGeneTherapyProducts/ApprovedProducts/UCM279612.pdf

## 11.8 OLD AGE DISEASES AND AGING

We are living in an aging society. The United Nations estimates that the world will have two billion people over the age of 60 by 2050. With the aging population, there are old age diseases that we have to face and treat with more effective therapies. Hypertension, strokes, Alzheimer's disease, heart diseases, type 2 diabetes, Parkinson's disease, and osteoporosis are some examples (Exhibit 11.8).

For some of these diseases, such as hypertension and heart disease, drugs such as diuretics, ACE inhibitors, calcium channel blockers and $\beta$-blockers are available for treatment. For some other diseases, such as Alzheimer's disease, more effective drugs have yet to be discovered (refer to Section 11.18). For stroke, recent drug such as dabigatran (Pradaxa) lowers the risk of stroke. Vorapaxar (Zontivity) is another latest drugs approved by FDA (May 2014) to reduce the risk of heart attacks and stroke in high-risk patients (Exhibit11.9)

It is expected that, in the coming decades, there will be more research on aging. Exhibit 11.10 describes some recent findings concerning aging.

## 11.9 LIFESTYLE DRUGS

As society becomes more affluent, there are demands for "lifestyle" drugs to treat non-life threatening conditions, or even to make a person feel more confident or look better. It is likely that there will be a proliferation of lifestyle drugs in the future.

**Exhibit 11.8 Old Age Diseases**

**Hypertension**

Hypertension, or high blood pressure, is the elevation of arterial blood pressure. For an adult, a systolic pressure above 140 mmHg or diastolic pressure above 90 mmHg is considered hypertension. The cause for hypertension may be due to narrowing or hardening of blood vessels, kidney diseases, or other unknown origins. Some commonly used drugs for the treatment of hypertension are angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor blockers, and calcium antagonists (calcium-channel blockers). The mechanism is such that ACE inhibitors block the enzyme from hydrolyzing angiotensin I to angiotensin II; angiotensin II receptor blockers stop the effects of angiotensin while calcium antagonists are used to reduce heart rate and relax blood vessels.

A five-year randomized clinical trial in US found that in spite of the availability of hypertensive medication, awareness promotions and guidelines, only a third of all the hypertensive patients have their blood pressure under effective control due to non-compliance in medication. More tailored behavioral management intervention may help to improve compliance and have better control.

*Source*: Data from osworth, HB *et al*. 2007, 'The Take Control of your Blood Pressure Study: Study Design and Methodology', *Contemporary Clinical Trials*, 28, pp. 33–47.

**Stroke**

A stroke occurs when there is an interruption of blood supply to the brain. An ischemic stroke occurs when a clot prevents blood flow in the brain. A hemorrhagic stroke is when there is a rupture of a blood vessel in the brain. In either case, the brain cells in the affected area die. This area is called an infarct. Medical treatment is required to arrest the damage. More effective treatment can be administered within 6 hours of the onset of stroke. A stroke may result in weakness, paralysis, impairment of speech and memory, or even death. Medical treatment includes the use of anticoagulants to treat stroke victims.

**Alzheimer's disease**

This disease is due to the accumulation of $\beta$-amyloid protein in the brain. The protein is believed to trigger brain degeneration through cell deaths of the neurons. Alzheimer's disease is characterized by loss of memory and intellectual performance, and slowness in thought. In the US, a class of drugs called cholinesterase inhibitors is approved to treat Alzheimer's disease. Both Europe and US have approved a drug called memantine for treatment of Alzheimer's disease.(refer to Section 11.18).

*Source*: Data from Food and Drug Administration 2013, *Namenda*, viewed June 9, 2014, http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/021487s010s012s014,021627s008lbl.pdf

### Diabetes

Refer to Exhibit 4.14 for a description of diabetes.

A new drug approved by FDA for diabetes mellitus type II is Tanzerum. It is a glucagon-like peptide-1 (GLP-1) receptor agonist, a hormone that helps to normalize blood sugar levels.

*Source*: Data from Food and Drug Administration 2014, FDA News Release, *FDA approves Tanzeum to treat type 2 diabetes*, viewed June 19, 2014, http://www.fda.gov/newsevents/newsroom/pressannouncements/ucm393289.htm.

### Parkinson's disease

Parkinson's disease is a progressive neurological disorder that develops most often after the age of 50. It is due to the degeneration of neurons in the part of the brain that controls movement. The degeneration of neurons causes a decrease in the level of dopamine, a neurotransmitter chemical necessary for the proper transmission of signals. Patients experience tremors in limbs, rigidity, difficulty in movements, and loss of facial expression.

*Source*: Data from PubMed Health 2013, *Parkinson's disease*, viewed June 9, 2014, http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0001762/.

### Osteoporosis

Osteoporosis is the loss of structural bony tissue, and gives rise to brittleness in bones. This may lead to fractures of hips, spines and wrists. Osteoporosis can begin at a young age if a person does not receive enough calcium and vitamin D. A person reaches maximum bone strength between 25 and 30 years of age; after that, the bone strength decreases by about 0.4% per year. After menopause, bone strength reduces by about 3% per year. Drugs such as estrogen, calcitonin, alendronate, raloxifene, and risedronate are approved for the treatment of postmenopausal osteoporosis.

*Source*: Data from PubMed Health 2012, *Osteoporosis* – overview, viewed June 9, 2014, http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0001400/

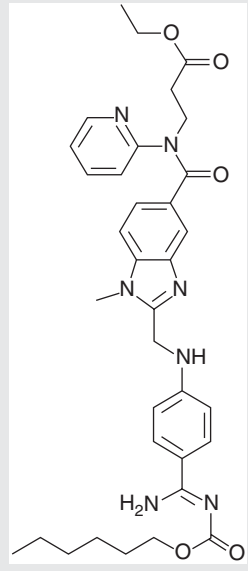
The major areas for lifestyle drugs are:

- Obesity treatment
- Aging: enhance muscular tone and youthful vitality
- Aging: anti-wrinkles

**Exhibit 11.9 Pradaxa and Zontivity**

**Pradaxa**

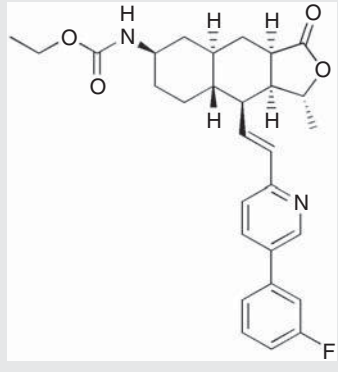
Pradaxa, active ingredient dabigatran etexilate mesylate, is a blood thinner medication to reduce the chance of blood clots formation. The empirical formula is $C_{34}H_{41}N_7O_5.CH_4O_3S$ and the molecular weight is 723.86 (mesylate salt), 627.75 (free base). The structural formula is:



In the clotting process thrombin enables soluble fibrinogen to be converted into insoluble strands of fibrin which cross-link and form clots. The active ingredient, dabigatran, is a thrombin inhibitor and interferes with the clotting process, thus reducing the risk of stroke and systemic embolism in patients with atrial fibrillation.

**Zontivity**

Zontivity (vorapaxar sulphate) is a protease-activated receptor-1 (PAR-1) antagonist. The empirical formula is $C_{29}H_{33}FN_2O_4{\cdot}H_2SO_4$, and its molecular weight is 590.7. The structural formula is:



Zontivity is an anti-platelet drug used to decrease the chance for platelets clump together and form blood clots. Through this action, Zontivity decreases the risk of heart attack and stroke.

*Source*: Food and Drug Administration 2014, *Pradaxa*, viewed June 10, 2014, http://www.fda.gov/safety/medwatch/safetyinformation/ucm250657.htm. Food and Drug Administration 2014, FDA *approves Zontivity to reduce the risk of heart attacks and stroke in high-risk patients*, viewed June 10, 2014, http://www.fda.gov/newsevents/newsroom/pressannouncements/ucm396585.htm

- Memory enhancement
- Sexual dysfunction
- Smoking cessation
- Hair loss therapy.

We have witnessed lifestyle drugs in the form of orlistat (Xenical, Roche) prescribed for obesity management (Exhibit 2.13), and growth hormones have been promoted for enhancement of muscle tone and youthful vitality (Exhibit 4.12). Botulinum toxin (Botox) is being injected as an anti-wrinkle treatment (Exhibit 11.11), and a vitamin A derivative (Retin-A gel, Tretinoin) is prescribed for the treatment of facial wrinkles. Drugs such as tacrine and donepezil, which work by attacking enzymes that breakdown

**Exhibit 11.10 Aging**

Aging is another research area that challenges scientists to understand and perhaps devise means to slow the process. An increasing number of scientists believe that aging is due to the prolonged process of oxidative damage. It has been found that oxygen radicals attack cell proteins and membranes, with the mitochondria being the most susceptible.

An antioxidant enzyme, superoxide dismutase (SOD), breaks down oxygen radicals and renders them harmless. Fruit flies and rats with mutated genes that express SOD live 40% longer than normal. Can this antioxidant really prolong human lifespan? There are no proven data to show that consuming copious amounts of antioxidant will help. One possible reason is that the human body can only accept a certain level of antioxidant; excessive amounts are excreted.

Proposed path of extending human life span includes:

- Curing diseases and replacing damaged body parts with stem cell therapies
- Slowing aging process on the cellular and molecular levels.

*Source*: Data from 1. Olshansky, SJ, Hayflick, L and Carnes, BA 2002, 'No truth to the fountain of youth', *Scientific American*, June, pp. 92–95; 2. Harmon, K 2012, 'How We All Will Live to Be 100', *Scientific American*, September, vol. 307, pp. 54–57.

**Exhibit 11.11 Botox**

Botox is a toxin produced by the bacterium *Clostridium botulinum*. When Botox is injected into facial tissues, it is absorbed by the nerve endings of muscle fibers. Nerve transmissions are interrupted and consequently the muscle relaxes. The relaxed muscle is then no longer effective to pull the facial lines to show the wrinkles.

Treatment with Botox is temporary. Once the nerve endings return to normal, the muscle will resume its contractual pull on the wrinkles.

acetylcholine, have been approved for boosting memory. Sildenafil (Viagra, Pfizer) is used to treat sexual dysfunction (Exhibit 3.18).

Bupropion (Zyban, GlaxoSmithKline) and varenicline (Chantix in US, Champix in other countries, Pfizer) are approved drugs for use as aids to smoking cessation, and finasteride (Propecia, Merck) has been approved by FDA for the treatment of male baldness. Based on the current trend, we can only expect that more lifestyle drugs will be developed and approved in the coming decades.

## 11.10 PERFORMANCE-ENHANCING DRUGS

In this competitive world, especially in the sports field, athletes try their best to outperform each other for glory and wealth. Unfortunately, some athletes resort to the use of performance-enhancing drugs to attain a competitive edge. Table 11.2 is a list of banned performance-enhancing drugs published by the World Anti-Doping Association (WADA) and the International Olympic Committee (IOC).

Despite measures such as regular screenings and threats of suspensions, some athletes continue to take risks and consume these banned drugs. It is clear that to wrestle with cases of banned drugs requires better detection technology together with more stringent monitoring and legal control. Undoubtedly, stamping out banned drugs in the sports arena will be very difficult and protracted, as evidenced by cases of continued doping scandals at the Olympics and other sporting events such as the Tour de France.

## 11.11 CHEMICAL AND BIOLOGICAL TERRORISM

The anthrax case in the United States (11 infected, 5 deaths) in late 2001, ricin (a potent poison that inhibits protein synthesis) found in early 2003 in the United Kingdom and France, and sarin (an organophosphate nerve gas) poisoning in Japan in 1995 (11 deaths, 5,500 people affected) highlighted that terrorism with biological and chemical materials is real. Exhibit 11.12 provides some basic information about anthrax and sarin. On the other hand chemical agents can be used for other purposes than incitement of terror, as exemplified by the Russian authority's use of a gas based on opiate fentanyl, as

**TABLE 11.2 List of Drugs Banned in Sports**

| Substances Prohibited at All Times | | |
|---|---|---|
| Anabolic Agents | Anabolic Androgenic Steroids (AAS) | Specific Substances and Derivatives |
| | a. Exogenous AAS | 1-androstendiol, 1-androstendione, bolandiol, bolasterone, boldenone, boldione, calusterone, clostebol, danazol, dehydrochlormethyltestosterone, desoxymethyltestosterone, drostanolone, ethylestrenol, fluoxymesterone, formebolone, furazabol, gestrinone, 4-hydroxytestosterone, mestanolone, mesterolone, metenolone, methandriol, methasterone, methyldienolone, methyl-1-testosterone, methylnortestosterone, methyltestosterone, metribolone, mibolerone, nandrolone, 19-norandrostenedione, norboletone, norclostebol, norethandrolone, oxabolone, oxandrolone, oxymesterone, oxymetholone, prostanozol, quinbolone, stanozolol, stenbolone, 1-testosterone, tetrahydrogestrinone, trenbolone |
| | b. Endogenous AAS | androstenediol, androstenedione, dihydrotestosterone, prasterone, testosterone and their metabolites and isomers |
| | Other Anabolic Agents | clenbuterol, tibolone, zeranol, zilpaterol |
| Peptide hormones, growth factors and related substances | | 1. Erythropoietin (EPO), darbepoetin (dEPO), hypoxia-inducible factor (HIF) stabilizers, methoxy polyethylene glycol-epoetin beta (CERA), peginesatide (Hematide)<br>2. Chorionic Gonadotrophin (CG) and Luteinizing Hormone (LH)<br>3. Corticotrophins<br>4. Growth hormone (GH), Insulin-like growth factor-1 (IGF-1)<br>5. Fibroblast growth factor, Hepatocyte growth factor, Mechano growth factors, Platelet-derived growth factor, vascular-endothelial growth factor |

(*continued*)

**TABLE 11.2 (*Continued*)**

| Substances Prohibited at All Times | | |
|---|---|---|
| Anabolic Agents | Anabolic Androgenic Steroids (AAS) | Specific Substances and Derivatives |
| Beta-2 agonists | | all beta-2 agonists including isomers, except formosterol, salbutamol, salmeterol and terbutaline |
| Hormone and metabolic modulators | | 1. Aromatase inhibitors (incl. aminoglutethimide, anastrozole, androsta-1,4,6-triene-3,17-dione, 4-androstene-3,6,17 trione, exemestane, formestane, letrozole, testolactone<br>2. Selective estrogen receptor modulators: raloxifene, tamoxifen, toremifene<br>3. Clomiphene, cyclofenil, fulvestrant<br>4. Agents modifying myostatin functions<br>5. Metabolic modulators: Insulins, peroxysome proliferator activated receptor $\delta$ |
| Diuretics and other masking agents | | Diuretics, desmopressin, plasma expanders, probenecid |
| Substances prohibited in-competition | | |
| Stimulants | | adrafinil, amfepramone, amphetamine, amphetaminil, amiphenazole, benzfetamine, benflurex, benzylpiperazine, bromantan, cathine, clobenzorex, cocaine, cropropamide, crotetamide, dimethylamphetamine, ephedrine, etamivan, etilamphetamine, etilefrine, famprofazone, fenbutrazate, fencamfamin, fencamine, fenetylline, fenfluramine, fenproporex, fonturacetam, furfenorex, heptaminol, isometheptene, levmethamfetamine, meclofenoxate, mefenorex, mephentermine, mesocarb, methamphetamine, methylenedioxyamphetamine, methylenedioxymethamphetamine, p-methylamphetamine, methylephedrine, methylphenidate, modafinil, nikethamide, norfenefrine, norfenfluramine, octopamine, oxilofrine, pemoline, pentetrazol, phendimetrazine, phenmetrazine, phenpromethamine, phenterimine, prolintane, propylhexedrine, pseudoephedrine, selegiline, sibutramine, strychnine, tauminoheptane, trimetazidine, tuaminoheptane |

**TABLE 11.2 (*Continued*)**

| Substances Prohibited at All Times | | |
|---|---|---|
| Anabolic Agents | Anabolic Androgenic Steroids (AAS) | Specific Substances and Derivatives |
| Narcotics | | buprenorphine, dextromoramide, diamorphine (heroin), fentanyl and derivatives, hydromorphone, methadone, morphine, oxycodone, oxymorphone, pentazocine, pethidine |
| Cannobinoids | | |
| Glucocorticosteroids | | |
| Alcohol | | |
| Beta-blockers | | |

*Source*: Data from World Anti-Doping Agency 2014, *The World Anti-Doping Code*, The 2014 Prohibited List – International Standard, viewed Jun 10, 2014, http://www.wada-ama.org/Documents/World_Anti-Doping_Program/WADP-Prohibited-list/2014/WADA-prohibited-list-2014-EN.pdf.

**Exhibit 11.12 Anthrax and Sarin**

**Anthrax**

Anthrax is a toxin with three separate components: a 'protective antigen' (PA), an 'edema factor' (EF) and a 'lethal factor' (LF).

The LF is the most disruptive to cellular functions, and disables intracellular signaling molecules. It prevents macrophages from releasing tumor necrosis factor (TNF) and interleukin cytokines, although the production of TNF and cytokines in the macrophages is not impeded. The host's immune system is compromised and is unable to eliminate the anthrax bacillus.

Ultimately, the macrophages die, releasing the enormous built-up stores of TNF and cytokines, triggering a septic shock-like collapse of multiple organ systems.

Currently the FDA approved treatment for anthrax is Ciprofloxacin, doxycycline, and penicillin. BioThrax is an anthrax vaccine approved by FDA (refer to Appendix 6).

*Source*: Data from Food and Drug Administration 2001, *Questions and Answers about Anthrax Prevention and Treatmen*t, viewed June 10, 2014, http://www.fda.gov/drugs/emergencypreparedness/bioterrorismanddrugpreparedness/ucm132658.htm

**Sarin**

Pure sarin is a colorless, odorless, volatile, and highly lethal liquid. It inhibits the enzyme action of cholinesterase, causing the production of excessive amounts of acetylcholine, which in turn affects the central nervous system.

Diazepam and pralidoxime iodide are prescribed for victims affected by sarin.

an incapacitating agent, to secure the release of hostages held by Chechen rebels in a Moscow theatre in late 2002.

Both chemical and biological terrorism can cause tremendous medical, social, commercial, legal, and political upheavals and problems. Governments in many countries are collaborating to examine ways to improve response preparedness in the event of chemical and biological terrorism. Both FDA and EMA have implemented counter-terrorism strategies. These include streamlining of regulatory approvals for vaccines and therapeutics for diseases that are possible targets of biological weapons, such as anthrax, botulism, smallpox, plague, tularemia, and hemorrhagic fevers.

In June 2002, FDA amended its regulations for the approval of certain drugs based only on animal efficacy data. For those drugs that are intended to protect or treat individuals exposed to lethal or disabling toxic substances or organisms, marketing approval may be granted based on evidence of effectiveness from appropriate animal studies when human efficacy studies are not ethical or feasible. Under this "animal efficacy rule", FDA approved pyridostigmine bromide for US military personnel. This drug increases survival rate after exposure to Soman nerve gas poisoning. Another medication approved is a lotion called Reactive Skin Decontamination Lotion, which is a liquid decontamination lotion for topical application to remove or neutralize chemical warfare agents and T-2 fungal toxin. As part of FDA's counter-terrorism measure, the agency awards grants to support clinical trials for the development of drugs against plagues (bubonic, pneumonic, meningitic or septicemic).

Bioterrorism agents were assigned into three categories by the Centre for Disease Control and Prevention (CDC, Exhibit 11.13). Anti-terrorism solutions introduced by CDC include (i) improvement to the networks of health reports so that outbreaks can be detected early and precautionary measures taken within a short time, (ii) development of effective diagnostic tests to ascertain the nature of the chemical and biological threat, (iii) stockpiling of antibiotics and vaccines to mitigate terrorist attacks, although this is a complex matter because of the multitude of microorganisms and chemicals that may be required, and (iv) secure sources of funding needed to develop therapies and antidotes to presumed agents of terrorism.

## 11.12 TRANSGENIC ANIMALS AND PLANTS

The production of drugs under GMP conditions is costly, especially for protein-based drugs, due to low yields and the need for aseptic processes and stringent control. Some manufacturers have looked to transgenic animals and plants as possible "factories" for the production of cost-effective protein-based drugs.

To produce protein-based drugs, DNA genes that code for the expression of the desired protein are inserted into animals or plants. These animals or plants treat the foreign DNA as part of their own genome. As the animals or plants grow, the protein is expressed. Most of the proteins are collected in milk or in eggs of animals; and in fruits or tubers of plants. The proteins are then extracted, purified and formulated as the protein-based drugs. There are several potential issues. Firstly, animals or plants may produce proteins that have different protein sequences, structures and glycosylation

**Exhibit 11.13 Categories of Bioterrorism Agents**

FDA has assigned bioterrorism agents into three categories as below:

- *Category A*: Greatest potential for adverse public health impact with mass casualties; moderate to high potential for large scale dissemination. Examples are: *Variola major* (smallpox), *Bacillus anthracis* (anthrax), *Yersinia pestis* (plague), *Clostridium botulinum* (botulism), *Francisella tularensis* (tularemia), Filovirus and Arenaviruses, for example, *Ebola virus*, *Lassa virus* (viral hemorrhagic fevers).
- *Category B*: Some potential for large scale dissemination; expected to cause lower medical and health impact. Examples are: *Brucella spp*. (brucellosis), *Burkholderia mallei* (glanders), *Burkholderia pseudomallei* (melioidosis), *Coxiella burnetii* (Q fever), *Alphaviruses* (encephalitis), Rickettsia prowazekii (typhus fever), Toxins, for example, ricin, *Staphylococcal enterotoxin B* (toxic syndromes), *Clamydia psittaci* (psittacosis), Food safety, for example,. *Salmonella spp*., *Escherichia coli*, Water safety, for example, *Vibrio cholerae*, *Cryptosporidium parvum*.
- *Category C*: Agents not considered high bioterrorism threat currently but could emerge as future threats. Examples are: nipah virus, hantavirus.

*Source*: Data from Rotz, LD *et al*. 2002, 'Public Health Assessment of Potential Biological Terrorism Agents', *Emerging Infectious Diseases*, 8, pp. 225–230.

patterns than the proteins from human origin. This would render the drug less effective. Secondly, other biological materials from the animals or plants that are potential contaminants for humans may be present, requiring elaborate steps for their removal. Thirdly, transgenic animals or plants have to be separated from natural animals and plants to prevent cross-contamination. Fourthly, both the animals and plants have to be kept under close surveillance to ensure they are free of diseases.

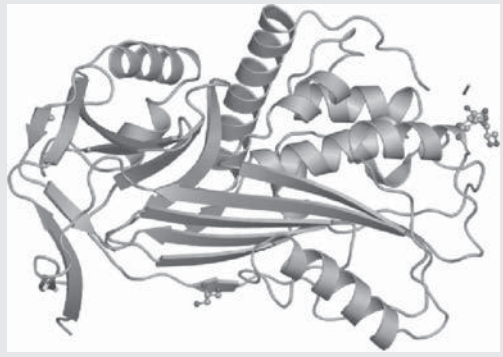
In August 2006 the EU approved A-Tryn for marketing (Exhibit 10.2, US approval in 2009). A-Tryn is a recombinant form of human antithrombin (anticoagulant), which is produced in the milk of genetically engineered goats. This approval showcased the entrance of the transgenic production of drugs. Another transgenic drug product approved by EMA is Ruconest (Exhibit 11.14, under review by FDA in 2014). It is a recombinant C1-inhibitor obtained from the milk of transgenic rabbits. Ruconest is used to treat hereditary angioedema in adults.

## 11.13 ANTIBIOTICS DRUG RESISTANCE

Antibiotics, including antibacterials and antifungals, are drugs used to destroy or slow the growth of microorganisms in our body. The mechanisms of action of these drugs on the microbes can be classified as follow:

**Exhibit 11.14 Ruconest**

Ruconest (conestat alfa, picture below) is a recombinant human component 1 (C1) esterase inhibitor with a molecular weight of 67 kDa. It is obtained from rabbit milk. C1-inhibitor controls the activation of certain proteins in the complement, coagulation, fibrinolytic and contact systems that are involved in inflammation. Patients with hereditary angioedema (HAE – swelling due to leakage of fluid from blood vessels into tissues) caused by C1-inhibitor deficiency experience recurrent attacks of angioedema. Replacement therapy with Ruconest relieves the symptoms and reduces the duration of these attacks.



*Source*: Protein Data Bank in Europe, *Crystal Structure of latent human C1-inhibitor*, viewed June 11, 2014, http://www.ebi.ac.uk/pdbe-srv/view/entry/2oay/experimental. Reproduced with permission of European Bioinformatics Institute

*Source*: Data from 1. European Medicines Agency 2010, CHMP assessment report, *Ruconest*, viewed June 10, 2014, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/001223/WC500098546.pdf; 2. European Medicines Agency 2010, *Ruconest*, viewed June 10, 2014, http://www.ema.europa.eu/docs/en_GB/document_library/Summary_of_opinion_-_Initial_authorisation/human/001223/WC500093934.pdf

- Interference with DNA synthesis
- Interference with protein synthesis
- Interference with cell wall synthesis
- Interference with cell membrane permeability
- Inhibition of enzyme/enzymes of the microorganism.

Unfortunately through misuse or overuse of antibiotics, such as suboptimal dosage and non-compliance in administrating the medication, the microbes are incompletely eradicated. As microbes survive, they grow and reproduce by dividing every few hours. Soon they evolve, mutate and adapt to the new environment where the progeny become

resistant to antibiotics. This is a natural selection process where the microbes with the resistance genes survive and pass them on to future generations.

For example methicillin-resistant *Staphylococcus aureus* (MRSA), is a strain of bacteria that has become resistant to methicillin, an antibiotic of the penicillin class. To healthy individuals the *Staphylococcus aureus* though present, does not cause active infection. But to the immune-suppressed and the elderly, *Staphylococcus aureus* infection can result in a morbidity and mortality. Worse still is the fact that MRSA is spread in healthcare institutions and community centers such as hospitals, medical centers, old folks home, childcare centers, gymnasiums, and confined living quarters. The terms HA-MRSA and CA-MRSA refer to hospital associated and community-associated MRSA.

Another microorganism, *Enterococci* bacteria, although less common than *Staphylococcus aureus*, can infect hospitalized patients and complicate diseases and prolong hospital stays. A particular strain, the vancomycin-resistant *Enterococcus faecium* (VRE) can be fatal and account for a third of the infections in intensive care units.

Hygiene and sanitation play an important role in the transmission of microbe infections. At the same time there are new and more effective antibiotics being developed:

- *Dalfopristin* (*Synercid IV*):Binds to bacterial ribosome and inhibits protein synthesis. It was approved under FDA's accelerated approval regulations for the treatment of patients with serious or life-threatening infections associated with the VRE bacteria.
- *Linezolid* (*Zyvox*): Stops bacterial protein synthesis in ribosome by inhibiting formation of initiation complex. It is indicated for the treatment of VRE and MRSA.
- *Daptomycin* (*Cubicin*): Causes membrane depolarization in bacteria and prevents membrane transport and permeability. It is prescribed for the treatment of complicated skin and skin structure infections and bloodstream infections by *Staphylococcus aureus*.

To encourage more effective antibiotics to be developed, the FDA's "Generating Antibiotics Now (GAIN) Act" has been implemented to provide incentives for pharmaceutical companies to develop antibiotics for the treatment of serious or life-threatening infections. Under the GAIN Act new antibiotics applications would be given priority review and for those antibiotics granted approval, an additional 5 years of exclusivity (refer to Section 11.15) is guaranteed.

## 11.14 REGULATORY ISSUES

Regulatory requirements are dynamic. They are introduced or amended as circumstances change. The amendment by FDA to the animal efficacy rule (Section 11.11), FDA's *Pathway to Global Product Safety and Quality* (Section 7.15), the initiative for a new risk-based approach to cGMP (Section 9.8) and the GAIN Act (Section 11.13) are examples of dynamic responses to changing environment and conditions. In Europe,

EMA sets out its *Road Map to 2015* to address the priority areas of: (i) addressing public health needs, (ii) facilitating access to medicines, and (iii) optimizing the safe and rational use of medicines. Another positive development in Europe is the recognition and adoption of regulatory controls for TM. The formation of the International Conference on Harmonization (ICH) in harmonizing drug regulations, such as the ICH Q7 document, has helped to establish a common denominator for regulatory requirements of GMP for many countries. The use of Common Technical Document for regulatory filing is adopted by most countries as the standard document to present data on preclinical, clinical and manufacturing when filing for marketing authorization. At the same time regulatory authorities have to legislate and implement regulatory controls against counterfeit drugs, which are prevalent in the developing countries in Asia, Latin America and Africa (refer to Section 11.19).

As gene therapy and stem cell research progress, we can expect more regulatory requirements to be developed to ensure proper safeguards are implemented. Exhibit 11.15 presents FDA's current oversight on gene therapy. Similarly, xenotransplantation and control of biopharmaceutical products will experience specific regulatory controls as new advances are made.

**Exhibit 11.15 FDA's Oversight on Gene Therapy**

FDA has not yet approved for sale any human gene therapy product. However, gene-related research and development is continuing to grow and FDA is very involved in overseeing this activity. The FDA draft *Guidance for Industry*: *Considerations for Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products*, 2013, sets up the requirements for assessment of safety, tolerability and feasibility of administering cellular or gene therapy products in clinical trials.

At the end of 2012 there were more than 1800 gene therapy trials that have been completed, ongoing, or approved worldwide in 31 countries. The top five countries for gene therapy clinical trials are: US (1174), UK (203), Germany (81), France (53), and Switzerland (50). The top five diseases targeted are: cancer (1186), monogenic diseases (161), cardiovascular diseases (155), infectious diseases (147), and neurological diseases (36). The top eight gene types that were transferred in the trials are: antigen (378), cytokine (340), tumor suppressor (153), suicide (149), deficiency (147), growth factor (139), receptor (132), and replication inhibitor (79).

*Source*: Data from 1. Food and Drug Administration 2013, *Guidance for Industry*: *Considerations for Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products*, viewed June 10, 2014, http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/UCM359073.pdf; 2. Ginn, SL *et al*. 2013, 'Gene therapy clinical trials worldwide to 2012 – an update', *Journal of Gene Medicine*, 15, pp. 65–77.

## 11.15 INTELLECTUAL PROPERTY RIGHTS AND MARKETING EXCLUSIVITIES

Intellectual property rights (IPRs) are important assets for the pharmaceutical industry. Most successful companies have suites of patents to protect their IPRs. The effect of IPR protection is amply demonstrated by the Prozac case. Fluoxetine (Prozac, Eli Lilly) was a blockbuster drug for many years. During the first half of 2001, when Prozac was still protected by patent, the sales were US$1.3 billion. Within one year of patent expiration, generics from other companies were released to the market. The sales of Prozac were reduced to US$380 million for the first half of 2002. A more recent example is that of Lipitor, which lost billions in sales when its patent expired in 2011 (refer to Section 1.4).

Pharmaceutical companies are adopting strategies to protect their products. These range from own generics, second brand, new indications, new dosage form, and reformulations or isolation of the effective enantiomer (refer to Section 3.6.). Product lifecycle management of drugs and IPR protection are strategies that pharmaceutical firms will focus on more closely in the coming decades. This is especially the case where pharmaceutical firms try to exclude the encroachment of generics for as long as possible. Exhibit 11.16 describes the legal battle between AstraZeneca, the manufacturer of Prilosec, and other companies trying to manufacture omeprazole generics. Another example is Genentech's set of Cabilly patents which cover key steps in the manufacture of recombinant antibodies. These patents were the subject of a number of legal proceedings and resulted in favor of Genentech, giving an effective lifetime coverage for this set of patents a total of 35 years, from 1983 to 2018 (Exhibit 11.7).

In addition patent terms and periods of marketing exclusivities are evolving to strike a balance between encouraging innovations and controlling monopolies. A further explanation of patents and exclusivities are as below:

***Patents:*** For US and Europe, patents are granted by the patent office for terms of 20 years.

**Exhibit 11.16 Prilosec's Legal Battle**

The AstraZeneca patent on omeprazole expired in October 2001. AstraZeneca went to court to seek extended patent protection for a special formulation of omeprazole. The special formulation consists of a subcoating layer inserted between the core of the drug's active ingredient, and the outer coating. The subcoating is formulated to protect the drug from being broken down quickly by the harsh acids in the stomach.

In October 2002, a US federal judge ruled that three generic companies have infringed on AstraZeneca's patent. However, a fourth company that has its own patent for coating the drug was cleared to market the drug in generic form.

*Source*: Data from Debaise, C 2002, *Wall Street Journal*, October 12

**Exhibit 11.17 Cabilly Patents**

The Cabilly patents consist of three patents issued to Shmuel Cabilly *et al*. and assigned to Genentech and City of Hope, a private, not-for-profit research organization in California. Details of these patents are shown below:

| Alias Name | Inventor | Assignee | Patent Number | Priority Date | Date of Grant | Status | Expiry Date |
|---|---|---|---|---|---|---|---|
| Cabilly I | Shmuel Cabilly *et al*. | Genentech and City of Hope | US4,816,567 | Apr 8, 1983 | Mar 28, 1989 | Expired | Mar 28, 2006 |
| Cabilly II | Shmuel Cabilly *et al*. | Genentech and City of Hope | US6,331,415 | Apr 8, 1983 | Dec 18, 2001 | In force | Dec 18, 2018 |
| Cabilly III | Shmuel *Cabilly et al*. | Genentech and City of Hope | US7,923,221 | Apr 8, 1983 | Apr 12, 2011 | In force | Dec 18, 2018 |

These patents relate to the expression of antibody heavy and light chains in the host cell system. There were several law suits regarding the claims and variations of these patents. The suits centered on whether some of the claims are obvious to those skilled in recombinant antibody manufacture, or indeed they are genuine inventions.

The US Patent and Trademark Office eventually ruled in favor of Genentech and due to the nature of the court proceedings, set an expiry date of December 18, 2018 for Cabilly II and III patents, and thus provided an effective lifetime coverage of 35 years from the priority date Cabilly I.

Genentech has licensed the patents to a number of pharmaceutical organizations that produce antibodies, and in 2008 Cabilly II alone generated US$237 millions in license fees to the company.

*Source*: Adapted from 1. Storz, U 2012, 'The Cabilly patents', *MAbs*, Mar-Apr, 4(2): pp. 274–280, viewed July 14, 2014, http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3361663/; 2. Waltz, E 2009, 'Genentech's Cabilly victory', *Nature Biotechnology*, 27, p. 307

In US the Patent Term Restoration (PTR) system is used to compensate patent term that was lost due to processing time to satisfy regulatory requirements (50% of the time spent on IND and 100% of the time spent on NDA). A maximum of restored period of 5 years is given to a patent, in addition to the 20 years granted initially. But the total patent life after drug product approval (NDA/BLA) cannot be more than 14 years.

To encourage medical innovation, Europe has introduced the Supplementary Protection Certificate (SPC) system. The SPC provides another 5 years extension to a patent that expires, and 5.5 years for those with pediatric application. A limit is set such that the

original patent term and extension should not exceed 15 years, and those for pediatrics 15.5 years after the approval of marketing authorization.

***Exclusivities:*** Marketing exclusivities are granted by regulatory authorities, for example, FDA and EMA to compensate pharmaceutical companies for the investments put into developing new medical products. This is the period of exclusive market for the innovator drug product from the day marketing approval is granted to the time that generics are allowed to enter the market.

In US the market exclusivity period is product dependant as below:

- Biologics – 12 years
- Orphan drugs – 7 years
- NCE – 5 years
- Other exclusivity, new indication – 3 years
- Pediatric exclusivity – an additional 6 months to existing exclusivity.

In Europe, EMA provides a 10 year marketing exclusivity. An additional 1 year is given to those products with new indications.

A graphical representation of IPR term, extensions and exclusivities is given in Figure 11.1.

After the expiry of patents and market exclusivities, generics are allowed to enter the market. In US generics follow the Abbreviated NDA (ANDA) path (refer to

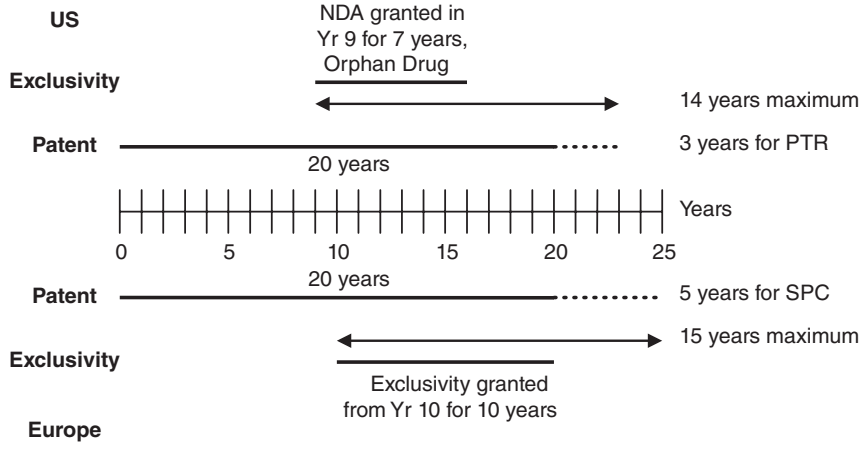



**Figure 11.1** Patent term, extensions and exclusivities. Note: Top section for US, Bottom section for Europe. Top section – As NDA (orphan drug) is granted in Yr 9 of patent, the 14 year rule means a maximum PTR of 3 years can be approved. Generics can come into the market on Yr 23. If NDA is granted in Yr 11 of patent, the 14 year rule means a maximum PTR of 5 years can be approved. Generics can then only come into the market on Yr 25. Bottom section – Similar reasoning applies to Europe. A drug for pediatric indication will have an additional 6 months for SPC.

**Exhibit 11.18 FDA's Rule on Generics**

To encourage generic production, FDA allows submission of an Abbreviated New Drug Application (ANDA) by a generic manufacturer before the expiration of the patented drug. For FDA to commence review of the ANDA, the generic drug applicant must certify that the patent for the existing drug is invalid and the generic product does not infringe it. The generic drug applicant must also notify the patent holder that it has filed the ANDA application. If the patent holder files an infringement suit against the generic applicant within 45 days of the ANDA notification, the patent holder is given a one-time "stay" of a generic drug's entry into the market for resolution of a patent challenge unless, before that time, the patent expires or is determined to be invalid or not infringed. This 30-month stay gives the patent holder time to assert its patent rights in court before a generic competitor is permitted to enter the market.

*Source*: Data from Food and Drug Administration 2003, *Guidance for Industry*: *Listed Drugs*, 30-month Stays, and Approval of ANDAs and 505(b)(2) Application Under Hatch-Waxman, as Amended by the Medicare Prescription Drug, Improvement, and Modernization Act of 2003, Questions and Answers, viewed June 10, 2014, http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm072887.pdf

Section 8.2.5) and in Europe, the Abridged National Authorization is applicable (refer to Section 8.3.2). There are certain rules that govern the ANDA application and review. A brief explanation of the rules is described in Exhibit 11.18.

There are arguments and counter arguments as to whether IPRs create monopolies for pharmaceutical companies to charge high prices for their products, and these contentions are likely to continue. Alternatives to patent rights have also been proposed, such as government sponsored prize to be awarded to products that pharmaceutical companies waive their IPRs and keep the prices affordable. In reality there are instances that pharmaceutical firms have waived their IPRs for a certain segment of the market due to commercial or political issues. This is shown in Exhibit 11.19. Another example is the decision by Novartis to exit from the Indian market when its IPR for Gleevec was not recognized by the Indian government. Under political pressure, some pharmaceutical companies may waive their premium pricing on patented drugs (Exhibit 11.20).

## 11.16 BIOETHICS

Bioethics is a discipline which deals with the moral issues of biological research and its application in medicine. The United Nations Educational, Scientific and Cultural Organization (UNESCO) in its 2005 Universal Declaration on Bioethics and Human Rights addresses ethical issues related to medicine, life sciences and associated technologies as applied to human beings. The aim of the declaration is to "provide a universal framework of principles and procedures to guide States in the formulation of their legislation, policies or other instruments in the field of bioethics".

**Exhibit 11.19 Sleeping Sickness in Africa**

Sleeping sickness is caused by the presence of parasitic protozoans (*Trypanosoma gambiense* or *T. rhodesiense*) in the blood. The parasite causes drowsiness, lethargy and eventually death if patient is untreated. As many as 55 million people in 36 African countries are exposed to this disease. In the late 1990s, WHO persuaded companies such as Bristol-Myers Squibb, Aventis and Bayer to waive their patent rights and donate drugs for treating sleeping sickness to Africans. In addition, these companies agreed to contribute US$5 million per year for 5 years for monitoring, treatment and research and development of sleeping sickness.

*Source*: Data from Wickware, P 2002, 'Resurrecting the resurrection drug', *Nature Medicine*, 8, pp. 908–909

**Exhibit 11.20 AIDS Drugs in the Third World**

GlaxoSmithKline, under pressure for not doing more to help the AIDS sufferers in South Africa, relented and handed over the licenses to four South African firms to produce and import zidovudine (AZT, Retrovir) and lamivudine (3TC, Lamivir). GlaxoSmithKline will charge no more than 5% royalty fee on the sale of these drugs. This action will help to supply badly needed drugs to the Third World countries; it also sets up the precedent of differential strategies for the manufacture and supply of drugs in the world.

*Source*: Data from BBC News 2003, *Glaxo responds to Aids drugs call*, viewed June 10, 2014, http://news.bbc.co.uk/2/hi/business/3306079.stm.

Bristol-Myers Squibb licensed its AIDS drug, Reyataz, free of charge to two generic manufacturers in South Africa and India. This decision made available the generic form of the drug to sub-Saharan Africa AIDS victims at affordable prices. Reyataz was first marketed in US in 2003 and its patent expires in 2017.

*Source*: Data from Kucharsky, D 2006, 'Bristol-Myers Squibb to provide royalty-free licenses for Reyataz in India, South Africa', *FirstWord Pharma*, viewed June 10, 2014, http://www.firstwordpharma.com/node/163706?tsid=17#axzz347dRwZGd

In many areas, bioethics will continue to pose vexing questions which communities and governments must face. In September 2007, the Human Fertilization and Embryology Authority (HFEA) of the UK approved the research of hybrid embryos which involves the insertion of human DNA into animal (cow or rabbit) cells where the genetic material has been removed. This is called cytoplasmic hybrid embryo and the research requires a license granted by the Authority. Proponents argue that the research is critical

to discovering treatments for genetic diseases such as Alzheimer's and Parkinson's, as there is a lack of human eggs for research. Opponents, however, dispute the ethicality of hybrid embryos, claiming research would lead to the formation of modified humans from traces of animal genes that may remain in the cell, even though the embryo is only allowed to grow for up to 2 weeks. The question is: where should the boundary of medical research be drawn? Any suggestion is certain to provoke arguments, but necessary to be defined.

## 11.17 CONCLUDING REMARKS

A reflection on the last 100 years shows the tremendous progress made in drug discovery and development. With better sanitation and health care, life expectancy has increased from an average in the 50s at the beginning of the 20th century, to an average in the 80s for developed nations in today's 21st century. However, there are startling differences in the life expectancy and disease types in rich and poor nations, and would require governments and WHO to address. Table 11.3 shows the leading causes of death in rich and poor countries. The poorer nations are still affected by diseases that are well-controlled, eradicated or treated in the developed countries.

The drug market continues to expand, even during times of economic downturn. With the introduction of many new technologies and processes, we can expect more effective and specific drugs in the decades ahead. However, a worrying trend has also appeared. It is the declining number of new drugs approved in the past 10 years, in spite of the billions of dollars spent on research and development (refer to Figure 1.2). In 2013, FDA approved 25 new molecular entity drugs (NMEs). In the same period, only two large molecule drugs were approved under the biologics license applications (BLAs) (Figure 11.2). An insight into drug regulations in the future can be seen from FDA's long term objectives as listed below:

- Sustain availability of safe and effective new and generic products by improving rapid, transparent, and predictable science-based review of marketing applications
- Increase the number of safe and effective new products available to patients, including products for unmet medical and public health needs, emerging infectious diseases and counterterrorism
- Improve safe and effective use of medical products with better information technology and effective risk/benefit communication
- Prevent harm from products by increasing the likelihood of detection and interception of substandard manufacturing processes and products
- Improve the infrastructure for problem detection and product information dissemination, to strengthen consumer protection and take timely, effective risk management actions with all FDA-regulated products.

Pharmaceutical firms have to re-examine their strategies to devise means to increase their drug pipelines for continuous streams of products. The high failure rates of Investigational New Drugs during clinical trials (Exhibit 5.8) necessitate the development

**TABLE 11.3 Leading Causes of Death in the World, 2012**

| The Ten Leading Causes of Death in The World (2012) | |
|---|---|
| All Countries | Deaths in Millions |
| Ischemic heart disease | 7.4 |
| Stroke | 6.7 |
| COPD | 3.1 |
| Lower respiratory infections | 3.1 |
| Trachea, bronchus, lung cancers | 1.8 |
| HIV/AIDS | 1.5 |
| Diarrheal diseases | 1.5 |
| Diabetes mellitus | 1.5 |
| Road injury | 1.3 |
| Hypertensive heart disease | 1.1 |
| **High Income Countries** | **Deaths per 100,000 Population** |
| Ischemic heart disease | 158 |
| Stroke | 95 |
| Trachea, bronchus, lung cancers | 49 |
| Alzheimer's disease and other dementia | 42 |
| COPD | 31 |
| Lower respiratory infections | 31 |
| Colon, rectum cancers | 27 |
| Diabetes mellitus | 20 |
| Hypertensive heart disease | 20 |
| Breast cancer | 16 |
| **Upper Middle Income Countries** | **Deaths per 100,000 Population** |
| Stroke | 126 |
| Ischemic heart disease | 107 |
| COPD | 50 |
| Trachea, bronchus, lung cancers | 31 |
| Diabetes mellitus | 23 |
| Lower respiratory infections | 23 |
| Road injury | 21 |
| Hypertensive heart disease | 20 |
| Liver cancer | 18 |
| Stomach cancer | 17 |
| **Lower Middle Income Countries** | **Deaths per 100,000 Population** |
| Ischemic heart disease | 95 |
| Stroke | 78 |
| Lower respiratory infections | 53 |
| COPD | 52 |
| Diarrheal diseases | 37 |
| Preterm birth complications | 28 |
| HIV/AIDS | 23 |
| Diabetes mellitus | 22 |
| Tuberculosis | 21 |
| Cirrhosis of the liver | 19 |

(*continued*)

**TABLE 11.3 (*Continued*)**

| The Ten Leading Causes of Death in The World (2012) All Countries | Deaths in Millions |
|---|---|
| **Low Income Countries** | **Deaths per 100,000 Population** |
| Lower respiratory infections | 91 |
| HIV/AIDS | 65 |
| Diarrheal diseases | 53 |
| Stroke | 52 |
| Ischemic heart disease | 39 |
| Malaria | 35 |
| Preterm birth complications | 33 |
| Tuberculosis | 31 |
| Birth asphyxia and birth trauma | 29 |
| Protein, energy and malnutrition | 27 |

*Source*: World Health Organization 2014, *The Top 10 Causes of Death (2012)*, viewed Jun 11, 2014, http://www.who.int/mediacentre/factsheets/fs310/en/. Reproduced by permission of the World Health Organization.

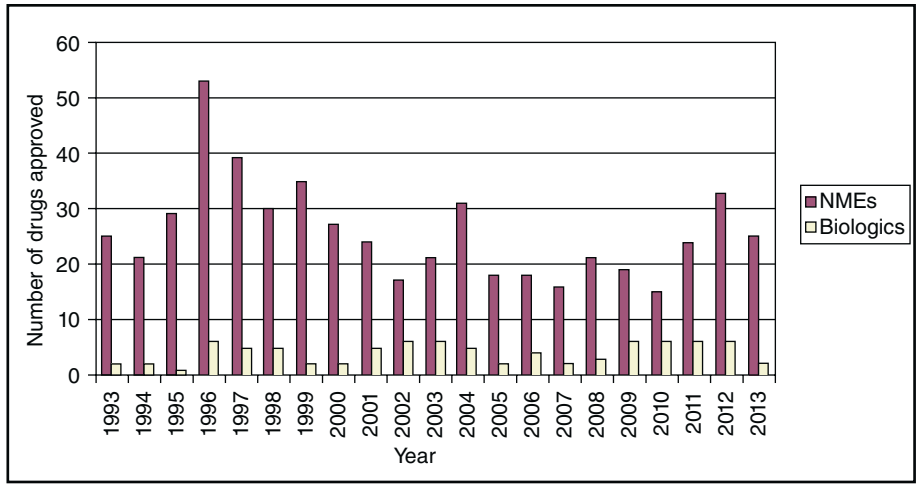



**Figure 11.2** New molecular entities and biologics approved by FDA (1993 to 2013). (*Source*: Mullard, A 2014, '2013 FDA drug approvals', *Nature Reviews Drug Discovery*, 13, pp. 85–89. Reproduced with permission of Macmillan Publishers Ltd.)

of better assay systems and animal models that correlate closely with human pharmacodynamics and pharmacokinetics. The study of pharmacogenomics will be crucial to address this issue.

In addition, the pharmaceutical industry is no longer isolated from society as a whole. It has to factor in considerations for social, political and ethical issues. The attitudes and concerns of society have to be taken into account. The cost of drugs and their availability to the Third World nations are other important aspects for consideration. Furthermore,

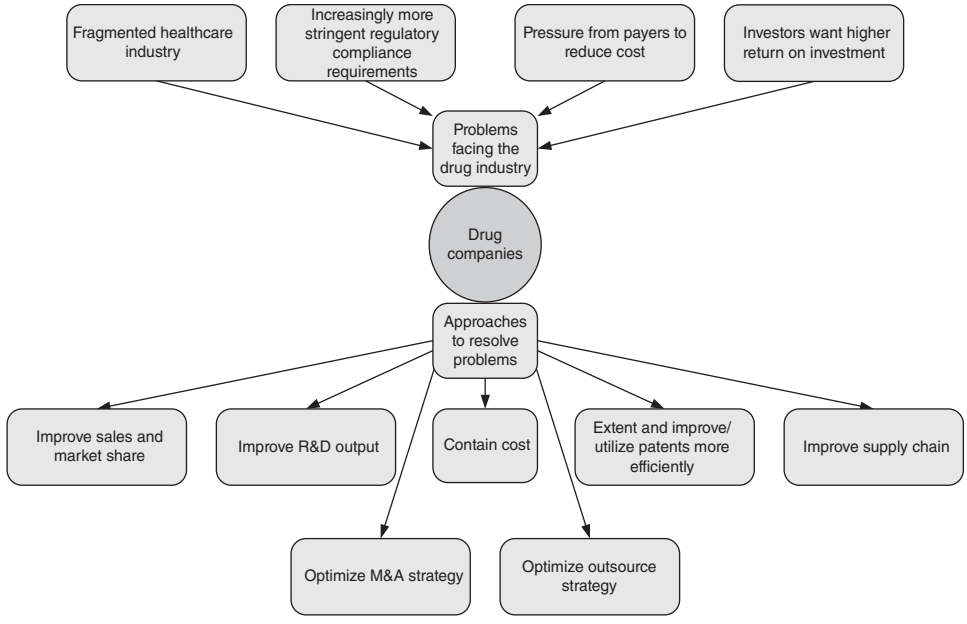



**Figure 11.3** Challenges facing the pharmaceutical companies. (*Source*: Belsey, MJ 2007, 'Drug developer strategies to boost competitiveness', *Nature Reviews Drug Discovery*, 6, pp. 265–266. Reproduced with permission of Macmillan Publishers Ltd.)

pharmaceutical firms need to be proactive and vigilant to comply with changes in regulations and government policies. New areas of research, such as gene therapy and stem cells, have opened up many ethical issues to be resolved. Production of protein-based drugs using transgenic animals and plants raises potential topics for ethical and political debates.

These challenges require investment, commitment and ingenious solutions, as shown in Figure 11.3. Measures such as licensing, alliance, mergers, acquisitions and outsourcing are needed to progress the companies and to focus on core competencies. Ultimately pharmaceutical firms and research organizations, together with government authorities, international organizations, and representatives from society, have to collaborate and address these challenges. The results will be the development of novel and efficacious drugs and therapies to treat patients and lead to improvements in the quality of life.

## 11.18 CASE STUDY #11.1

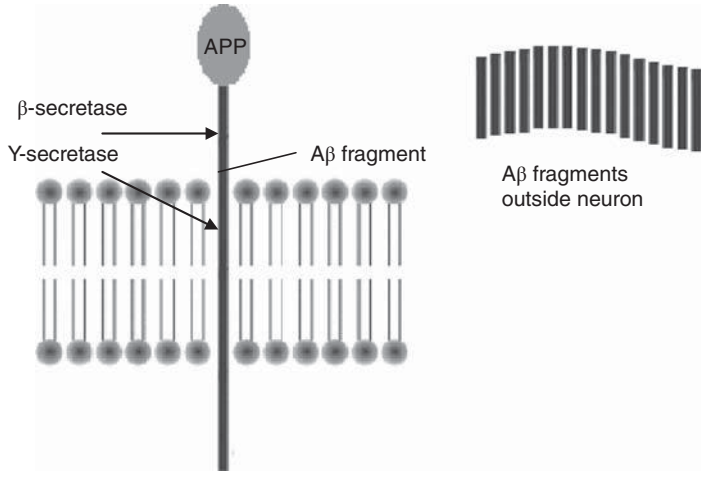
### 11.18.1 Alzheimer's Disease

Until now there is still no effective drug for the treatment of Alzheimer's disease. To understand the strategy used to develop a drug for its treatment, a necessary condition rests with an understanding of the cause of the disease.

Alzheimer's disease can result from mutations in one of three genes: *APP*, *PSEN1* or *PSEN2*. The key features of Alzheimer's disease as a result of gene mutations are the accumulation of $\beta$-amyloid (A$\beta$) protein outside neurons in the brain and the presence of tangled proteins, called tau, within neurons. The tau clumps together, and consequently neurons are unable to function normally and eventually die. It is found that A$\beta$ provides the initial insult to set off the disease.

Recent work shows further understanding as below:

- The amyloid precursor protein (APP) protrudes from the neuron membrane
- Both proteases, $\beta$-secretase and $\gamma$-secretase, cut out A$\beta$ from APP, using aspartic acid as a catalyst in a two stage process
- There are three genes believed to be involved in the cutting of the amyloids: one gene (*APP*) encodes for the APP while two (*PSEN1* and *PSEN2*) encode the enzymes $\beta$-secretase and $\gamma$-secretase
- The aggregation of A$\beta$ outside the neuron somehow alters tau proteins inside the neuron, perhaps through a mechanism which involves the kinase proteins.




From this knowledge, several probable solutions for developing drugs are being attempted to find a treatment for this disease:

### *Small Molecule Drug Solution:*

- Develop protease inhibitor – no known inhibitor for $\beta$-secretase yet; inhibitor of $\gamma$-secretase is known and development is currently in progress
- Design drug to prevent the aggregation of A$\beta$ outside of the neuron
- Devise antagonist to block kinases, thus mitigating the effect of A$\beta$ on tau.

***Large Molecule Drug Solution:***

- Provide vaccine to act prophylatically – use A$\beta$ or its fragments as an antigen to elicit antibody response; trial conducted shows antibodies were generated as predicted but for unknown reasons patients suffered encephalitis; other vaccines are contemplated which may use different parts or fragments of A$\beta$ to evoke immune response and to reduce the encephalitis adverse reaction (see Exhibit 4.6)
- Generate therapeutic antibodies to target A$\beta$ for destruction, similar to the example of Herceptin's mechanism of action; but it may be difficult to deliver large protein molecules across the blood-brain barrier of the tightly-packed endothelial cells
- Use of gene therapy by surgically implanting "healthy" gene with nerve growth factor to cure the three errant genes.

There are no drugs currently available that can treat Alzheimer's disease effectively. The current approved drugs are to assist Alzheimer's patients maintain their cognitive functions and carry on the daily activities. Donepezil (Aricept), rivastigmine (Exelon), tacrine (Cognex), and galantamine (Razadyne) are prescribed to treat mild to moderate symptoms. The latest approved drug is memantine (Namenda), it is prescribed to treat moderate to severe cases. With concerted efforts it is hopeful that better and more effective medication for treatment of Alzheimer's disease would be available in the not too distant future.

## 11.19 CASE STUDY #11.2

### 11.19.1 Counterfeit Drugs

One of the many challenges facing regulatory authorities worldwide is that of counterfeit drugs. The World Health Organization (WHO) defined counterfeit drugs as:

> "Those products manufactured below established standards of quality and therefore dangerous to patients' health and ineffective for the treatment of diseases. They are deliberately and fraudulently mislabeled and may contain the correct ingredients but fake packaging, or with the wrong ingredients, or without active ingredients or with insufficient active ingredients."

The counterfeit drugs market in 2010 was estimated to be about US$ 80 billion. Most of the counterfeit drugs are found in developing countries where enforcement of the regulatory authorities is weak or ineffective. But in reality counterfeit drugs are present globally. In general the distributions of counterfeit drugs as proportions of the total market are as below:

- *Industrialized and Developed Countries with Effective Regulatory Systems*: less than 1% of total market; mainly lifestyle drugs such as hormones, steroids and antihistamines.

- *Some Developing Countries in Asia*, *Latin America and Africa*: may range from 10% to 30% of total market
- *Former Soviet Countries*: estimated to be about 20% of total market
- Online purchase from the Internet with dubious source with as high as 50% of purchases being counterfeits.

In 2012 there were more than 2,000 incidents of counterfeiting. Of these 40% were of commercial size (>1,000 dosage units), 52% non-commercial and 7% unknown. The distributions of incidents are:

- Asia – 952
- Europe – 454
- Latin America – 423
- N. America – 217
- Eurasia 210
- Near East – 153
- Africa – 45.

The factors identified by WHO that encourage counterfeiting of medicines are:

- Lack of political will and commitment
- Lack of appropriate drug legislation
- Absence of or weak drug regulation
- Weak enforcement and penal sanctions
- Corruption and conflict of interest
- Demand exceeding supply
- High prices of medicines
- Inefficient cooperation between stakeholders
- Lack of regulation by exporting countries and within free trade zones
- Trade through several intermediaries.

WHO has set up the International Medicinal Products Anti-Counterfeiting Taskforce (IMPACT) with all Member States to combat counterfeit drugs. Five key areas were identified:

- *Legislative and Regulatory Infrastructure*: strengthen legislations, increase penal sanctions, and empower law enforcement agencies
- *Regulatory Implementation*: improve control on safety and efficacy of drugs, and the distribution channels; develop better coordination between local, regional and central authorities
- *Enforcement*: Monitors and track borders for counterfeit activities by working with World Customs Agency, INTERPOL and other enforcement networks

- *Technology*: involve pharmaceutical companies and distributors to develop innovative solutions, such as radio-frequency identification (RFID) to track movements of drugs, tamper-proof packaging to deter tampering and printing technologies aimed at end user compliance
- *Risk Communication*: IMPACT to develop and coordinate effective mechanisms to alert and respond to counterfeit drug activities; inform and educate users and healthcare professionals to be alert and report suspicious cases.

*Source*: 1. World Health Organization 2014, *General Information on counterfeit medicines*, viewed June 11, 2014, http://www.who.int/medicines/services/counterfeit/overview/en/index1.html; 2. Pharmaceutical Security Institute 2014, *Counterfeit Situation*, *Incident Trends*, viewed June 11, 2014, http://www.psi-inc.org/incidentTrends.cfm; 3. Pharmaceutical Security Institute 2014, *Counterfeit Situation*, *Geographic Distribution*, viewed June 11, 2014, http://www.psi-inc.org/geographicDistributions.cfm

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# APPENDIX 1

# HISTORY OF DRUG DISCOVERY AND DEVELOPMENT

## A1.1 EARLY HISTORY OF MEDICINE

Drug discovery and development has a long history and dates back to the early days of human civilization. In those ancient times, drugs were not just used for physical remedies but were also associated with religious and spiritual healing. Sages or religious leaders were often the administrators of drugs. The early drugs or folk medicines were mainly derived from plant products, and supplemented by animal materials and minerals. These drugs were most probably discovered through a combination of trial and error experimentation and observation of human and animal reactions as a result of ingesting such products.

Although these folk medicines probably originated independently in different civilizations, there are a number of similarities, for example, in the use of same herbs for treating similar diseases. This is likely to be a contribution by ancient traders, who in their travels might have assisted the spread of medical knowledge.

Folk medicines were the only available treatments until recent times. Drug discovery and development started to follow scientific techniques in the late 1800s. From then on, more and more drugs were discovered, tested, and synthesized in large-scale manufacturing plants, as opposed to the extraction of drug products from natural sources in relatively small batch quantities. After World War I, the modern pharmaceutical industry came into being, and drug discovery and development following scientific principles was firmly established.

*Drugs: From Discovery to Approval*, Third Edition. Rick Ng.

Although pharmaceutical drugs are now widely used worldwide, many ethnic cultures have retained their own folk medicines. In certain instances, these folk medicines exist side by side and are complemented by pharmaceutical drugs.

The following are some snapshot examples of how drugs were discovered from the early human civilizations.

### A1.1.1 Chinese Medicine

Traditional Chinese medicine (TCM) is believed to have originated in the times of the legendary emperor Shennong 5,000 years ago. The dynasty system and meticulous recording have helped to preserve the TCM scripts of old China. Some important medical writings are *Shang Han Lun* (Discussion of Fevers), *Huang Di Nei Jing* (The Internal Book of Emperor Huang), and *Shennong Ben Cao Jing* (The Pharmacopoeia of Shennong – a legendary emperor). Exhibit A1.1 relates a legend about the discovery of a herb for treating injuries.

The Chinese pharmacopoeia is extensive. Some of the active ingredients from Chinese herbs have been used in "Western" drugs; for example, reserpine from *Rauwouofia* for antihypertensive and emotional and mental control, and the alkaloid ephedrine from *Mahuang* for the treatment of asthma.

### A1.1.2 Egyptian Medicine

Ancient papyrus provided written records of early Egyptian medical knowledge. The Ebers papyrus (from around 3,000 BC) provided 877 prescriptions and recipes for internal medicine, eye and skin problems, and gynecology. Another record, from the Kahun papyrus of around 1,800 BC, detailed treatments for gynecological problems. Medications were based mainly on herbal products such as myrrh, frankincense, castor oil, fennel, sienna, thyme, linseed, aloe, and garlic.

### A1.1.3 Indian Medicine

The Indian folk medicine, called Ayurvedic medicine, can be traced back 3,000–5,000 years, and was practiced by the Brahmin sages of ancient times. The treatments were

**Exhibit A1.1 A Legend about San Qi**

Chinese legend described that the legendary emperor Shennong one day tried to kill a snake by beating it. The snake returned a few days later, apparently none the worse after the beating. He beat it again and left it mortally injured. Again, the snake returned several days later. This time, after the beating, he observed that the snake crawled back into the bush and ate a plant material. This plant is now called San Qi (*Panax notoginseng*) and is used for treating external injuries. It is an ingredient for the well-known TCM herbal formula known as Yunnan Bai Yao.

*Source*: Data from Reid, D 1996, *Chinese Herbal Medicine*, Shambhala Publications, Boston, MA.

set out in sacred writings called Vedas. The material medica are extensive and most are based on herbal formulations. Some of the herbs have appeared in Western medicines, such as cardamom and cinnamon. Susruta, a physician in the fourth century AD, described the use of henbane as antivenom for snakebites.

### A1.1.4 Greek Medicine

Some of the Greek medical ideas were derived from the Egyptians, Babylonians, and even the Chinese and Indians. Castor oil was prescribed as a laxative; linseed or flex seed were used as a soothing emollient, laxative, and antitussive. Other treatments include fennel plant for relief of intestinal colic and gas, and asafetida gum resin as an antispasmodic. The greatest Greek contribution to the medical field is perhaps to dispel the notion that diseases are due to supernatural causes or spells. The Greeks established that diseases result from natural causes. Hippocrates, the father of medicine, at about 400 BC is credited with laying down the ethics for physicians. Exhibit A1.2 describes the mythology of Asclepius, the Greek God of Medicine.

### A1.1.5 Roman Medicine

As great administrators, the Romans instituted hospitals, although these were used mainly to cater for the needs of the military. Through this work, organized medical care was made available. The Romans also extended the pharmacy practice of the Greeks. Dioscorides and Galen were two noted physicians in Roman days. Dioscorides' *Materia Medica* contains descriptions of treatments based on 80% plant, 10% animal, and 10% mineral products.

## A1.2 DRUG DISCOVERY AND DEVELOPMENT IN THE MIDDLE AGES

The Middle Ages, from around AD 400 to 1500, witnessed the decline of the Roman influence. This was also the time when plagues scourged many parts of Europe. Diseases such as bubonic plague, leprosy, smallpox, tuberculosis, and scabies were rampant. Many millions of people succumbed to these diseases.

**Exhibit A1.2 Asclepius: Greek God of Medicine**

In Greek mythology, Asclepius, the god of medicine, studied medicine under Chiron. He excelled over Chiron, and his medical skills were reputed to be able to bring back the dead. This incurred the wrath of Hades, the god of the underworld, and the envy of other gods. They complained to Zeus, who also thought that he alone should have the power of life and death. Zeus slew Asclepius with a thunderbolt. However, Asclepius' daughters, Panacea and Hygeia, survived and carried on to tend to the sick.

*Source*: Data from Wikipedia, *Asclepius*, viewed June 11, 2014, http://en.wikipedia.org/wiki/Asclepius

### A1.2.1 The Early Church

There are some references to herbs in the Bible. However, the Church's main contribution to medicines is the preservation and transcription of Greek medical manuscripts and treatises. This enabled the knowledge developed in the ancient times to be continued and later used in the Renaissance period.

### A1.2.2 Arabian and Persian Medicine

Through trades with many regions, the Arabians learned and extended medical knowledge. Their major contribution is perhaps the knowledge of medical preparations and distillation methods, although the techniques were probably derived from the practices of alchemists. Avicenna, a Persian, around AD 900–1000, recorded a vast encyclopedia of medical description and treatment. Another noted Persian physician was Rhazes, who accurately described measles and smallpox.

## A1.3 FOUNDATION OF CURRENT DRUG DISCOVERY AND DEVELOPMENT

The Renaissance period laid the foundation for scientific thoughts in medicinal preparations and medical treatments. There were many advances made in anatomy, physiology, surgery, and medical treatments, including public health care, hygiene, and sanitation.

### A1.3.1 Smallpox

In 1796, Edward Jenner successfully experimented with smallpox inoculations (Exhibit A1.3). This paved the way for the use of vaccination against some infectious diseases.

### A1.3.2 Digitalis

In the late 1700s, William Withering introduced digitalis, an extract from the plant foxglove, for treatment of cardiac problems.

### A1.3.3 Scurvy

James Lind (1753) noted that scurvy was caused by the lack of vitamin C. He prescribed the consumption of lemon juice to treat scurvy.

### A1.3.4 Rabies

Louis Pasteur (1864) discovered that microorganisms cause diseases, and he devised vaccination against rabies. This was achieved through the use of attenuated rabies virus.

## A1.4 BEGINNINGS OF MODERN PHARMACEUTICAL INDUSTRY

Despite the advances made in the 1800s, there were only a few drugs available for treating diseases at the beginning of the 1900s. These were the following:

**Exhibit A1.3 Edward Jenner's Smallpox Vaccine**

In the late 1700s, Jenner heard that people who worked with cattle and had caught the cowpox disease (a mild disease related to smallpox) were immune and never caught smallpox. In 1796, he proceeded to inoculate a boy using the fluid from the blister of a woman with cowpox. The boy developed cowpox. Two months later, Jenner inoculated the boy with fluids from the blister of a smallpox sufferer. The boy became immune and did not get smallpox.

Through his work, Jenner invented vaccination, and saved many lives. However, in today's regulatory control, Jenner's method would not have been approved.

*Source*: Data from Wikipedia, *Edward Jenner*, viewed June 11, 2014, http://en.wikipedia.org/wiki/Edward_Jenner

- *Digitalis*: extracted from a plant called foxglove, digitalis stimulates the cardiac muscles, and was used to treat cardiac conditions
- *Quinine*: derived from the bark of the Cinchona tree, and used to treat malaria
- *Ipecacuanha*: extracted from the bark or root of the Cephaelis plant, and used to treat dysentery
- *Aspirin*: extracted from bark of willow tree, and used for the treatment of fever
- *Mercury*: used to treat syphilis.

More systematic research was being performed to discover new drugs from the early 1900s.

Paul Ehrlich used an arsenic compound, arspheamine, to treat syphilis. Gerhard Domagh found that the red dye Prontosil was active against streptococcal bacteria. Later, French scientists isolated the active compound to be sulfanilamide, and this gave rise to a new range of sulfa drugs against hosts of bacteria.

### A1.4.1 Penicillin

In 1928, Alexander Fleming discovered that *Penicillium* mould was active against staphylococcus bacteria. Ernst Chain rediscovered this fact some 10 years later, when he collaborated with Howard Florey. By 1944, large-scale production of penicillin was available through the work of Howard Florey and Ernst Chain. This work foreshadowed the commencement of biotechnology, where microorganisms were used to produce drug products. A description of the discovery and large-scale manufacturing of penicillin is given in Exhibit A1.4.

## A1.5 EVOLUTION OF DRUG PRODUCTS

In the early days, until the late 1800s, most drugs were based on herbs or extraction of ingredients from botanical sources.

The synthetic drugs using chemical methods were heralded at the beginning of the 1900s, and the pharmaceutical industry was founded. Many drugs were researched and

**Exhibit A1.4 The Development of Penicillin**

In the 1930s, Howard Florey and Ernst Chain worked with a team of scientists at Oxford University in Britain. Ernst Chain discovered an earlier paper by Alexander Fleming on the antibacterial properties of penicillin.

The Florey–Chain team's investigation showed that penicillin interferes with the cell wall of bacteria. Bacteria cells ruptured instead of continuing to grow. In 1938, their animal test, on eight mice given lethal doses of infectious bacteria, showed stunning results. The four mice with penicillin survived, whereas four controls with no medication died. Their first human patient who suffered from infection showed early improvement with penicillin, but died subsequently when the stock of penicillin was exhausted.

The team worked on the technology for large-scale production of penicillin. Commercial quantities were available before the end of World War II and saved millions of lives, especially soldiers wounded in the war.

*Source*: Data from Torok, S, *Howard Florey—the Story*: *Maker of the Miracle Mould*, viewed June 11, 2014, http://www.abc.net.au/science/slab/florey/story.htm

manufactured, but mostly they were used for therapeutic purposes rather than completely curing the diseases.

From the early 1930s, drug discovery concentrated on screening natural products and isolating the active ingredients for treating diseases. The active ingredients are normally the synthetic version of the natural products. These synthetic versions, called new chemical entities (NCEs) have to go through many iterations and tests to ensure they are safe, potent, and effective.

In the late 1970s, development of recombinant DNA products utilizing knowledge of cellular and molecular biology commenced. The biotechnology industry became a reality.

The pharmaceutical industry, together with the advances in gene therapy and understanding of mechanisms of causes of diseases, and the research results from the Human Genome Project, have opened up a plethora of opportunities and made possible the development and use of drugs specifically targeting the sites where diseases are caused.

## A1.6 FURTHER READING

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# APPENDIX 2

# CELLS, NUCLEIC ACIDS, GENES, AND PROTEINS

## A2.1 CELLS

Cells are the basic units for all living organisms. Every cell is bounded by a membrane which protects the cell from the outside environment. The membrane is made up of a lipid bilayer structure as shown in Figure A2.1. The function of the membrane is to control materials that enter and exit the cell and enable biochemical reactions to take place within the cell. For plants, bacteria, and fungi, there is a cell wall outside the membrane and this provides additional structural support and mechanical strength to the cell.

### A2.1.1 Prokaryote Cell

Simple single-cell organisms, such as bacteria and blue-green algae, are called prokaryotes (refer to Figure A2.2). Prokaryotes do not have a well-defined nucleus.

The genetic material, deoxyribonucleic acid (DNA), is concentrated in the nuclear region. DNA controls the functions of the cell. Ribosomes, granular structures that consist of ribonucleic acid (RNA) and proteins, are distributed in the cytosol (soluble part of the cell excluding the nuclear region).

Prokaryote cells divide and grow into two daughter cells. In the division process, the DNA replicates and each daughter cell receives one copy.

### A2.1.2 Eukaryote Cell

Complex multicellular cells, such as those of plants and humans, are termed eukaryotes. The cell structure is considerably more complex than that of the prokaryote cells

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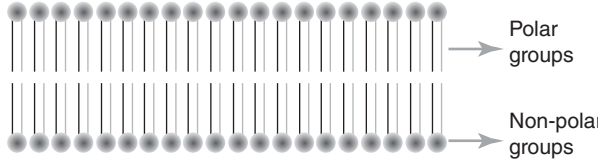



**Figure A2.1** Lipid bilayer cell membrane.

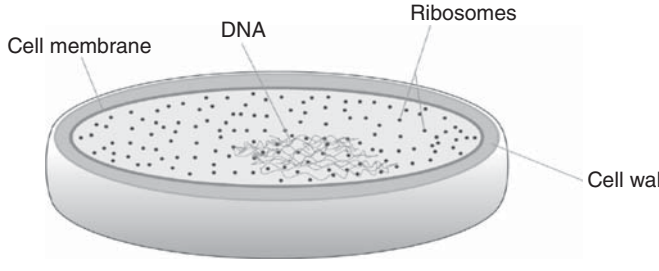



**Figure A2.2** A prokaryote cell.

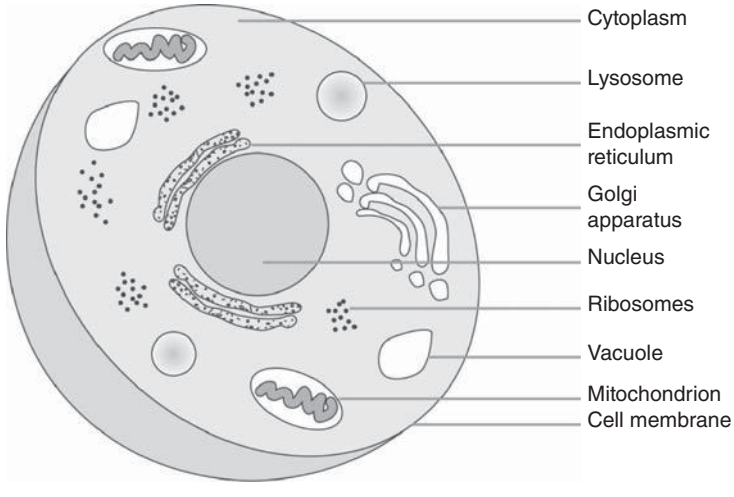



**Figure A2.3** A human eukaryote cell.

(see Figure A2.3 for a human eukaryote cell; plant cells are not shown: they have well-defined cell wall and different structure).

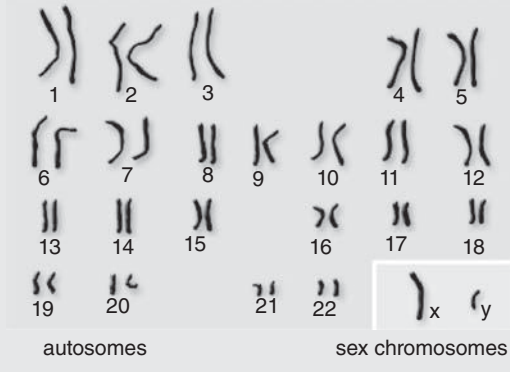
Within the cell membrane is the cytoplasm. This is where many biochemical reactions take place. The most important structure within the human cell is the nucleus. It is bounded by a nuclear membrane and is separated from other organelles (noncellular structures in a cell that serve specific functions) in the cytoplasm.

**Exhibit A2.1 Cells and Chromosomes**

Cells can be divided into germ cells and somatic cells. Germ cells are reproductive cells, for example, ova or sperm. Germ cells contain genetic characteristics that are passed on to the next generation. Somatic cells do not contribute their genes to future generations; they are the tissue cells such as nerve cells and muscle cells.

Within the cell is the nucleus with the chromosomes. DNA strands are housed within the chromosomes, together with some proteins. The 46 human chromosomes are grouped into 22 pairs and two sex chromosomes. Numbering of chromosomes is based on sizes, chromosome 1 being the largest and 22 the smallest.

In addition to the 22 pairs, a female cell contains two X chromosomes and a male cell contains an X and a Y chromosome. When a female egg (carrying an X chromosome) combines with male sperm having an X chromosome, a female offspring is born. When the egg combines with a sperm having a Y chromosome, a male offspring results.




*Source*: National Institutes of Health, *Genetics Home Reference*, How many chromosomes do people have?, viewed November 12, 2014, http://ghr.nlm.nih.gov/handbook/basics/howmanychromosomes

DNA is organized into strands within the chromosomes inside the nucleus. There are 46 chromosomes in the human cell, 23 from maternal (egg) and 23 from paternal (sperm) origin (refer to Exhibit A2.1). All human cells contain a full set of chromosomes and identical genes. However, different sets of genes are expressed, or turned on, in different cells, leading to the various types of cells, such as nerve cells and muscle cells.

Organelles in the cytoplasm are the following:

- *Mitochondrion*: the powerhouse of the cell, where oxidation processes take place to provide energy for the cell

- *Endoplasmic reticulum* (*ER*): a single membrane system of two distinct types, rough and smooth. The rough ER has ribosomes attached to the membrane, whereas the smooth ER does not
- *Ribosomes*: sites where protein synthesis takes place
- *Golgi apparatus*: involves in gathering and dispatching of proteins and lipids
- *Lysosomes*: membrane-bound sacs filled with enzymes for processing nutrients
- *Vacuole*: a space within the cytoplasm that consists of wastes and materials taken in by the cell, for example, bacteria engulfed by a white blood cell.

## A2.2 NUCLEIC ACIDS

### A2.2.1 DNA

DNA is a polymer composed of monomeric nucleic acids called nucleotides. A nucleotide consists of a nitrogenous base, sugar, and phosphoric acid (whereas a nucleoside consists of only the base and sugar, refer to Figure A2.4).

There are two types of bases: pyrimidines and purines (Figure A2.5). The pyrimidine bases include cytosine, thymine, and uracil. Cytosine is found in both DNA and RNA. Thymine only occurs in DNA, and uracil is substituted for thymine in RNA. The purine bases are adenine and guanine, both of which are found in DNA and RNA.

Nucleotides are joined into a chain formation, as illustrated in Figure A2.6(a). In DNA, two nucleotide chains intertwine around each other in a double helix formation (Figure A2.6(b)). The backbone of the two strands is the phosphate–sugar linkage.

Alignment of the two strands is via the interactions of the bases: adenine (A) of one strand pairs up with thymine (T) of the complementary strand (with two hydrogen

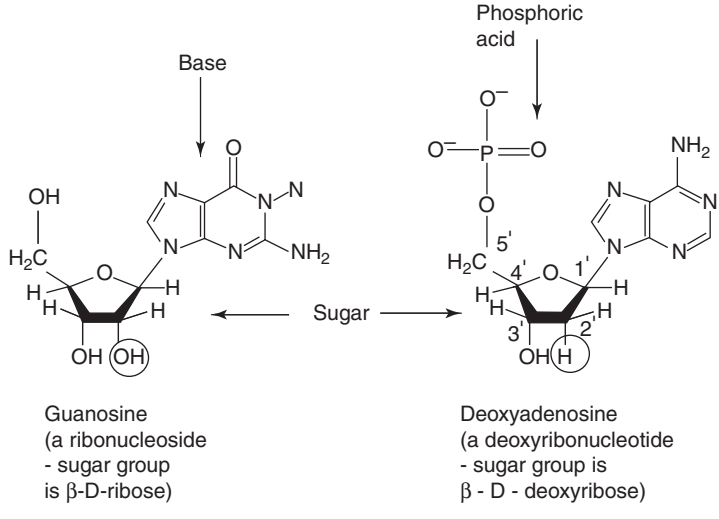



**Figure A2.4** Nucleoside and nucleotide. Circled areas show the presence and absence of oxygen atom in the ribose and deoxy ribose sugars.

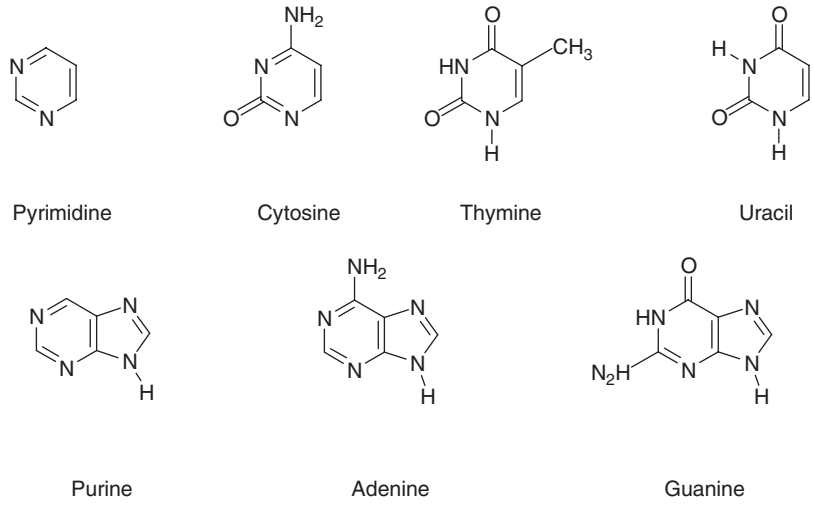



**Figure A2.5** Nucleic acid bases.

bonds); similarly, guanine (G) pairs up with cytosine (C) (with three hydrogen bonds) as shown in Figure A2.6(c).

There are 10 base pairs in a complete turn of the helix, which spans a distance of 3.4 nm. The outside diameter of the helix is about 2 nm. By convention, the double stranded DNA sequence is written from left to right; the 5′ end (position 5 of the sugar group) is assigned to the top left-hand strand.

### A2.2.2 RNA

RNA is made up of nucleotides similar to DNA, except that the sugar is $\beta$-D-ribose compared to DNA's $\beta$-D-deoxyribose (Figure 2.4). There are two-stranded RNAs, but normally RNA exists in single strand.

There are three kinds of RNA: messenger RNA (mRNA), transfer RNA (tRNA), and ribosomal RNA (rRNA). All three RNAs are involved in the synthesis of proteins using amino acids.

Information for making a protein is passed from the DNA to the mRNA. This is likened to the master copy (DNA) of a building plan residing in a document room (nucleus) being photocopied onto a duplicate (mRNA). The duplicated plan (mRNA) is then taken to a building site (ribosome) for protein construction. DNA determines the nucleotide sequence of the mRNA; the process of transferring the order of sequence is called transcription. Amino acids (there are 20 naturally occurring amino acids; refer to Table A2.1) for the construction of protein are brought to the ribosome by tRNAs. The role of rRNA is to combine with protein to form ribosomes, the site where protein synthesis takes place. The order of amino acids in the protein is controlled by mRNA via a process called translation. A schematic representation of the protein synthesis process is shown in Figure A2.7.

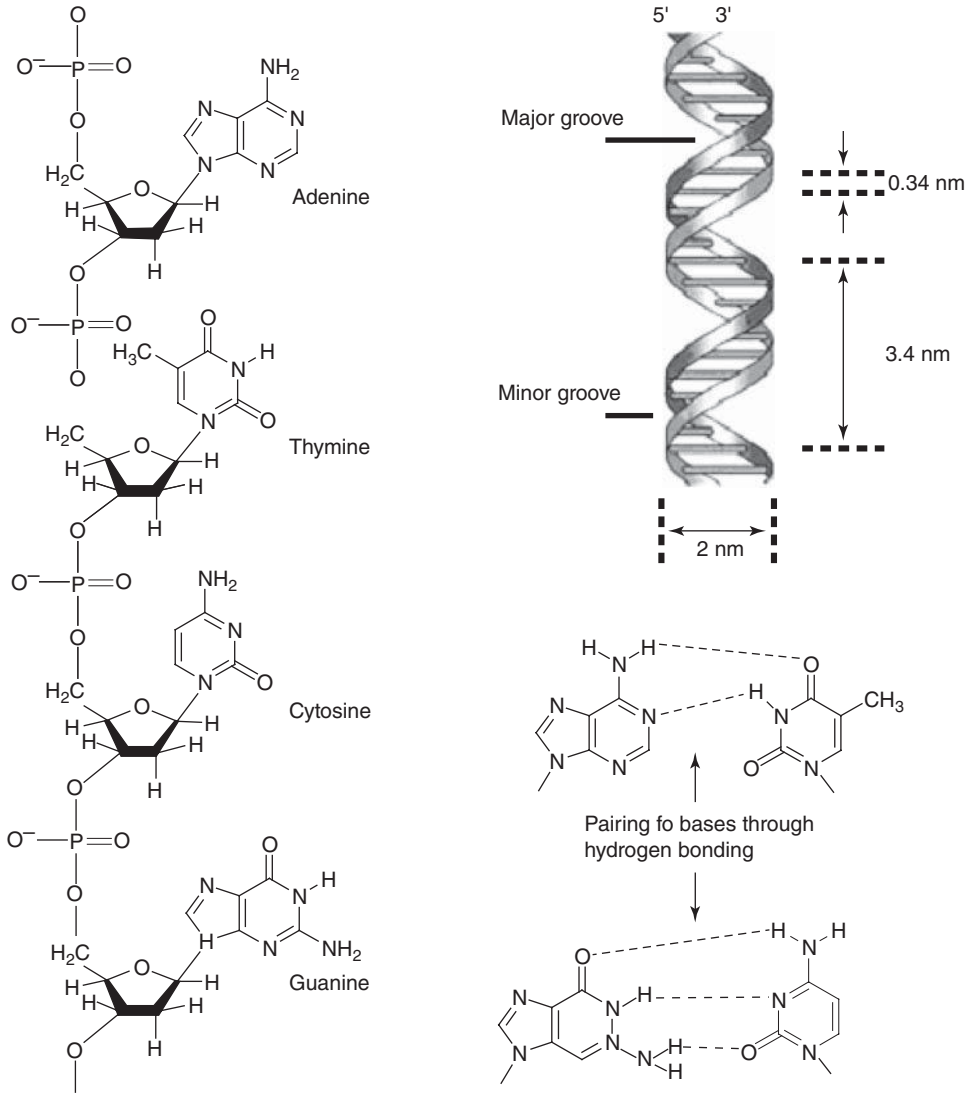



**Figure A2.6** (a) Single strand nucleotide, (b) DNA in double helix formation, and (c) the pairing up of bases on two separate strands via hydrogen bonding to form the double helix.

## A2.3 GENES AND PROTEINS

### A2.3.1 Genes

Genes are our hereditary units. Each gene contains the instruction for the synthesis of protein. The summation of all the genes within a cell is called the genome. Instructions for synthesis of proteins are stored in the DNA through specific arrangements of the base sequence. The sequence is made up of the four bases: adenine, thymine,

**TABLE A2.1 The 20 Naturally Occurring Amino Acids**

| Name | Abbreviation | Name | Abbreviation |
|---|---|---|---|
| Alanine | Ala | Leucine | Leu |
| Arginine | Arg | Lysine | Lys |
| Asparagine | Asn | Methionine | Met |
| Aspartic Acid | Asp | Phenylalanine | Phe |
| Cysteine | Cys | Proline | Pro |
| Glutamic Acid | Glu | Serine | Ser |
| Glutamine | Gln | Threonine | Thr |
| Glycine | Gly | Tryptophan | Trp |
| Histidine | His | Tyrosine | Tyr |
| Isoleucine | Ile | Valine | Val |

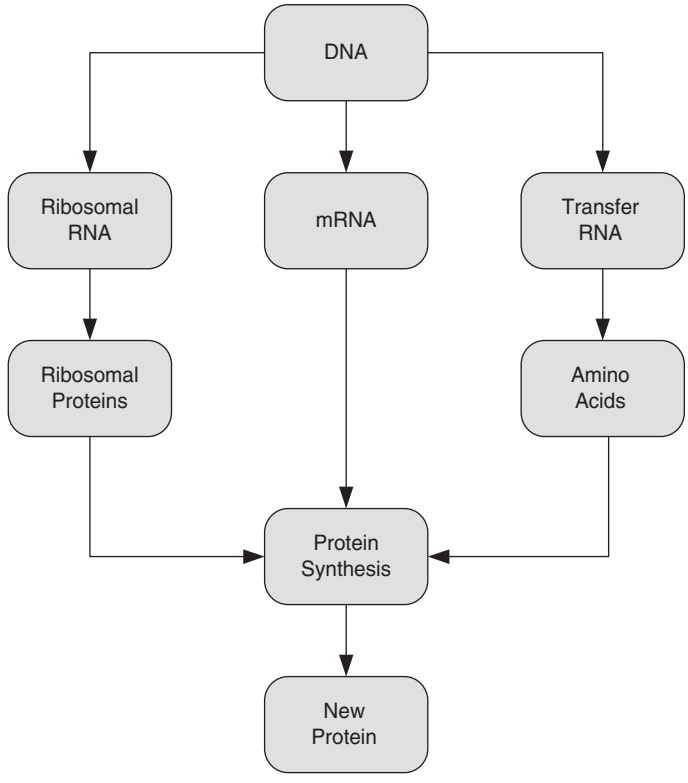



**Figure A2.7** Protein synthesis process.

guanine, and cytosine (A, T, G, and C). These instruction codes are arranged in three-letter words called codons. Each word specifies which amino acid is to be used for constructing the proteins. Genes are not found in a continuous fashion in the DNA sequence. Sequences that are expressed (used to make proteins) are called exons. Intervening sequences, which do not code for proteins, are called introns. Promoters and repressors are present along the DNA sequence to control the expression or

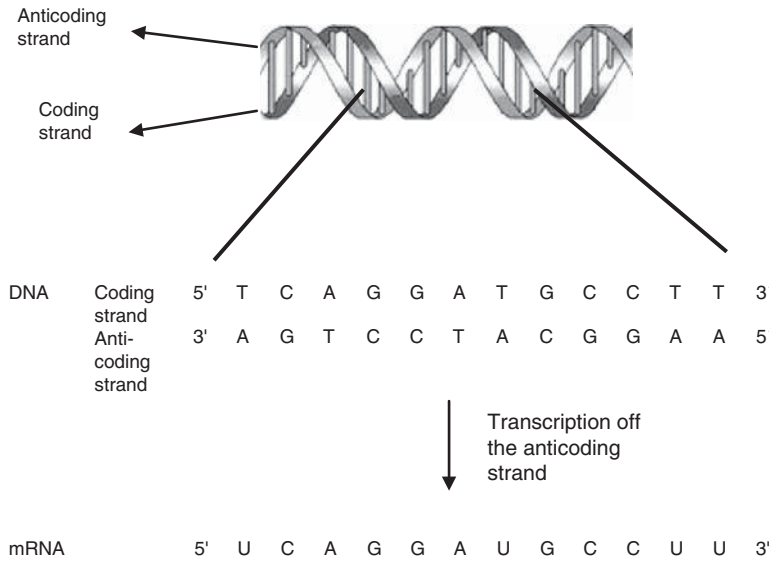



**Figure A2.8** Transcription from DNA to mRNA.

suppression of a gene. Information from the exons is transcribed from DNA to mRNA. There are many ways to process transcription, and so one gene can code for multiple versions of mRNA, leading to multiple proteins.

To transcribe information from DNA to mRNA, one strand of the DNA is used as a template. This is called the anticoding, or template, strand and the sequence of mRNA is complementary to that of the template DNA strand (Figure A2.8) (i.e., C→G, G→C, T→A, and A→U; note that T is replaced by U in mRNA). The other DNA strand, which has the same base sequence as the mRNA, is called the coding, or sense, strand. There are 64 ($4 \times 4 \times 4$) possible triplet codes of the four bases; 61 are used for coding amino acids and three for termination signals. As there are 20 amino acids for the 61 codes, some triplets code for the same amino acid. A table of the genetic code is presented in Exhibit A2.2.

In situations where there are errors in the chromosomes or genes, genetic disorders may results as shown in Exhibit A2.3.

### A2.3.2 Proteins

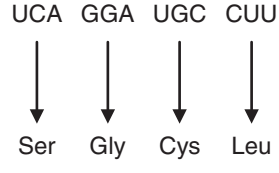
Proteins are the workhorses in our bodies, and carry out all the essential processes and functions. They may come in the forms of enzymes for catalyzing reactions, hormones for transmitting information between cells, receptors for receiving signals, and antibodies for defending us from invading organisms. Proteins are made up of chains of amino acids. Amino acids have a carboxyl group ($COO^-$) at one end and an amino group ($NH_3^+$) at another. Peptide bonds are formed by joining one carboxyl group with an amino group (Figure A2.9).

**Exhibit A2.2 The Genetic Code Dictionary**

The triplet codon genetic codes of mRNA are translated into amino acids as shown below.

| | | | |
|---|---|---|---|
| UUU→Phe | UCU→Ser | UAU→Tyr | UGU→Cys |
| UUC→Phe | UCC→Ser | UAC→Tyr | UGC→Cys |
| UUA→Leu | UCA→Ser | UAA→Stop | UGA→Stop |
| UUG→Leu | UCG→Ser | UAG→Stop | UGG→Trp |
| CUU→Leu | CCU→Pro | CAU→His | CGU→Arg |
| CUC→Leu | CCC→Pro | CAC→His | CGC→Arg |
| CUA→Leu | CCA→Pro | CAA→Gln | CGA→Arg |
| CUG→Leu | CCG→Pro | CAG→Gln | CGG→Arg |
| AUU→Ile | ACU→Thr | AAU→Asn | AGU→Ser |
| AUC→Ile | ACC→Thr | AAC→Asn | AGC→Ser |
| AUA→Ile | ACA→Thr | AAA→Lys | AGA→Arg |
| AUG→Met | ACG→Thr | AAG→Lys | AGG→Arg |
| GUU→Val | GCU→Ala | GAU→Asp | GGU→Gly |
| GUC→Val | GCC→Ala | GAC→Asp | GGC→Gly |
| GUA→Val | GCA→Ala | GAA→Glu | GGA→Gly |
| GUG→Val | GCG→Ala | GAG→Glu | GGG→Gly |

The translation of genetic information from the mRNA data in Figure A2.8 gives rise to the amino sequence shown below, using the translation table from Exhibit A2.2 as a guide:




Through the formation of polypeptide bonds between amino acids, very long chains of sequences are obtained. Generally, proteins consist of hundreds and thousands of amino acids. For example, human hemoglobin has four polypeptide chains, of which two are $\alpha$-chains and two are $\beta$-chains. There are 141 amino acids in each $\alpha$-chain with a sequence of:

Val Leu Ser Pro Ala … … … …. Thr Ser Lys Tyr Arg

The $\beta$-chain has 146 amino acids with the sequence:

Val His Leu Thr Pro … … … … Ala His Lys Tyr His

Proteins are not linear molecules. From the linear sequences (primary structures) of amino acids, the interactions of various components of the amino acids via H-bonding,

**Exhibit A2.3 Examples of Selected Genetic Disorders**

**Down Syndrome**

People with Down Syndrome have three chromosomes, a trisomy, of Chromosome 21. The result is lower cognitive ability and physical stature. The disorder also causes affected people with higher incidence of heart, intestinal, and thyroid problems.

**Edwards Syndrome**

A genetic disorder with three chromosomes in Chromosome 18 due to abnormality in the egg or sperm before conception. Most fetuses do not survive and about 50% die in pregnancy and of those born; only 5–10% survives beyond 1 year. Babies have abnormally shaped head and other physical characteristics. In addition sufferers of Edwards Syndrome also have heart defects.

**Klinefelter's Syndrome**

This condition affects males where there are three chromosomes, XXY instead of XY in the sex chromosome. These males are normally infertile and have some female characteristics. There may be a greater risk in developing germ cell tumors.

**Turner Syndrome**

Instead of XX female sex chromosome, the female sufferers have only one X chromosome. Most fetuses do not survive. Those born are infertile and carry congenital defects in heart, kidney, and thyroid.

disulfide bonding and electrostatic influences can result in the proteins forming into helices or sheets (secondary structures). They can further become folded into large three-dimensional structures called tertiary structures. These tertiary structures may aggregate into even larger units through noncovalent bonding and give rise to quaternary structures (Figures A2.10 and 2.11).

### A2.3.3 Human Genome Project, Genomics and Proteomics

The Human Genome Project was launched in 1990. It was a US$3 billion project involving 350 laboratories around the world. In 2001, a draft copy of the sequence of the three billion base pairs was published. By April 2003, 99% of the human genome had been sequenced. The project goals were the following:

- *Identify* all the approximately 20,500 genes in human DNA
- *Determine* the sequences of the three billion chemical base pairs that make up human DNA
- *Store* this information in databases
- *Improve* tools for data analysis

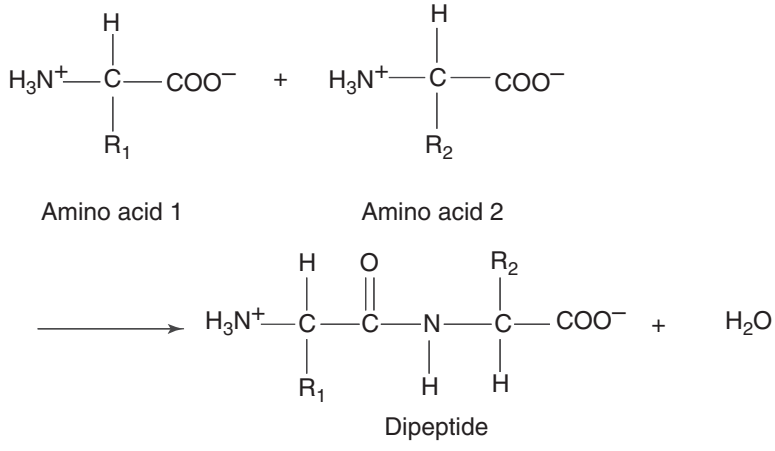



**Figure A2.9** Formation of a peptide bond.

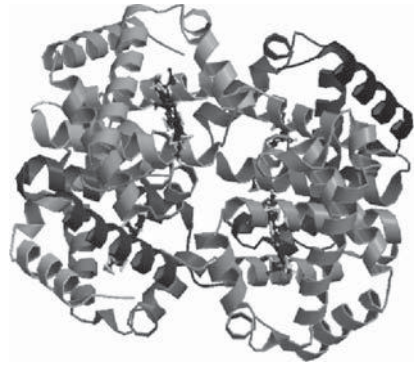


**Figure A2.10** Protein structure: Human deoxyhemoglobin. (*Source*: Reprinted with permission from Protein Data Bank, Chatake T *et al*. 2007, 'Protonated states of buried histidine residues in human deoxyhemoglobin revealed by neutron crystallography', *Journal of the American Chemical Society*,, 129, pp. 14840–14841, http://www.rcsb.org/pdb/explore/explore.do?structureId=2DXM) .

- *Transfer* related technologies to the private sector
- *Address* the ethical, legal, and social issues that may arise from the project.

Scientists are continuing to determine the 20,500 genes that control our lives. Genomics helps scientist to understand biochemistry in our body and thereby develop better drugs to treat diseases.

The real value in the genome sequence is to find out the regions of the genome that encode proteins. Proteomics, the study of the structures and functions of proteins, further enhances our understanding of proteins and their functions, leading to insights on

**Figure A2.11** Protein structure: HIV-1 Protease complexed with a tripeptide inhibitor. (*Source*: Reprinted with permission from Protein Data Bank, Louis, JM, Dyda F, Nashed, F *et al*. 1998, 'Hydrophilic peptides derived from the transframe region of Gag-Pol inhibit the HIV-1 protease', *Biochemistry*, 37. pp. 2105, http://www.rcsb.org/pdb/explore/explore.do?structureId=1A30).

how they are affected in normal and disease conditions. Exhibit A2.3 shows some of the medical conditions due to genetics problems.

## A2.4 FURTHER READING

Campbell, JJ 2008, *Understanding Pharma: The Professional's Guide to How Pharmaceutical Companies Really Works*, Pharmaceutical Institute, Inc., Raleigh, NC.

Campbell, MK and Farrell, SO 2014, *Biochemistry*, 8th edn., Cengage Learning, Stamford, CT.

# APPENDIX 3

# SELECTED DRUGS AND THEIR MECHANISMS OF ACTION

| Drug | Indication | Mechanism of Action |
|---|---|---|
| Abilify (aripiprazole) | Schizophrenia, bipolar disorder, major depressive disorder | Mediates through a combination of partial agonist activity at D2 and 5-$HT_{1A}$ receptors and antagonist activity at 5-$HT_{2A}$ receptors |
| Adderall (amphetamine aspartate; amphetamine sulfate; dextroamphetamine saccharate; dextroamphetamine sulfate) | Attention Deficit Hyperactivity Disorder (ADHD), Narcolepsy | Blocks reuptake of norepinephrine and dopamine into the presynaptic neuron and increases the release of these monoamines into the extraneuronal space |
| Advair Discus (fluticasone propionate and salmeterol | Asthma | Fluticasone propionate is a glucocorticoid receptor agonist with potent antiinflammatory activity; salmeterol is a $beta_2$-adrenergic agonist |

(*continued*)

*Drugs: From Discovery to Approval*, Third Edition. Rick Ng.

| Drug | Indication | Mechanism of Action |
|---|---|---|
| Afinitor (everolimus) | HER2-negative breast cancer, neuroendocrine tumors, advanced renal cell carcinoma | Inhibits rapamycin (mTOR) kinase; the mTOR pathway is dysregulated in several human cancers |
| Alimta (pemetrexed disodium) | Nonsmall cell lung cancer, mesothelioma | Disrupts folate-dependent metabolic processes essential for cell replication |
| Altace (ramipril) | Hypertension | Inhibits ACE, decreases peripheral arterial resistance |
| Anafranil (clomipramine hydrochloride) | Obsessive-compulsive disorder | Affects neuronal transmissions; inhibits reuptake of 5-HT |
| AndroGel (testosterone gel) | Deficiency or absence of endogenous testosterone | Supplements deficiency in testosterone |
| Aralen (chloroquine phosphate) | Malaria | Inhibits protein synthesis by inhibiting DNA and RNA polymerase |
| Aranesp (darbepoetin alfa) | Anemia due to chronic kidney disease and chemotherapy | Stimulates erythropoiesis |
| Atacand (candesartan cilexetil-hydrochloro thiazide) | Hypertension | Acts as an angiotensin II receptor antagonist |
| Atripla (efavirenz/emtricitabine/ tenofovir disoproxil fumarate) | HIV infection | Efavirenz is a non-nucleoside reverse transcriptase inhibitor of HIV-1; emtricitabine inhibits the activity of the HIV-1 reverse transcriptase; tenofovir diphosphate inhibits the activity of HIV-1 reverse transcriptase |
| Augmentin (amoxicillin/clavulanate potassium) | Pneumonia, acute bacterial sinusitis | Inhibits bacterial cell wall synthesis |
| Avastin (bevacizumab) | Metastatic colorectal cancer, nonsmall cell lung cancer, metastatic breast cancer, metastatic renal cell carcinoma | Binds to VEGF and prevents VEGF binding to its receptors and inhibits formation of new blood vessels |
| Avonex (interferon beta-1a) | Relapsing forms of multiple sclerosis | Activates numerous interferon-induced gene products and markers, increase levels of interleukin 10 |

(*continued*)

| Drug | Indication | Mechanism of Action |
|---|---|---|
| Benadryl (diphenhydramine) | Antihistamine for hayfever or other respiratory allergies, runny nose and sneezing due to common cold | Blocks the actions of histamine on $H_1$ receptor |
| Benicar (olmesartan medoxomil) | Hypertension | Inhibits angiotensin II converting enzyme (ACE) |
| Betaseron (interferon beta-1b) | Relapsing forms of multiple sclerosis | Reduces neuron inflammation, increases production of nerve growth factor |
| Cardura (doxazosin mesylate) | Benign prostatic hyperplasia, hypertension | Blocks $\alpha_1$-adrenergic receptor, resulting in decrease of blood pressure |
| Celebrex (celecoxib) | Osteoarthritis, rheumatoid arthritis, juvenile rheumatoid arthritis, ankylosing spondylitis, acute pain, primary dysmenorrhea | COX-2 selective nonsteroidal antiinflammatory drug (NSAID); inhibits transformation of arachidonic acid to prostaglandin precursors |
| Cialis (tadalafil) | Erectile dysfunction, benign prostatic hyperplasia | Enhances the effect of nitric oxide needed for the erection mechanism |
| Claritin (loratadine) | Allergic rhinitis, chronic idiopathic urticaria | Acts as $H_1$ histamine antagonist |
| Codeine Sulfate | Mild to moderately severe pain | Binds to opiate receptors and blocks pain pathway |
| Combivent Respimat (ipratropium bromide and albuterol sulfate) | Chronic obstructive pulmonary disease (COPD) | Ipratropium bromide is an anticholinergic agent; albuterol sulfate activates $beta_2$-adrenergic receptors leading to smooth muscle relaxation |
| Complera (emtricitabine, rilpivirine, tenofovir disoproxil fumarate) | HIV | Emtricitabine, rilpivirine and tenofovir are reverse transcriptase inhibitors; they inhibit the enzyme that copies HIV RNA into new viral DNA |
| Copaxone (glatiramer acetate) | Relapsing forms of multiple sclerosis | Modifies immune processes responsible for pathogenesis of multiple sclerosis |

(*continued*)

| Drug | Indication | Mechanism of Action |
|---|---|---|
| Crestor (rosuvastatin calcium) | Hyperlipidemia, dyslipidemia, hypertriglyceridemia, atherosclerosis | Inhibitor of HMG-CoA reductase that converts coenzyme A to a cholesterol precursor |
| Cymbalta (duloxetine HCl) | Depressive disorder, anxiety disorder, neuropathic pain, fibromyalgia, and chronic musculoskeletal pain | Serotonergic and noradrenergic reuptake inhibitor in the CNS |
| Daytrana (methylphenidate) | ADHD | Blocks reuptake of norepinephrine and dopamine into the presynaptic neuron and increases the release of these monoamines into the extraneuronal space |
| Dexilant (dexlansoprazole) | Erosive esophagitis, nonerosive gastroesophageal reflux disease | Suppresses gastric acid secretion by specific inhibition of the ($H^+$, $K^+$)-ATPase in the gastric parietal cell |
| Diovan (valsartan) | Hypertension | Blocks the binding of angiotensin II to its receptors |
| Dutoprol (metoprolol succinate-extended release/ hydrochlorothiazide) | Hypertension | A $beta_1$-selective (cardioselective) adrenergic receptor-blocking agent, also inhibits $beta_2$-adrenoreceptors |
| Enbrel (etanercept) | Rheumatoid arthritis, polyarticular juvenile idiopathic arthritis, psoriatic arthritis, ankylosing spondylitis, plaque psoriasis | TNF receptor that binds to TNF and renders TNF inactive |
| Epivir (lamivudine) | HIV | Inhibits HIV reverse transcriptase and DNA polymerase |
| Epogen (epoetin alfa) | Anemia due to chronic kidney disease, HIV and cancer patients undergoing chemotherapy | Stimulates erythropoiesis |
| Evista (raloxifene hydrochloride) | Osteoporosis | Estrogen receptor modulator with estrogenic actions on bone and antiestrogenic actions on uterus and breast |

(*continued*)

| Drug | Indication | Mechanism of Action |
|---|---|---|
| Fenofibrate (fenobirate) | Hyperlipidemia, dyslipidemia, hypertriglyceridemia | Increases lipolysis and elimination of triglyceride-rich particles from plasma by activating lipoprotein lipase and reducing production of apoprotein C-III |
| Flovent (fluticasone propionate) | Asthma | Agonist at the glucocorticoid receptor with potent antiinflammatory effects |
| Fosamax (alendronate sodium) | Osteoporosis | Binds to hydroxyapatite in bone and inhibits osteoclast-mediated bone resorption |
| Gilenya (fingolimod) | Relapsing forms of multiple sclerosis | Reduction of lymphocyte migration into the central nervous system |
| Gleevec (imatinib mesylate) | Chronic myeloid leukemia, acute lymphoblastic lukemia | Inhibits tyrosine kinase (bcr-abl) and induces apoptosis in bcr-abl positive cell lines |
| Herceptin (trastuzumab) | Breast cancer, gastric cancer | Inhibits proliferation of tumor cells with HER2 gene |
| Humalog (insulin lispro) | Diabetes mellitus | Regulation of glucose metabolism |
| Humira (adalimumab) | Rheumatoid arthritis, juvenile idiopathic arthritis, psoriatic arthritis, ankylosing spondylitis, Crohn's disease, ulcerative colitis, plaque psoriasis | Binds to TNF-$\alpha$ and blocks its interaction with p55 and p75 cell surface TNF receptors; lyses surface TNF expressing cells in presence of complement |
| Invega Sustenna (paliperidone palmitate) | Schizophrenia | Dopamine Type 2 (D2) and serotonin Type 2 (5HT2A) receptor antagonism. |
| Isentress (raltegravir) | HIV | Targets integrase, an enzyme that integrates viral genetic material into human chromosome |
| Janumet (sitagliptin/metformin HCl) | Type II diabetes mellitus | Regulation of glycemic control |
| Januvia (sitagliptin) | Type II diabetes mellitus | Inhibits the enzyme dipeptidyl peptidase 4 and leads to the increase secretion of insulin |
| Lanoxin (digoxin) | Mild to moderate heart failure | Inhibits Na/K/ATPase, increases intracellular calcium and increases ventricular contractibility |

(*continued*)

| Drug | Indication | Mechanism of Action |
|---|---|---|
| Lantus (insulin glargine) | Type I and Type II diabetes mellitus | Regulates glucose metabolism by stimulating glucose uptake and inhibit glucose production |
| Levemir (insulin detemir) | Diabetes mellitus | Regulation of glucose metabolism |
| Levo-T (levothyroxine sodium) | Hypothyroidism, euthyroid goiters | Synthetic form of thyroid hormone |
| Lipitor (atorvastatin calcium) | Hypercholesterolemia | Inhibits the enzyme HMG-CoA reductase and reduces the biosynthesis of cholesterol |
| Lovaza (omega-3-acid ethyl esters) | Hypertriglyceridemia | Reduces synthesis of triglycerides in liver |
| Lovenox (enoxaparin sodium) | Deep vein thrombosis, ischemic complications of unstable angina | Exerts antithrombotic properties |
| Lucentis (ranibizumab) | Age-related macular degeneration, macular edema, diabetic macular edema | Binds to receptor binding site of VEGF-A, reduces endothelial cell proliferation and new blood vessel formation |
| Lunesta (eszopiclone) | Insomnia | Interacts with GABA receptor |
| Lyrica (pregabalin) | Neuropathic pain, post herpetic neuralgia, fibromyalgia | Binds to alpha$_2$-delta site in CNS tissues |
| Motrin (ibuprofen) | Rheumatoid arthritis, osteoarthritis | Inhibits cyclooxygenase (COX), inhibits production of inflammatory mediators |
| Namenda (memantine HCl) | Moderate to severe dementia of Alzheimer's type | Acts as low affinity *N*-methyl-D-aspartate (NMDA) receptor antagonist |
| Nasonex (mometasone furoate monohydrate) | Allergic rhinitis, nasal polyps | Glucocorticosteroid with potent anti-inflammatory properties |
| Neulasta (pegfilgrastim) | Decrease incidence of infection in patients undergoing chemotherapy | Stimulate bone marrow to produce more neutrophils to fight infection in patients undergoing chemotherapy |
| Nexium (esomeprazole magnesium) | Erosive esophagitis, gastroesophageal reflux disease, gastric ulcer, *H. pylori* triple therapy (Nexium with amoxicillin and clarithromycin) | Proton pump inhibitor that suppresses gastric acid secretion by specific inhibition of the $H^+/K^+$-ATPase in the gastric parietal cell |
| NovoLog (insulin aspart) | Diabetes mellitus | Regulation of glucose metabolism |

(*continued*)

| Drug | Indication | Mechanism of Action |
|---|---|---|
| Neupogen (filgrastim) | Decrease incidence of infection in cancer patients under chemotherapy, bone marrow transplant; patients with severe chronic neutropenia | Stimulates the proliferation and differentiation of granulocytes |
| Orencia (abatacept) | Rheumatoid arthritis, juvenile idiopathic arthritis | Fusion proteins that binds to CD80 and CD86, and inhibits T cell activation |
| Ortho Evra (norelgestromin/ethinyl estradiol) | Transdermal contraceptive patch | Inhibition of ovulation |
| OxyContin (oxycodone hydrochloride) | Pain management | An opioid agonist which inhibits the release of neurotransmitters |
| Platinol (cisplatin) | Metastatic testicular tumors, metastatic ovarian tumors, bladder cancer | Binds to DNA and prevents separation of the helical strands and interferes with cell division |
| Pradaxa (dabigatran etexilate mesylate) | Reduction of risk of stroke and systemic embolism in nonvalvular atrial fibrillation, deep vein thrombosis | Exerts antithrombotic properties through binding to active sites in thrombin |
| Premarin (conjugated estrogens) | Moderate to severe vasomotor symptoms due to menopause | Replacement of estrogens for the development and maintenance of the female reproductive system and secondary sexual characteristics |
| Prevnar 13 (Pneumococcal 13-valent Conjugate Vaccine) | Vaccine against *Streptococcus pneumoniae*, ottitis media and pneumonia | Contains pneumococcal polysaccharides conjugated to a carrier protein (CRM197) to elicit T-cell dependent immune response |
| Prezista (darunavir) | HIV | Acts as a HIV-1 protease inhibitor and interferes with HIV life cycle |
| Prilosec (omeprazole) | Duodenal ulcer, gastric ulcer, gastroesophageal reflux disease | Proton pump inhibitor with specific inhibition of $H^+K^+$-ATPase |
| ProAir HFA (albuterol sulphate) | Bronchospasm | $Beta_2$-adrenergic agonist leading to muscle relaxation |

(*continued*)

| Drug | Indication | Mechanism of Action |
|---|---|---|
| Procrit (epoetin alfa) | Anemia (chronic renal failure patients, Zidovudine treated HIV patients, cancer patients on chemotherapy, surgery patients) | Stimulates erythropoiesis |
| Prozac (fluoxetine hydrochloride) | Major depressive disorder, obsessive compulsive disorder, bulimia nervosa, panic disorder | Inhibits reuptake of 5-hydroxytryptamine (serotonin) into central nervous system neurons |
| Rebif (interferon beta-1a) | Relapsing forms of multiple sclerosis | Reduction of neuron inflammation |
| Remicade (infliximab) | Crohn's disease, ulcerative colitis, rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, plaque psoriasis | Binds to TNF-$\alpha$ and inhibits binding of TNF-$\alpha$ to its receptors |
| Renvela (sevelamer carbonate) | Control of serum phosphorus in patients with chronic kidney disease | Binds to phosphate in gastrointestinal tract to lower phosphate absorption |
| Restasis (cyclosporine) | Increase tear production | Acts as partial immunomodulator to relieve ocular inflammation which suppresses tear production |
| Reyataz (atazanavir sulphate) | HIV | Acts as a HIV-1 protease inhibitor and interferes with HIV life cycle |
| Ritalin (hydrochloride methylphenidate hydrochloride) | Attention deficit disorders | Blocks reuptake of norepinephrine |
| Rituxan (rituximab) | Non-Hodgkin's lymphoma, chronic lymphocytic leukemia, rheumatoid arthritis | Binds to antigen CD20 on B-cell of non-Hodgkin's lymphoma and leads to B cell lysis |
| Roferon-A (interferon alfa-2a) | Chronic hepatitis C, Philadelphia chromosome positive myelogenous leukemia | Inhibits replication of viruses or tumor cells |
| Sensipar (cinacalcet) | Hyperparathyroidism in patients with chronic kidney disease | A calcimimetic that mimics action of calcium on tissues; increases sensitivity of calcium receptors on parathyroid cells to reduce parathyroid hormone levels |

(*continued*)

| Drug | Indication | Mechanism of Action |
|---|---|---|
| Seroquel (quetiapine fumarate) | Schizophrenia, bipolar disorder | Antagonism of dopamine type 2 (D2) and serotonin type 2 (5HT2) receptors |
| Spiriva (tiotropium bromide) | Broncospasm in COPD, chronic bronchitis and emphysema | Antimuscarinic agent that leads to bronchodialation |
| Stelara (ustekinumab) | Psoriasis, psoriatic arthritis | Monoclonal antibody that binds with p40 protein used by IL-12 and IL-13 cytokines; these cytokines are involved in inflammatory and immune responses |
| Stribild (elvitegravir, cobicistat, emtricitabine, tenofovir disoproxil fumarate) | HIV | Combination drug that suppresses viral reproduction |
| Suboxone (buprenorphine and naloxone) | Opioid addiction | Buprenorphine is a partial agonist at the mu-opioid receptor and an antagonist at the kappa-opioid receptor. Naloxone is a potent antagonist at mu-opioid receptors and produces opioid withdrawal signs and symptoms in individuals physically dependent on full opioid agonists |
| Symbicort (budesonide and formoterol fumarate dihydrate) | Asthma | Budesonide is an anti-inflammatory corticosteroid and formoterol fumarate is a long-acting selective $beta_2$-adrenergic agonist that dilates that respiratory tract |
| Synagis (palivizumab) | Prevention of serious respiratory tract disease caused by respiratory syncytial virus (RSV) in children | Monoclonal antibody with anti-RSV activity |
| Synthroid (levothyroxine sodium) | Hypothyroidism, euthyroid goiters | Synthetic thyroid hormone as supplement for thyroid deficiency |

(*continued*)

| Drug | Indication | Mechanism of Action |
|---|---|---|
| Tagamet (cimetidine) | Duodenal ulcer, benign gastric ulcer, gastroesophageal reflux disease | Acts as a histamine $H_2$ receptor antagonist and reduces secretion of gastric acid and pepsin output |
| Tamiflu (oseltamivir phosphate) | Influenza infection | Inhibits influenza virus neuraminidase and affects release of viral particles |
| Taxol (paclitaxel) | Ovarian cancer, breast cancer, Kaposi's sarcoma | Inhibits tumor cell division |
| Tecfidera (dimethyl fumarate) | Relapsing forms of multiple sclerosis | The mechanism by which dimethyl fumarate (DMF) exerts its therapeutic effect in multiple sclerosis is unknown. DMF and the metabolite, monomethyl fumarate, have been shown to activate the nuclear factor (erythroid-derived 2)-like 2 (Nrf2) pathway *in vitro* and *in vivo* studies |
| Tenormin (atenolol) | Hypertension | Competitive blocker of $\alpha$-adrenergic receptors in heart and blood vessels |
| Tylenol (acetaminophen) | Temporary relief of minor aches and pains | Acts as an analgesic and antipyretic agent |
| Uceris (budesonide) | Ulcerative colitis | Reduces the severity of inflammation and helps to heal the lining of the colon |
| Valium (diazepam) | Anxiety disorders | Enhances effect of neurotransmitter GABA and leads to central nervous system depression |
| Ventolin HFA (albuterol sulphate) | Bronchospasm | $Beta_2$-adrenergic agonist leading to muscle relaxation |
| Viagra (sildenafil citrate) | Erectile dysfunction | Enhances the effect of nitric oxide needed for the erection mechanism |
| Victoza (liraglutide) | Type II diabetes mellitus | Increases intracellular cyclic AMP leading to insulin release |

(*continued*)

| Drug | Indication | Mechanism of Action |
|---|---|---|
| Vytorin (ezetimibe/simvastatin) | Hyperlipidemia, hypercholesterolemia | Ezetimibe reduces blood cholesterol by inhibiting the absorption of cholesterol by the small intestine; Simvastatin is a specific inhibitor HMG-CoA reductase, the enzyme that catalyzes the conversion of HMG-CoA to mevalonate, leading to the formation of cholesterol |
| Vyvanse (lisdexamfetamine dimesylate) | Attention Deficit Hyperactivity Disorder (ADHD) | Blocks reuptake of norepinephrine and dopamine into the presynaptic neuron and increases the release of these monoamines into the extraneuronal space |
| Xarelto (rivaroxaban) | Reduction of risk of stroke and systemic embolism in nonvalvular atrial fibrillation, deep vein thrombosis | Inhibitor of Factor X and prothrombinase, reduces platelet aggregation to form blood clots |
| Xeloda (capecitabine) | Colorectal cancer, breast cancer | Inhibits cancer cell division |
| Xgeva (denosumab) | Bone metastasis from solid tumors | Binds to RANKL, a transmembrane protein essential for the formation, function, and survival of osteoclasts, the cells responsible for bone resorption. |
| Xolair (omalizumab) | Asthma | Inhibits binding of IgE to receptor mast cells and basophils |
| Xylocaine (lidocaine HCl) | Anesthesia | Stabilizes the neuronal membrane by inhibiting the ionic fluxes required for the initiation and conduction of impulses thereby effecting local anesthetic action |
| Xyntha (antihemophilic factor) | Bleeding in hemophilia A disorder | Factor VIII which is required for clotting process |

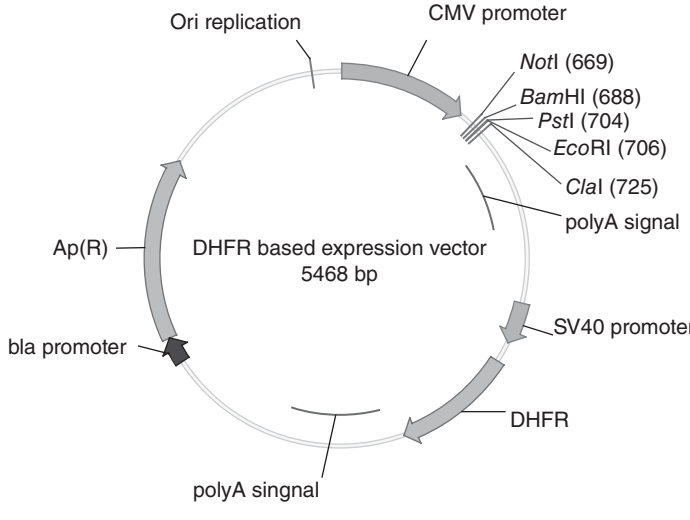
(*continued*)

| Drug | Indication | Mechanism of Action |
|---|---|---|
| Zetia (ezetimibe) | Hypercholesterolemia | Inhibits absorption of cholesterol by the small intestine |
| Zostavax (Zoster vaccine, live) | Prevention of shingles (herpes zoster) | Live, attenuated zoster vaccine to boost varicella zoster virus-specific immunity |
| Zovirax (acyclovir) | Herpes zoster, genital herpes, chickenpox | Inhibits the replication of viral DNA - herpes simplex virus types 1 (HSV-1), 2 (HSV-2), and varicella-zoster virus (VZV) |
| Zytiga (abiraterone acetate) | Metastatic castration-resistant prostate cancer | Inhibits 17 $\alpha$-hydroxylase/C17,20-lyase (CYP17), an enzyme expressed in testicular, adrenal, and prostatic tumor tissues and is required for androgen biosynthesis |

*Source*: Food and Drug Administration website, http://www.fda.gov/

# APPENDIX 4

# A DHFR PLASMID VECTOR




This DHFR-based expression vector has 5468 base pairs of double-stranded DNA. The functions of the components are the following:

- *ori Replication*: Starting point for DNA replication

*Drugs: From Discovery to Approval*, Third Edition. Rick Ng.

**TABLE A4.1 Restriction Endonucleases**

| Restriction Endonuclease | Bacteria | Sequence Recognized | Ends |
|---|---|---|---|
| NotI | *Nocardia otitis-caviarum* | GC^GGCCGC | Sticky |
| BamHI | *Bacillus amyloliquefaciens* | G^GATCC | Sticky |
| PstI | *Providencia stuartii 164* | CTGCA^G | Sticky |
| EcoRI | *Escherichia coli RY13* | G^AATTC | Sticky |
| ClaI | *Caryophanon latum L* | AT^CGAT | Sticky |

*Source*: Courtesy of Dr Matthias Brand.

Note: ^ represents cleavage site and "sticky end" means a stretch of unpaired nucleotides at the end of a DNA molecule.

- *CMV Promoter*: Promoter for the expression of gene of interest in mammalian cells; it allows the binding of RNA polymerase to initiate transcription
- *NotI–ClaI*: Multiple cloning sites for cloning of foreign genes of interest through the use of restriction endonucleases inserted at the locations shown; inserted foreign genes are joined to the plasmid through the use of ligases (see Table A4.1 for each type of endonuclease and the sequence recognized)
- *PolyA Signal*: Polyadenylation signal for attachment of the polyA tail to generate mature mRNA, important for transcription termination
- *SV40 Promoter*: Promoter for the expression of DHFR gene
- *DHFR*: The enzyme dihydrofolate reductase (DHFR) is required for nucleotide (thymine) synthesis and cell growth and serves as selection marker in mammalian cells
- *bla Promoter*: Promoter for the expression of beta-lactamase gene
- *Ap*(*R*): This selectable marker gene is for the resistance of ampicillin (beta-lactamase) which degrades penicillins to enable selection of the desired DHFR vector.

Table A4.1 presents the origins and sequence recognition sites for the restriction endonucleases in the plasmid vector:

# APPENDIX 5

# VACCINE PRODUCTION METHODS

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| Years | Cell Culture | Recombinant DNA, Virus-like Particles | Reverse Vaccinology | Conjugation | Combinations | New Adjuvants |
|---|---|---|---|---|---|---|
| 1980s | Rabies | Hepatitis B | | *H. influenzae* type b | — | — |
| 1990s | Japanese encephalitis virus, varicella zoster, hepatitis A, rotavirus | Acellular pertussis, Lyme disease | — | Meningococcus (c) | Diphtheria–tetanus –pertussis–*H. influenzae* type b, *H. influenzae* type b-hepatitis B, DTaP-*H. influenzae* type b | Influenza |
| 2000s | Avian influenza, live influenza, rotavirus, varicella zoster, H1N1 influenza, smallpox | HPV | — | Pneumococcus (7-, 10, 13-valent), meningococcus (A, C, W135, Y) | Hepatitis B-hepatitis A, diphtheria–tetanus –acellular pertussis–poliovirus –hepatitis B, meningococcus (A, C, W135, Y), MMRV | HPV, H1N1 influenza |
| 2010s | Yellow fever virus | — | Meningococcus B, *Staphylococcus aureus* | Group B streptococcus, typhoid | Meningococcus (A, B, C, W135, Y) | Vaccines with superior alum formulation, TLR agonists |

Note: *H. influenzae*, *Haemophilus influenzae*; HPV human papilloma virus, MMRV, measles–mumps–rubella–varicella; TLR, Toll-like receptor
*Source*: Rappuoli, R, Mandl, CW, Black, S and De Gregorio, E 2011, 'Vaccines for the twenty-first century society', *Nature Reviews Immunology*, 11, pp. 865–872. Reproduced with permission of Macmillan Publishers Ltd.

# APPENDIX 6

# VACCINES APPROVED BY FDA

- *Adenovirus Type 4 and Type 7 Vaccine, Live, Oral*, Barr Labs, Inc.
- *Anthrax Vaccine Adsorbed*, Emergent BioDefense Operations Lansing, Inc. (Biothrax)
- *BCG Live*, Organon Teknika Corp (TICE BCG, BCG Vaccine)
- *Diphtheria & Tetanus Toxoids Adsorbed*, STN 103919 Sanofi Pasteur, Inc.
- *Diphtheria & Tetanus Toxoids & Acellular Pertussis Vaccine Adsorbed*, Sanofi Pasteur, Inc. (Tripedia); GlaxoSmithKline Biologicals (Infanrix); Sanofi Pasteur, Ltd (Daptacel)
- *Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed, Hepatitis B (Recombinant) and Inactivated Poliovirus Vaccine Combined*, GlaxoSmithKline Biologicals (Pediarix)
- *Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed and Inactivated Poliovirus Vaccine*, GlaxoSmithKline Biologicals (Kinrix)
- *Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed, Inactivated Poliovirus and Haemophilus b Conjugate (Tetanus Toxoid Conjugate) Vaccine*, Sanofi Pasteur, Ltd (Pentacel)
- *Haemophilus b Conjugate Vaccine (Tetanus Toxoid Conjugate*), Sanofi Pasteur, SA (ActHIB); GlaxoSmithKline Biologicals, S.A., (Hiberix)
- *Haemophilus B Conjugate Vaccine (Meningococcal Protein Conjugate*), Merck Sharp & Dohme Corp. (PedvaxHIB)

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- *Haemophilus b Conjugate (Meningococcal Protein Conjugate) and Hepatitis B (Recombinant) Vaccine*, Merck & Co, Inc. (Comvax)
- *Hepatitis A Vaccine, Inactivated*, GlaxoSmithKline Biologicals (Havrix); Merck & Co, Inc. (Vaqta)
- *Hepatitis A Inactivated and Hepatitis B (Recombinant) Vaccine*, GlaxoSmithKline Biologicals (Twinrix)
- *Hepatitis B Vaccine (Recombinant)*, Merck & Co, Inc. (Recombivax HB); GlaxoSmithKline Biologicals (Engerix-B)
- *Human Papillomavirus Vaccine*, Merck & Co, Inc. (Gardasil), GlaxoSmithKline Biologicals (Cervarix)
- *Influenza A (H1N1) 2009 Monovalent*, CSL Limited; MedImmune LLC; ID Biomedical Corporation of Quebec; Novartis Vaccines and Diagnostics Limited; Sanofi Pasteur, Inc.
- *Influenza Virus Vaccine, H5N1 (for National Stockpile)*, Sanofi Pasteur, Inc.
- *Influenza A (H5N1) Virus Monovalent Vaccine, Adjuvanted*, ID Biomedical Corporation of Quebec
- *Influenza Virus Vaccine, Quadrivalent, Types A and Types B*, MedImmune, LLC (FluMist Quadrivalent); GlaxoSmithKline Biologicals (Fluarix Quadrivalent); Sanofi Pasteur Inc. (Fluzone Quadrivalent); ID Biomedical Corporation of Quebec (FluLaval Quadrivalent)
- *Influenza Virus Vaccine, Trivalent, Types A and B*, Multiple manufacturers
- *Japanese Encephalitis Vaccine, Inactivated, Adsorbed*, Intercell Biomedical (Ixiaro)
- *Japanese Encephalitis Virus Vaccine Inactivated*, Research Foundation for Microbial Diseases of Osaka University (JE-Vax)
- *Measles, Mumps and Rubella Virus Vaccine*, **Live**, Merck & Co, Inc. (M-M-R II)
- *Measles, Mumps, Rubella and Varicella Virus Vaccine Live*, Merck & Co, Inc. (ProQuad)
- *Meningococcal (Groups A, C, Y, and W-135) Oligosaccharide Diphtheria CRM197 Conjugate Vaccine*, Novartis Vaccines and Diagnostics, Inc. (Menveo)
- *Meningococcal Groups C and Y and Haemophilus b Tetanus Toxoid Conjugate Vaccine*, GlaxoSmithKline Biologicals (MenHibrix)
- *Meningococcal (Groups A, C, Y, and W-135) Polysaccharide Diphtheria Toxoid Conjugate Vaccine*, Sanofi Pasteur, Inc. (Menactra)
- *Meningococcal Polysaccharide Vaccine, Groups A, C, Y, and W-135 Combined*, Sanofi Pasteur, Inc. (Menomune-A/C/Y/W-135)
- *Pneumococcal Vaccine, Polyvalent*, Merck & Co, Inc. (Pneumovax 23)
- *Pneumococcal 7-valent Conjugate Vaccine (Diphtheria $CRM_{197}$ Protein)*, Wyeth Pharmaceuticals, Inc. (Prevnar)
- *Pneumococcal 13-valent Conjugate Vaccine (Diphtheria $CRM_{197}$ Protein)*, Wyeth Pharmaceuticals, Inc. (Prevnar 13)
- *Poliovirus Vaccine Inactivated (Monkey Kidney Cell)*, Sanofi Pasteur, SA (IPOL)

- *Rabies Vaccine*, Sanofi Pasteur, SA (Imovax); Novartis Vaccines and Diagnostics, Ltd (RabAvert)
- *Rotavirus Vaccine, Live, Oral*, GlaxoSmithKline Pharmaceuticals (Rotarix)
- *Rotavirus Vaccine, Live, Oral, Pentavalent*, Merck & Co, Inc. (RotaTeq)
- *Smallpox (Vaccinia) Vaccine, Live*, Sanofi Pasteur Biologics Co (ACAM2000)
- *Tetanus & Diphtheria Toxoids, Adsorbed*, Massachusetts Public Health Biologic Lab (No Trade Name)
- *Tetanus & Diphtheria Toxoids Adsorbed for Adult Use*, Sanofi Pasteur, Inc. (Decavac); Sanofi Pasteur, Ltd (Tenivac)
- *Tetanus Toxoid*, Sanofi Pasteur, Inc.
- *Tetanus and Diphtheria Toxoids Adsorbed, STN 103944*, Sanofi Pasteur, Inc.
- *Tetanus Toxoid, Reduced Diphtheria Toxoid and Acellular Pertussis Vaccine, Adsorbed*, Sanofi Pasteur, Ltd (Adacel); GlaxoSmithKline Biologicals (Boostrix)
- *Typhoid Vaccine Live Oral Ty21a*, Berna Biotech, Ltd (Vivotif)
- *Typhoid Vi Polysaccharide Vaccine*, Sanofi Pasteur SA (Typhim Vi)
- *Varicella Virus Vaccine, Live*, Merck & Co, Inc. (Varivax)
- *Yellow Fever Vaccine*, Sanofi Pasteur, Inc. (YF-Vax)
- *Zoster Vaccine, Live*, Merck & Co, Inc. (Zostavax)

*Source*: Food and Drug Administration 2014, *Vaccines Licensed for Immunization and Distribution in the US with Supporting Documents*, viewed June 19, 2014, http://www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts/ucm093830.htm

# APPENDIX 7

# PHARMACOLOGY/TOXICOLOGY REVIEW FORMAT

*Pharmacology*

Primary pharmacodynamics:

Mechanism of action:

Drug activity related to proposed indication:

Secondary pharmacodynamics:

Pharmacology summary:

Pharmacology conclusions:

Safety pharmacology:

Neurological effects:

Cardiovascular effects:

Pulmonary effects:

Renal effects:

Gastrointestinal effects:

Abuse ability:

Other:

Safety pharmacology summary:

Safety pharmacology conclusions:

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*Pharmocokinetics/Toxicokinetics*:
Pharmacokinetic parameters:
Absorption:
Distribution:
Metabolism:
Excretion:
Other studies:
Pharmacokinetic/toxicokinetic summary:
Pharmacokinetic/toxicokinetic conclusions:
*Toxicology*:
Study title:
Key study finding:
Methods:
Dosing:
Species/strain:
#sex/group or time point (main study):
Satellite groups used for toxicokinetics or recovery:
Age:
Weight:
Doses in administered units:
Route, form, volume, and infusion rate:
Observations and times:
Clinical signs:
Body weights:
Food consumption:
Ophthalmoscopy:
Electrocardiography:
Hematology:
Clinical chemistry:
Urinalysis:
Gross pathology:
Organs weighed:
Histopathology:
Toxicokinetics:
Other:
Results:
Mortality:
Clinical signs:
Body weights:

- Food consumption:
- Ophthalmoscopy:
- Electrocardiography:
- Hematology:
- Clinical chemistry:
- Urinalysis:
- Organ weights:
- Gross pathology:
- Histopathology:
- Toxicokinetics:

Summary of individual findings:
Toxicology summary:
Toxicology conclusions:

*Genetic Toxicology*:

Study title:
Key findings:
Methods:

- Strains/species/cell line:
- Dose selection criteria:
  - Basis of dose selection:
  - Range finding studies:
- Test agent stability:
- Metabolic activation system:
- Controls:
  - Vehicle:
  - Negative controls:
  - Positive controls:
  - Comments:
- Exposure conditions:
  - Incubation and sampling times:
  - Doses used in definitive study:
  - Study design:
- Analysis:
  - Number of replicates:
  - Counting method:
- Criteria for positive results:

Summary of individual study findings:

- Study validity:
- Study outcome:

Genetic toxicology summary:
Genetic toxicology conclusions:

*Carcinogenicity:*

Study title:
Key study findings:
Study type:
Species/strain:
Number/sex/group; age at start of study:
Animal housing:
Formulation/vehicle:
Drug stability/homogeneity:
Methods:
Doses:
Basis of dose selection:
Restriction paradigm for dietary restriction studies:
Route of administration:
Frequency of drug administration:
Dual controls employed:
Interim sacrifices:
Satellite pharmacokinetic or special study groups:
Deviations from original study protocol:
Statistical methods:
Observations and times:
Clinical signs:
Body weights:
Food consumption:
Hematology:
Clinical chemistry:
Organ weights:
Gross pathology:
Histopathology:
Toxicokinetics:
Results:
Mortality:
Clinical signs:
Body weights:
Food consumption:
Hematology:
Clinical chemistry:

Organ weights:
Gross pathology:
Histopathology:
Nonneoplastic:
Neoplastic:
Toxicokinetics:
Summary of individual study findings:
Adequacy of the carcinogenicity study and appropriateness of the test model:
Evaluation of tumor findings:
Carcinogenicity summary:
Carcinogenicity conclusions:
Recommendations for further analysis:
Labeling recommendations:

*Reproductive and Developmental Toxicology*

Study title:
Key study findings:
Methods:
Species/strain:
Doses employed:
Route of administration:
Study design:
Number/sex/group:
Parameters and endpoints evaluated:
Results:
Mortality:
Clinical signs:
Body weights:
Food consumption:
Toxicokinetics:
For fertility studies:
In-life observations:
Terminal and necroscopic evaluations:
For embryo fetal development studies:
In-life observations:
Terminal and necroscopic evaluations:
Dams:
Offspring:
For peri-postnatal development studies:
In-life observations:

Dams:
Offspring:
Terminal and necroscopic evaluations:
Dams:
Offspring:
Summary of individual study findings:
Reproductive and developmental toxicology summary:
Reproductive and developmental toxicology conclusions:
Labeling recommendations:

*Source*: Information for the following pages is adapted from the *Food and Drug Administration Guidance for Reviewers, Pharmacology/Toxicology Review Format*, 2001, available at http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm072976.pdf

# APPENDIX 8

# EXAMPLES OF GENERAL BIOMARKERS

Laboratory Tests for Hematology

| Laboratory Test | Normal Range |
|---|---|
| WBC (white blood cell count) | $3.6–9.8 \times 10^3/\mu L$ |
| RBC (red blood cell count) | Male: $4.2–6.2 \times 10^6/\mu L$<br>Female: $3.7–5.5 \times 10^6/\mu L$ |
| Hemoglobin | Male: 12.9–17.9 g/dL<br>Female: 11.0–15.6 g/dL |
| Hematocrit | Male: 38–53%<br>Female: 33–47% |
| WBC classification: | |
| Band neutrophil | 0–3% or 0–5% |
| Segmented neutrophil | 45–70% |
| Lymphocyte | 25–40% or $2.4 \pm 0.8 \times 10^3/\mu L$ |
| Monocyte | 2–8% |
| Eosinophil | 1–3% or 70–400/μL |
| Basophil | 0–0.5% |
| MCV (mean corpuscular volume) | 82–98fL (i.e., $10^{-15}$L) |
| MCH (mean corpuscular hemoglobin) | 27–32pg (i.e., $10^{-12}$g) |

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Laboratory Tests for Hematology

| Laboratory Test | Normal Range |
|---|---|
| MCHC (mean corpuscular hemoglobin concentration) | 31–36% |
| Platelet count | 120–400 × $10^3$/μL |
| RDW (red cell distribution width) | 11.5–14.5 |
| MPV (mean platelet volume) | 9.8 ± 1.2 fL |
| Reticulocyte count | 0.5–1.5% |
| ESR (erythrocyte sedimentation rate) | Male: < 10 mm/hour<br>Female: < 20 mm/hour |
| Bleeding time | 3–10 minutes |
| Clotting time | 8–10 minutes |
| PT (prothrombin time) | 10–13 seconds |
| APTT (activated partial thromboplastin time) | 26–36 seconds |
| G-6-PD (glucose-6-phosphatase dehydrogenase) | 4.10–7.90 IU/g Hb |
| Fibrinogen | 200–400 mg/dL |
| FDP (fibrinogen degradation product) | < 10 μg/mL |

Laboratory Tests for Clinical Chemistry

| Laboratory Test | Normal Range |
|---|---|
| Liver function tests: | |
| ALP (alkaline phosphatase) | 65–272 IU/L |
| AST/SGOT (serum glutamic oxaloacetin transaminase) | 15–35 IU/L |
| ALT/SGPT (serum glutamic pyruvate transaminase) | 3–30 IU/L or 8–45 IU/L |
| γ-GT (gamma glutamyl transferase) | 5-40 IU/L |
| Bilirubin | 0.3–1.0 mg/dL |
| LDH (lactic acid dehydrogenase) | 150–400 IU/dl |
| Total protein | 6.6–8.1 gm/dL |
| Albumin | 3.9–5.1 gm/dL |
| Globulin | 2.3–3.5 gm/dL |
| Renal function tests: | |
| BUN (blood urea nitrogen) | 5–20 mg/dL |
| Creatinine | 0.7–1.5 mg/dL |
| Creatinine clearance | Male: 62–108 mL/minute<br>Female: 57–78 mL/minute |
| Electrolytes: | |
| Sodium ($Na^+$) | 135–140 mmol/L |
| Potassium ($K^+$) | 3.5–5.0 mmol/L |
| Chloride ($Cl^-$) | 98–108 mmol/L |
| Calcium ($Ca^{2+}$) | 2.1–2.6 mmol/L |
| Phosphorus (P) | 2.5–4.5 mg/dL |
| Magnesium ($Mg^{2+}$) | 1.9–2.5 g/dL |

Laboratory Tests for Clinical Chemistry

| Laboratory Test | Normal Range |
|---|---|
| Uric acid | Male: 3.5–7.9 mg/dL<br>Female: 2.6–6.0 mg/dL |
| CPK (creatinine phosphokinase) | 37–289 IU/L |
| Aldolase | 1.7–4.9 units/L |
| Amylase | Serum: 30–200 IU/L<br>Urine: 4–30 IU/2hour |
| Lipase | <200 units/L |
| Cholesterol: | |
| Total cholesterol | 130–200 mg/dL |
| HDL-cholesterol | 35–65 mg/dL |
| LDL-cholesterol | <130 mg/dL |
| Apo A-1 (apolipoprotein) | Male: 66–151 mg/dL<br>Female: 75–170 mg/dL |
| Apo B (apolipoprotein B) | Male: 49–124 mg/dL<br>Female: 26–119 mg/dL |
| Triglycerides | <250 mg/dL |
| Glucose: | |
| AC glucose | 70–110 mg/dL |
| 30 PC glucose | 90–160 mg/dL |
| 1-hour PC glucose | 90–160 mg/dL |
| 2-hour PC glucose | 75–125 mg/dL |
| 3-hour PC glucose | 70–110 mg/dL |
| $HbA_{1C}$ (glycosylated hemoglobin) | 4–7% |
| Serum iron | Male: 89–200 μg/dL<br>Female: 70–180 μg/dL |
| Ferritin | Male: 27–300 ng/mL<br>Female: 10–130 ng/mL |
| Acid P-tase (acid phosphatase) | Male: 4.7 IU/L<br>Female: <3.7 IU/L |
| Protein electrophoresis: | |
| Total protein | 5.9–8.0 g/dL |
| Albumin | 4.0–5.5 g/dL |
| Alpha-1 globulin | 0.15–0.25 g/dL |
| Alpha-2 globulin | 0.43–0.75 g/dL |
| Beta globulin | 0.50–1.00 g/dL |
| Gamma globulin | 0.60–1.30 g/dL |

| Laboratory Tests for Clinical Chemistry | |
|---|---|
| Laboratory Test | Normal Range |
| Lipoprotein electrophoresis | |
| Pre-beta | 20 ± 6% |
| Beta | 50 ± 5% |
| Alpha | 36 ± 7% |
| Hemoglobin electrophoresis: | |
| $H_bA$ | 97% |
| $H_bA_2$ | 1.5–3.5% |
| $H_bF$ | <2.0% |
| $H_bC$ | 0 |
| $H_bS$ | 0 |
| Osmolality | 280–295 mOsm/kg |

| Laboratory Tests for Urinalysis | |
|---|---|
| Laboratory Test | Normal Range |
| Dipstick test: | |
| pH | 4.6–8.0 |
| Protein | <8 mg/dL |
| Glucose | |
| Ketone | |
| Occult blood | |
| Urobilinogen | 0.1–1.0 EU/dL |
| Leukocyte esterase | |
| Nitrite | $<10^5$ colony/mL |
| Sediment: | |
| RBC | |
| WBC | |
| Epithelial cells | |
| Casts | <5/HPF |
| Crystal | <5/HPF |
| Microorganisms | 0 |
| Parasites | 0/LPF |
| Spermatozoa | |
| Specific gravity: | |
| Gram stain | |
| Bence-Jones protein | |
| Paraguat test | |
| Porphobilinogen | 1.016–1.022 |
| Myoglobin | |
| Pregnancy test | |
| Fractional urinalysis | |

# APPENDIX 9

# TOXICITY GRADING

FDA has set out guidance documents for grading of toxicity in the conduct of clinical trials. The following is an adaptation from a recent document: Guidance for Industry – Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials. The other guidelines are reference of the end of this appendix.

**Tables for Clinical Abnormalities**

| Local Reaction to Injectable Product | Mild (Grade 1) | Moderate (Grade 2) | Severe (Grade 3) | Potentially Life Threatening (Grade 4) |
|---|---|---|---|---|
| Pain | Does not interfere with activity | Repeated use of nonnarcotic pain reliever >24 hours or interferes with activity | Any use of narcotic pain reliever or prevents daily activity | Emergency room (ER) visit or hospitalization |
| Tenderness | Mild discomfort to touch | Discomfort with movement | Significant discomfort at rest | ER visit or hospitalization |
| Erythema/ redness* | 2.5–5 cm | 5.1–10 cm | > 10 cm | Necrosis or exfoliative dermatitis |
| Induration/ swelling† | 2.5–5 cm and does not interfere with activity | 5.1–10 cm or interferes with activity | > 10 cm or prevents daily activity | Necrosis |

*In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.
†Induration/swelling should be evaluated and graded using the functional scale as well as the actual measurement.

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| Vital Signs* | Mild (Grade 1) | Moderate (Grade 2) | Severe (Grade 3) | Potentially Life Threatening (Grade 4) |
|---|---|---|---|---|
| Fever (°C)†(°F)* | 38.0–38.4 100.4–101.1 | 38.5–38.9 101.2–102.0 | 39.0–40 102.1–104 | > 40 > 104 |
| Tachycardia – beats per minute | 101–115 | 116–130 | > 130 | ER visit or hospitalization for arrhythmia |
| Bradycardia – beats per minute‡ | 50–54 | 45–49 | < 45 | ER visit or hospitalization for arrhythmia |
| Hypertension (systolic) – mm Hg | 141–150 | 151–155 | > 155 | ER visit or hospitalization for malignant hypertension |
| Hypertension (diastolic) – mm Hg | 91–95 | 96–100 | > 100 | ER visit or hospitalization for malignant hypertension |
| Hypotension (systolic) – mm Hg | 85–89 | 80–84 | < 80 | ER visit or hospitalization for hypotensive shock |
| Respiratory rate – breaths per minute | 17–20 | 21–25 | > 25 | Intubation |

*Subject should be at rest for all vital sign measurements.
†Oral temperature; no recent hot or cold beverages or smoking.
‡When resting heart rate is between 60–100 beats per minute. Use clinical judgment when characterizing bradycardia among some healthy subject populations, for example, conditioned athletes.

| Systemic Illness | Mild (Grade 1) | Moderate (Grade 2) | Severe (Grade 3) | Potentially Life Threatening (Grade 4) |
|---|---|---|---|---|
| Illness or clinical adverse event (as defined according to applicable regulations) | No interference with activity | Some interference with activity not requiring medical intervention | Prevents daily activity and requires medical intervention | ER visit or hospitalization |

| Systemic (General) | Mild (Grade 1) | Moderate (Grade 2) | Severe (Grade 3) | Potentially Life Threatening (Grade 4) |
|---|---|---|---|---|
| Nausea/ vomiting | No interference with activity or one to two episodes/24 hours | Some interference with activity or >two episodes/ 24 hours | Prevents daily activity, requires outpatient IV hydration | ER visit or hospitalization for hypotensive shock |
| Diarrhea | 2–3 loose stools or <400 g/24 hours | 4–5 stools or 400–800 g/24 hours | 6 or more watery stools or >800 g/24 hours or requires outpatient IV hydration | ER visit or hospitalization |
| Headache | No interference with activity | Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity | Significant; any use of narcotic pain reliever or prevents daily activity | ER visit or hospitalization |
| Fatigue | No interference with activity | Some interference with activity | Significant; prevents daily activity | ER visit or hospitalization |
| Myalgia | No interference with activity | Some interference with activity | Significant; prevents daily activity | ER visit or hospitalization |

**Tables for Laboratory Abnormalities**

| Serum* | Mild (Grade 1) | Moderate (Grade 2) | Severe (Grade 3) | Potentially Life Threatening (Grade 4)† |
|---|---|---|---|---|
| Sodium – hyponatremia mEq/L | 132–134 | 130–131 | 125–129 | < 125 |
| Sodium – hypernatremia mEq/L | 144–145 | 146–147 | 148–150 | > 150 |
| Potassium – hyperkalemia mEq/L | 5.1–5.2 | 5.3–5.4 | 5.5–5.6 | > 5.6 |
| Potassium – hypokalemia mEq/L | 3.5–3.6 | 3.3–3.4 | 3.1–3.2 | < 3.1 |
| Glucose – hypoglycemia mg/dL | 65–69 | 55–64 | 45–54 | < 45 |
| Glucose – hyperglycemia Fasting – mg/dL Random – mg/dL | 100–110 110–125 | 111–125 126–200 | >125 >200 | Insulin requirements or hyperosmolar coma |

**Tables for Laboratory Abnormalities**

| Serum* | Mild (Grade 1) | Moderate (Grade 2) | Severe (Grade 3) | Potentially Life Threatening (Grade 4)† |
|---|---|---|---|---|
| Blood urea nitrogen BUN mg/dL | 23–26 | 27–31 | > 31 | Requires dialysis |
| Creatinine – mg/dL | 1.5–1.7 | 1.8–2.0 | 2.1–2.5 | > 2.5 or requires dialysis |
| Calcium – hypocalcemia mg/dL | 8.0–8.4 | 7.5–7.9 | 7.0–7.4 | < 7.0 |
| Calcium – hypercalcemia mg/dL | 10.5–11.0 | 11.1–11.5 | 11.6–12.0 | > 12.0 |
| Magnesium – hypomagnesemia mg/dL | 1.3–1.5 | 1.1–1.2 | 0.9–1.0 | < 0.9 |
| Phosphorous – hypophosphatemia mg/dL | 2.3–2.5 | 2.0–2.2 | 1.6–1.9 | < 1.6 |
| CPK – mg/dL | 1.25–1.5 × ULN‡ | 1.6–3.0 × ULN | 3.1 –10 × ULN | > 10 × ULN |
| Albumin – hypoalbuminemia g/dL | 2.8–3.1 | 2.5–2.7 | < 2.5 | |
| Total protein – hypoproteinemia g/dL | 5.5–6.0 | 5.0–5.4 | < 5.0 | |
| Alkaline phosphate – increase by factor | 1.1–2.0 × ULN | 2.1–3.0 × ULN | 3.1–10 × ULN | > 10 × ULN |
| Liver function tests –ALT, AST increase by factor | 1.1–2.5 × ULN | 2.6–5.0 × ULN | 5.1–10 × ULN | > 10 × ULN |
| Bilirubin – when accompanied by any increase in liver function test increase by factor | 1.1–1.25 × ULN | 1.26–1.5 × ULN | 1.51–1.75 × ULN | > 1.75 × ULN |
| Bilirubin – when liver function test is normal; increase by factor | 1.1–1.5 × ULN | 1.6–2.0 × ULN | 2.0–3.0 × ULN | > 3.0 × ULN |
| Cholesterol | 201–210 | 211–225 | > 226 | |
| Pancreatic enzymes – amylase, lipase | 1.1–1.5 × ULN | 1.6–2.0 × ULN | 2.1–5.0 × ULN | > 5.0 × ULN |

*The laboratory values provided in the tables serve as guidelines and are dependent on institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

†The clinical signs or symptoms associated with laboratory abnormalities might result in characterization of the laboratory abnormalities as Potentially Life Threatening (Grade 4). For example, a low sodium value that falls within a grade 3 parameter (125–129 mE/L) should be recorded as a grade 4 hyponatremia event if the subject had a new seizure associated with the low sodium value.

‡ULN is the upper limit of the normal range.

| Hematology* | Mild (Grade 1) | Moderate (Grade 2) | Severe (Grade 3) | Potentially Life Threatening (Grade 4) |
|---|---|---|---|---|
| Hemoglobin (female) – gm/dL | 11.0–12.0 | 9.5–10.9 | 8.0–9.4 | < 8.0 |
| Hemoglobin (female) change from baseline value – gm/dL | Any decrease–1.5 | 1.6–2.0 | 2.1–5.0 | > 5.0 |
| Hemoglobin (male) – gm/dL | 12.5–13.5 | 10.5–12.4 | 8.5–10.4 | < 8.5 |
| Hemoglobin (male) change from baseline value – gm/dL | Any decrease–1.5 | 1.6–2.0 | 2.1–5.0 | > 5.0 |
| WBC increase – cell/mm$^3$ | 10,800–15,000 | 15,001–20,000 | 20,001–25, 000 | > 25,000 |
| WBC decrease – cell/mm$^3$ | 2,500–3,500 | 1,500–2,499 | 1,000–1,499 | < 1,000 |
| Lymphocytes decrease – cell/mm$^3$ | 750–1,000 | 500–749 | 250–499 | < 250 |
| Neutrophils decrease – cell/mm$^3$ | 1,500–2,000 | 1,000–1,499 | 500–999 | < 500 |
| Eosinophils – cell/mm$^3$ | 650–1500 | 1501–5000 | > 5000 | Hypereosinophilic |
| Platelets decrease – cell/mm$^3$ | 125,000–140,000 | 100,000–124,000 | 25,000–99,000 | < 25,000 |
| PT – increase by factor (prothrombin time) | 1.0–1.10 × ULN† | 1.11–1.20 × ULN | 1.21–1.25 × ULN | > 1.25 ULN |
| PTT – increase by factor (partial thromboplastin time) | 1.0–1.2 × ULN | 1.21–1.4 × ULN | 1.41–1.5 × ULN | > 1.5 × ULN |
| Fibrinogen increase – mg/dL | 400–500 | 501–600 | > 600 | |
| Fibrinogen decrease – mg/dL | 150–200 | 125–149 | 100–124 | < 100 or associated with gross bleeding or disseminated intravascular coagulation (DIC) |

*The laboratory values provided in the tables serve as guidelines and are dependent on institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.
†ULN is the upper limit of the normal range.

*Source*: Food and Drug Administration 2007, *Guidance for Industry – Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials*, viewed June 25, 2014, http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/ucm091977.pdf

# APPENDIX 10

# HEALTH SYSTEMS IN SELECTED COUNTRIES

*Drugs: From Discovery to Approval*, Third Edition. Rick Ng.

| | Australia | Canada | China | France | Germany | India | Japan | United Kingdom | USA |
|---|---|---|---|---|---|---|---|---|---|
| Population (2012) | 23,050,000 | 34,838,000 | 1,384,770,000 | 63,937,000 | 82,800,000 | 1,236,687,000 | 127,250,000 | 62,783,000 | 317,505,000 |
| GNI*/capita, US$ (2012) | 43,300 | 42,530 | 9,040 | 36,720 | 42,230 | 3,910 | 36,300 | 37,340 | 52,610 |
| % GDP on healthcare (2011) | 9.0 | 10.9 | 5.1 | 11.6 | 11.3 | 3.9 | 10.0 | 9.4 | 17.7 |
| Per capita total health expenditure, US$ (2011) | 3,890 | 4,541 | 423 | 4,128 | 4,474 | 146 | 3,415 | 3,364 | 8,467 |
| Hospital bed/10,000 population (2006–2012) | 39 | 27 | 38 | 64 | 82 | 7 | 137 | 29 | 29 |
| Doctors/10,000 population (2006–2013) | 32.7 | 20.7 | 14.6 | 31.8 | 38.1 | 7.0 | 23 | 27.9 | 24.5 |

| | Australia | Canada | China | France | Germany | India | Japan | United Kingdom | USA |
|---|---|---|---|---|---|---|---|---|---|
| Healthcare system | **Public**: Federal government funds universal medical services scheme (Medicare). State Government finances health services, incl. hospitals **Private**: Individuals purchase private insurance through government subsidies and regulation | **Public**: Medicare funded through tax to provide comprehensive medical services **Private**: Out-of-pocket payments for pharmaceuticals, home, and community-based services, dental and optical services | **Public**: Government insurance scheme, labor insurance scheme, and cooperative medical are public systems **Private**: Private insurance introduced in 1982 and now growing | **Public**: Universal cover for most population through salary-related social contributions **Private**: Private insurance supplements charges and services not covered | **Public**: Statutory Health Insurance (SHI) compulsory for all citizens below threshold income. Those above have options to stay with SHI or opt for private insurance **Private**: About 10% of population on private insurance | **Public**: State Insurance Scheme and Government Health Scheme cover state and government employees and dependants. Hospitals and services vary in quality from state to state **Private**: Voluntary private health insurance is limited. Majority is based on out-of-pocket expenses | **Public**: Health schemes are segmented and comprehensive **Private**: Hospitals are mostly privately-owned | **Public**: National Health Service (NHS) provides free and comprehensive care, finance is by taxation revenue **Private**: 12% population under private schemes | **Public**: Medicare covers those >65 and Medicaid for low income people **Private**: Private insurance provided by employers or individually purchased. More than 64% population covered by private health insurance |

*GNI, Gross National Income; GDP + net revenue (interests and dividends) from overseas.

*Source*: Data from World Health Organization 2014, *World Health Statistics*, viewed May 29, 2014, http://apps.who.int/iris/bitstream/10665/112738/1/9789240692671_eng.pdf?ua=1

# ACRONYMS

| | |
|---|---|
| ABS | acrylonitrile butadiene styrene |
| ACE | angiotensin-converting enzyme |
| ADME | absorption, distribution, metabolism, and excretion |
| AIDS | acquired immune deficiency syndrome |
| ANDA | Abbreviated New Drug Application |
| APC | antigen-presenting cell |
| API | active pharmaceutical ingredient |
| ARTG | Australian Register of Therapeutic Goods |
| AUC | area under curve |
| BGTD | Biologics and Genetic Therapies Directorate (Canada) |
| BHK | baby hamster kidney |
| BLA | Biologics License Application |
| BPC | bulk pharmaceutical chemical |
| BSL | biosafety level |
| CAM | complementary or alternative medicine |
| CBER | Center for Biologics Evaluation and Research (FDA) |
| CD | cluster of differentiation |
| CDE | Center for Drug Evaluation (China) |
| CDER | Center for Drug Evaluation and Research (FDA) |
| CDSCO | Central Drugs Standard Control Organization (India) |
| cDNA | complementary DNA |
| CFDA | China State Food and Drug Administration |
| CFR | Code of Federal Regulation (FDA) |

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| | |
|---|---|
| CFTR | cystic fibrosis transmembrane conductance regulator |
| CFR | colony forming unit |
| cGMP | current Good Manufacturing Practice |
| CIP | clean-in-place |
| CMC | chemistry, manufacturing, and control |
| COP | clean-out-of-place |
| CPAC | Central Pharmaceutical Affairs Council (Japan) |
| CHMP | Committee for Human Medicinal Products (EMEA) |
| CRF | case report form |
| CRO | clinical research organization |
| CTC | Clinical Trial Certificate |
| CTD | Common Technical Document |
| CTN | Clinical Trial Notification |
| CTX | Clinical Trial Exemption |
| CVMP | Committee for Veterinary Medicinal Products (EMEA) |
| DDCR | Department of Drug and Cosmetics Registration (China) |
| DIN | Drug Identification Number (Canada) |
| DMF | Drug Master File |
| DNA | deoxyribose nucleic acid |
| DQ | design qualification |
| ED | effective dose |
| EIR | Establishment Inspection Report |
| ELA | Establishment License Application |
| ELISA | enzyme linked immunosorbent assay |
| EMEA | European Medicines Agency |
| EPA | Environmental Protection Agency |
| EPAR | European Public Assessment Report |
| EPCB | end of production cell bank |
| EPO | erythropoietin |
| ERMP | European Risk Management Strategy |
| EST | expressed sequence tag |
| ESTRI | Electronic Standards for Transmission of Regulatory Information |
| EU | European Union |
| Fab | antigen-binding fragment |
| FACS | fluorescence-activated cell sorter |
| FBS | fetal bovine serum |
| Fc | constant fragment |
| FDA | Food and Drug Administration (United States) |
| FDCA | Food, Drug, and Cosmetic Act (United States) |
| Fv | variable fragment |
| GAMP | Good Automated Manufacturing Practice |
| GAP | Good Agricultural Practice |
| GCP | Good Clinical Practice |
| GDEA | Generic Drug Enforcement Act (United States) |
| GLP | Good Laboratory Practice |
| GM-CSF | granulocyte macrophage colony stimulating factor |

| | |
|---|---|
| GMP | Good Manufacturing Practice |
| GPCR | G-protein coupled receptor |
| GQP | Good Quality Practice |
| GRAS | generally recognized as safe |
| GVP | Good Vigilance Practice |
| HAMA | human antimouse antibody |
| HEPA | high-efficiency particulate air |
| hGH | human growth hormone |
| HGP | Human Genome Project |
| HIV | human immunodeficiency virus |
| HPLC | high-performance liquid chromatography |
| HTS | high-throughput screening |
| HVAC | heating, ventilation, and airconditioning |
| ICDRA | International Conference of Drug Regulatory Authorities |
| ICH | International Conference on Harmonization |
| IDDM | insulin-dependent diabetes mellitus |
| IEC | independent ethics committee |
| IFN | interferon |
| IGF | insulin-like growth factor |
| IgG | immunoglobulin |
| IHR | International Health Regulations |
| IL | interleukin |
| IMP | investigational medicinal product |
| IMPACT | International Medicinal Products Anti-Counterfeiting Taskforce |
| IND | Investigational New Drug |
| INN | International Nonproprietary names |
| INTERPOL | International Criminal Police Organization |
| IOC | International Olympic Committee |
| IPR | intellectual property right |
| IQ | installation qualification |
| IRB | Independent Review Board |
| ISO | International Organization for Standardization |
| LAL | limulus amebocyte lysate |
| LD | lethal dose |
| LIMS | laboratory information management system |
| MA | marketing authorization |
| MAb | monoclonal antibody |
| MAC | maximum allowable carryover |
| MCB | master cell bank |
| M-CSF | macrophage colony stimulating factor |
| MedDRA | Medical Dictionary for Regulatory Activities Terminology |
| MHLW | Ministry of Health, Labor and Welfare (Japan) |
| MHPD | Marketed Health Products Directorate (Canada) |
| MHRA | Medicines and Healthcare Products Regulatory Agency (United Kingdom) |
| mRNA | messenger RNA |

| | |
|---|---|
| MRSA | methicillin-resistant *Staphylococcus aureus* |
| MSE | bovine spongiform encephalopathy |
| NCE | New Chemical Entity |
| NDA | New Drug Application |
| NDS | New Drug Submission |
| NHPD | Natural Health Products Directorate (Canada) |
| NICPBP | National Institute for the Control of Pharmaceutical & Biological Products (China) |
| NIDDM | noninsulin-dependent diabetes mellitus |
| NIH | National Institutes of Health (United States) |
| NME | new molecular entity |
| NMR | nuclear magnetic resonance |
| NOC | Notice of Compliance (Canada) |
| NOE | nuclear overhauser effects |
| NSAID | nonsteroidal antiinflammatory drug |
| OOS | out of specification |
| OPSR | Organization for Pharmaceutical Safety and Research (Japan) |
| OQ | operational qualification |
| OSHA | Occupational Safety and Health Administration |
| ORA | Office of Regulatory Affairs |
| OTC | over-the-counter |
| PAFSC | Pharmaceutical Affairs and Food Sanitation Council (Japan) |
| PAI | preapproval inspection |
| PAT | process analytical technology |
| PCR | polymerase chain reaction |
| PCT | Patent Cooperation Treaty |
| PD | pharmacodynamics |
| PDGF | platelet-derived growth factor |
| PDUFA | Prescription Drug User Fee Act (United States) |
| PFSB | Pharmaceutical and Food Safety Bureau (Japan) |
| PGHS | prostaglandin $H_2$ synthase |
| PHSA | Public Health Service Act (United States) |
| PI | Pharmaceutical Inspectorate |
| PIC/S | Pharmaceutical Inspection Cooperation Scheme |
| PK | pharmacokinetics |
| PLA | Product License Application |
| PMDA | Pharmaceutical and Medical Devices Agency (Japan) |
| POU | point of use |
| PQ | performance qualification |
| PTC | point to consider |
| QA | quality assurance |
| QC | quality control |
| QPCR | quantitative PCR |
| rDNA | recombinant DNA |
| RFID | radiofrequency identification |
| RLD | reference listed drug |

| | |
|---|---|
| RNA | ribose nucleic acid |
| RO | reverse osmosis |
| SAR | structure–activity relationship |
| SARS | severe acute respiratory syndrome |
| SCID | severe combined immune deficiency |
| SNP | single nucleotide polymorphism |
| SOD | superoxide dismutase |
| SOP | standard operating procedure |
| SPA | scintillation proximity assay |
| SPC | summary of product characteristics (EMEA) |
| SSM | standard safety margin |
| TCM | traditional Chinese medicine |
| TGA | Therapeutic Goods Administration (Australia) |
| TM | traditional medicine |
| TNF | tumor necrosis factor |
| TOC | total organic carbon |
| TPD | Therapeutic Products Directorate (Canada) |
| tRNA | transfer RNA |
| UHTS | ultra high throughput screening |
| UNESCO | United Nations Educational, Scientific and Cultural Organization |
| UNICEF | United Nations Children Fund |
| URS | user requirement specification |
| WADA | World Anti-Doping Association |
| WCB | working cell bank |
| WFI | water-for-injection |
| WHO | World Health Organization |
| WTO | World Trade Organization |

# GLOSSARY

**Adrenaline:** A hormone that prepares the body for "fright, flight or fight"; also called epinephrine.

**Adverse event:** An unanticipated event that involves risk to the subject and that results in harm to the subject or others.

**Affinity:** A measure of the binding of an antibody to an antigen.

**Amide:** An organic compound containing an (O–C–N) group.

**Amine:** An organic compound with the general formula of $R_{3-x}NH_x$, where R is a hydrocarbon group and $0 < x < 3$.

**Amino acid:** An organic compound containing an amino group ($–NH_2$) and a carboxyl group (–COOH).

**Aminotransferase:** An enzyme that catalyzes the transfer of an amino group to an acid.

**Amyloid:** A glycoprotein that is deposited extracellularly in tissues.

**Angiotensin:** A peptide. There are two forms of angiotensin I and II. Angiotensin I is converted to angiotensin II by an enzyme, angiotensin-converting enzyme. Angiotensin II constricts blood vessels to increase blood pressure.

**Antibody:** A protein secreted by B cells when they are stimulated by an antigen. Antibodies act specifically against particular antigens in an immune response.

**Ascites:** Abnormal accumulation of serous fluid in the spaces between tissues and organs in the cavity of the abdomen.

**Aseptic:** Preventing infection, free or free from pathogenic microorganisms

**Assay:** A test or trial.

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**Avidity:** Strength of binding, especially the binding of an antibody to an antigen.

**Bacillus:** Rod-shaped bacteria.

**Bacteriophage:** A type of virus that destroys bacteria; also called phage.

**Cell line:** A collection of cells that will proliferate indefinitely when provided with appropriate space to grow and fresh medium to feed on.

**Cyclase:** An enzyme that forms a cyclic compound.

**Cytochrome:** A substance that contains iron and acts as a hydrogen carrier for the eventual release of energy in aerobic respiration.

**Cytometry:** The method of counting cells using a cytometer.

**Dalton:** A unit of measurement equal to the mass of a hydrogen atom.

**Electrophoresis:** The differential movement of molecules through a gel under the influence of an electric field.

**Endotoxin:** A poison release by a bacterium when the cell wall is broken.

**Entropy:** A measure of disorder.

**Epinephrine:** See *adrenaline*.

**Esophagitis:** Inflammation of the esophagus.

**Ethical drugs:** Patented prescription drugs.

**Etiology:** The cause or causes of a disease or abnormal condition.

***Ex vivo*:** Outside a living body.

**Expression:** Information from a gene is transcribed and translated, which results in the production of a protein.

**Generics:** Copies of drugs for which the patents have expired.

**Genome:** The entire DNA of a cell.

**Genomics:** The study of genes and gene function.

**Glycoconjugate:** A carbohydrate that is linked to a lipid or protein.

**Glycoprotein:** See *glycosylation*.

**Glycosylation:** During and after protein synthesis, the protein molecule can undergo modifications. Glycosylation is the attachment of a carbohydrate to the –OH group of serine and threonine (O-glycosylation) or the amide $-NH_2$ group of asparagine (N-glycosylation) of the protein, to form a glycoprotein.

**Hepatic:** Relating to the liver.

**Hydrophilic:** Soluble in water.

**Hydrophobic:** Insoluble in water.

**IgG:** Immunoglobulin G, a class of antibody.

***In vitro*:** Within a glass, in a test tube – an artificial environment.

***In vivo*:** Within a living body.

**Intercellular:** Between cells.

**Intracellular:** Within cell.

**Ischemia:** A low supply of oxygen due to low blood flow.

**Kinase:** An enzyme that catalyzes the transfer of a phosphate group.

**Ligand:** A molecule that binds to another molecule.

**Ligase:** An enzyme involved in DNA replication.

**Limulus amebocyte lysate:** A reagent for determining the quantity of bacterial endotoxins. It is obtained from the aqueous extracts of circulating amebocytes of the horseshoe crab.

**Liposome:** A spherical vesicle formed by a lipid enclosing an aqueous part.

**log *P*:** Logarithmic function of the partition coefficient.

**Lupus erythematosus:** A chronic inflammatory disease of connective tissue, affecting the skin and internal organs.

**Lymphoma:** A malignant tumor of the lymph nodes.

**Malaise:** An indefinite feeling of debility or lack of health often indicative of or accompanying the onset of an illness.

**Multiple sclerosis:** A disease of the nervous system.

**Myclodysplasia:** Abnormal or defective formation of the bone marrow.

**Mycoplasma:** Minute primitive bacteria without a rigid cell wall. *Mycoplasma pneumoniae* causes atypical pneumonia in humans.

**Myeloma cells:** Malignant tumor cells.

**Nucleic acid:** A molecule composed of nucleotides joined together.

**Nucleotide:** A compound consisting of a nitrogen-containing base, a sugar, and a phosphate group.

**Oligonucleotide:** Molecule containing up to 20 nucleotides joined by phosphodiester bonds. Above this length, the term "polynucleotide" is used.

**Otitis media:** Inflammation of the middle ear.

**Pathogenesis:** The mechanism and cellular events leading to the development of a disease.

**Peroxidase:** An enzyme that catalyzes the oxidation of substances in the presence of hydrogen peroxide.

**pH:** The negative logarithm of $H_3O^+$ ion concentration. The scale ranges from 1 to 14; less than 7 is acidic and more than 7 is basic.

**Phagocytosis:** The engulfment of a particle or a microorganism by leukocytes.

**Pharmacogenomics:** The study of how an individual's genetic inheritance affects the body's response to drugs.

**Phosphatase:** An enzyme that catalyzes the hydrolysis of phosphoric acid esters.

**Phosphorylation:** The addition of a phosphate group to a compound.

**Plasmid:** A cytoplasmic DNA that is capable of autonomous replication.

**Pneumococcus:** The bacterium *Streptococcus pneumoniae*, which is associated with pneumonia.

**Polypeptide:** A molecule consisting of many joined amino acids, but not as complex as a protein.

**Prophylactic:** An agent that is used to prevent the development of a disease or condition.

**Prostaglandin:** A protein that has many functions, including mediation of the inflammatory process.

**Protease:** An enzyme that acts on proteins.

**Proteomics:** The study of protein expression of normal and diseased cells and tissues.

**Pyrogen:** A substance or agent that causes fever.

***Restenosis*:** Recurrent stenosis, a condition where the blood vessel or heart valve is narrowed.

**Restriction enzyme:** An enzyme that cuts DNA into short segments.

**Retrovirus:** An RNA virus.

**Saccharide:** A carbohydrate.

**Single nucleotide polymorphism:** Difference at one nucleotide in a DNA sequence among individuals.

**Subcutaneous:** Beneath the outer skin.

**Target:** A specific protein or enzyme upon which a drug acts.

**Therapeutic index:** A measure of the relative desirability of a drug, the ratio is given by $LD_{50}/ED_{50}$.

**Therapeutic:** Treatment and healing of disease.

**Transcription:** The process of transfer of genetic information from DNA to RNA.

**Translation:** The process of transfer of information from RNA into manufacture of protein.

**Vector:** A carrier.

**Xenotransplantation:** The transplantation of cells, tissues, or organs from nonhuman animal sources into humans.

*Source*: 1. Martin, E.A. (ed.) 2010, *Concise Medical Dictionary*, 8th edn., Oxford University Press, Oxford, UK; 2. MedlinePlus website, http://www.nlm.nih.gov/medlineplus/

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