Chapter 1
Introduction

Global sales of prescription medicines reached nearly 1 trillion dollars in 2013 and show no sign of abating [1]. At first sight, this might give the impression that all is well with the biopharmaceutical industry; however, this hides the well-documented fact that company pipelines of innovative drugs are not full enough to keep up with the escalating costs and difficulty of bringing them to market [2]. There are many points in the drug development pipeline where improvements can be made to increase the chance of success. One such point lies at the beginning of the discovery process itself, the identification of drug targets with therapeutic potential. Organized drug target discovery was once almost exclusively undertaken in the pharmaceutical industry, but this situation is changing through stronger collaboration between companies and academia. Regardless of where drug target discovery is actually undertaken, there is a need for as much scientific information as possible to guide the research; this information is provided through biology, chemistry and medicine but is overwhelming in its totality. Individual pieces of information can be readily accessed in online databases, publications and verbal communication with colleagues, but it is difficult to present this totality of target opportunities in a format that is easily browsed. This book is designed to address this issue by presenting a large list of potential human drug targets in a physical form that is easy to browse through, rather like a catalogue; each entry contains just enough information to attract interest without adding undue clutter to the text while at the same time supplying the key information required to follow up online. This is a book about potential and actual human drug targets, not the drugs themselves; microbial targets are not included in order to keep the book within manageable proportions for the sake of both the reader and the author.

This chapter sets the scene for the rest of the book by describing the drug target concept from its origins in 19th century pharmacology through to the Human Genome Project and the present day.

1.1 Magic bullets

> If we picture an organism as infected by a certain species of bacterium, it will obviously be easy to effect a cure if substances have been discovered which have an exclusive affinity for these bacteria and act deleteriously or lethally on these alone, while at the same time they possess no affinity for the normal constituents of the body and can therefore have the least harmful, or other, effect on

Human Drug Targets: A Compendium for Pharmaceutical Discovery, First Edition. Edward D. Zanders.

> that body. Such substances would then be able to exert their full action exclusively on the parasite harboured within the organism and would represent, so to speak, magic bullets, which seek their target of their own accord.

These words were spoken in 1906 by Paul Ehrlich as part of an address to inaugurate the Georg-Speyer Haus, an institute devoted to chemotherapy research in Frankfurt, Germany [3]. His comments provide a useful summary of the concept of a drug target and are applicable to all diseases, not just those caused by infectious agents. Ehrlich's research represented a transition point between the beginnings of the modern pharmacology that emerged in the 19th century and the description and eventual isolation of defined receptors for synthetic drug molecules that occurred in the 20th.

The following sections present modern ideas about drug targets in a historical context, highlighting the relatively recent molecular characterization of receptors for drugs which, in many cases, have been used for over a century.

1.2 Background to modern pharmacology

Some of the following is taken from Prüll, Maehle and Halliwell's informative history of the development of the drug receptor concept [4].

Natural products have been isolated from living organisms to treat diseases for thousands of years, but a coherent understanding of the disease process itself and how the agents actually worked was lacking until only the last 200 years or so. Of the many examples of rational and quasi-religious theories propounded for disease and drug action, I rather enjoy that of the 18th-century Scottish physician John Brown; he suggested that illness was due to either a lack of bodily excitement or to overexcitement. The cure was a mixture of alcohol and opium for the former and a vegetable diet or bloodletting for the latter. Despite this being at odds with modern thinking, there is an air of familiarity about it, although nowadays the bloodletting is generally a side effect rather than a therapeutic intervention.

Pharmacology as a named discipline was born in France, through the work of François Magendie in Paris and later Rudolf Buchheim, who established the first laboratory for experimental pharmacology at the University of Dorpat in Estonia. Magendie and a collaborator, Alire Raffeneau-Delille, studied the toxic action in dogs of several drugs of vegetable origin, including *nux vomica*, marking the first experiments of modern pharmacology. The results suggested to Magendie that the action of natural drugs depended on the chemical substances they contain, and it should be possible to obtain these substances in a pure state. This emphasis on pure substances rather than compound remedies was a turning point in pharmaceutical research. Later in the 19th century, the first hints of structure–activity relationships between drugs and physiological responses were obtained as a result of advances in organic chemistry. For example, Sir Benjamin Ward Richardson showed that chemical modifications of amyl nitrate produced anaesthetics with varying degrees of activity in frogs. Alexander Crum Brown and Thomas Fraser presented a paper to the Royal Society of Edinburgh in 1868 entitled 'On the Connection between Chemical Constitution and Physiological Action; with Special Reference to the Physiological Action of the Salts of the Ammonium Bases Derived from Strychnia, Brucia, Thebata, Codeia, Morphia, and Nicotia'. They showed that whatever the normal effect of these alkaloids, the change of a tertiary nitrogen atom to the quaternary form invariably produced a curare-like paralysing action, thus providing the opportunity for making novel agents.

This early medicinal chemistry was not fully developed until the 20th century. In the meantime, it was necessary to develop theories of drug action that fitted the experimental observations made by pioneering pharmacologists, microbiologists and chemists. One important aspect of this related to the idea of *affinity* between a drug and the cells and tissues of the body. The title of Goethe's 1809 novel about understanding human relationships, *Wahlverwandtschaften* (*Elective Affinities*), was applied to pharmacology by Friedrich Sobernheim in terms of *specific elective affinities*. Another key aspect of drug action was that disease results from alterations in cellular structure and activity, an idea published by Rudolf Virchow in 1858. This observation, coupled with data showing that dyes would selectively bind to specific cell types and structures, created the groundwork for a receptor theory of drug action.

1.2.1 The receptor theory

Ehrlich worked on antibody-mediated haemolysis of red blood cells that stimulated his theory in which a countless number of side-chains would adapt to the "constantly changing chemistry" of the body. This chemistry would be influenced by race, sex, nutrition, energy, secretion and other factors, and so there were continuous changes taking place in the blood serum'. In 1900, Ehrlich and his collaborator Julius Morgenroth introduced the term 'receptor' for the first time: 'For the sake of brevity, that combining group of the protoplasmic molecule to which the introduced group is anchored will hereafter be termed receptor'.

Independent support for the receptor theory was provided by the Cambridge (UK) physiologist John Newport Langley with his concept of *receptive substances*. He interpreted these as 'atom-groups of the protoplasm' of the cell. When compounds bonded to the receptive atom groups, they would alter the protoplasmic molecule of the cell and in this way change the cell's function. In more differentiated cells, such as those of the muscles and glands, the receptive atom groups had undergone a 'special development' which enabled them to combine with hormones or with alkaloids. Due to those cells' connection with nerve fibres, these further developed atom groups tended to concentrate in the region of the nerve endings. In contrast, *fundamental atom groups* were essential for the cell's life. If a chemical substance bound to such a group, the cell would be damaged and die [4]. These comments bring to mind the modern distinction between genes coding for drug targets and those housekeeping genes that are essential for cellular viability.

The receptor theory was not immediately accepted (despite the support of Sir Arthur Conan Doyle, formerly an ophthalmologist but better known as the creator of Sherlock Holmes; this support was reciprocated, as Ehrlich was a great fan of detective stories [4]). The most prominent alternative to a chemical receptor theory was the idea that the physical properties of molecules and target tissues dictated drug action. This viewpoint, held notably by Walther Straub in Germany, was part of a major controversy in pharmacology, amazingly until as late as the 1940s.

Pharmacology was advancing rapidly in the early 20th century despite the aforementioned controversy at the end of the previous paragraph. The neurotransmitter acetylcholine was discovered through the work of Sir Henry Dale and Otto Loewi, earning them the Nobel Prize in 1936. Dale also discovered histamine, while the Japanese chemist Jokichi Takamine, working in the United States for the Parke–Davis and Company, purified adrenaline for the first time in 1900. For this latter feat, the Emperor of Japan donated fifteen imperial cherry trees to Parke–Davis which were planted outside their administrative offices [5].

Despite the ability of pharmacologists to affect cells and tissues with these chemically defined molecules, the idea of a specific receptor was still resisted; in a practical world, they were considered to be too theoretical, at least until the point where their existence could be proven experimentally.

One approach to this was to put pharmacology on a quantitative footing, whereas previously it had been almost entirely descriptive. In 1909, Archibald Hill described the action of nicotine and curare on the contraction or relaxation of frog muscle; through analysing the concentration–effect curves and the temperature dependence of the reactions, he deduced that the drug action was due to a chemical process. This pioneering quantitative work was taken up by Alfred Clark in London in the 1920s, using isolated tissues in a similar manner to Hill. The sigmoidal dose–response curves familiar to drug discovery scientists gave him insight into receptor function as well as the phenomenon of antagonism. To quote Clark, 'atropine and acetyl choline (*sic*), therefore, appear to be attached to different receptors in the heart cells and their antagonism appears to be an antagonism of effects rather than of combination'.

By the 1950s and 1960s, concepts such as agonists, affinity and drug efficacy were well known, but little of this knowledge had been applied to pharmaceutical discovery. This all changed with the identification and exploitation of adrenaline receptor subtypes by Raymond Alquist and Sir James Black, respectively.

The American pharmacologist Alquist embarked upon a study of sympathomimetic compounds designed to relax uterine muscles in the cases of dysmenorrhoea in the 1940s. Briefly, he determined the rank order of potency of a series of compounds (including adrenaline) on the excitation or inhibition of various tissues and in the process discovered two classes of adrenergic receptors which he named α- and β-receptors. This work was aided in part by having access to using sophisticated instruments developed from technology developed during the recent World War. Alquist's seminal work was published in 1948, but he considered the idea of receptors as a theoretical tool; the later subdivisions of adrenoreceptors

into α_1, α_2, β_1 and β_2 subtypes caused him anxiety (β_3 came much later). He believed that 'if there are too many receptors, something is obviously wrong' [4]. What he would have made of our current inventory of receptors and subtypes is probably best left to the imagination.

Sir James Black worked for the UK company Imperial Chemical Industries (now subsumed into AstraZeneca) in the late 1950s. His work on agents to treat angina pectoris led to the first 'beta blocker' drugs, pronethalol and propranolol, thus pioneering the exploitation of receptor subtypes that is now routine practice in biopharmaceutical companies.

By the 1960s, the receptor theory of drug action was accepted by pharmacologists, but still not understood at the molecular level. D.K. de Jongh's comments written in 1964 sum the situation up in a rather literary manner: 'To most of the modern pharmacologists the receptor is like a beautiful but remote lady. He has written her many a letter and quite often she has answered the letters. From these answers the pharmacologist has built himself an image of this fair lady. He cannot, however, truly claim ever to have seen her, although one day he may do so'. That day came soon enough after the application of cell and molecular biology to pharmacological problems.

1.2.2 Molecular pharmacology

The following is adapted from Halliwell's article published in *Trends in Pharmacological Sciences* [6]. Early work on drug receptors provided hints that they were located in specialized regions of tissues. R.P. Cook showed in 1926 that acetylcholine action on frog muscle was blocked by methylene blue dye before it stained the muscle tissue. This antagonist action was reversible, as demonstrated by washing away and reapplying the methylene blue even though the heart muscle retained the blue staining throughout. This suggested that methylene blue had reversibly bound to receptors located at the cell surface. Much later in the late 1960s, Eduardo Robertis and colleagues disrupted tissue with detergents and isolated synaptosomes by differential centrifugation. Synaptosomal membranes contain the nicotinic acetylcholine receptors that were the first receptor molecules to be purified. This purification was independently achieved in the early 1970s by Jean-Pierre Changeux and Ricardo Miledi using the electric organ of rays and eels as rich sources of receptor protein. This period saw the introduction of radioligand binding and affinity purification with potent ligands, in this case α-bungarotoxin. The receptor was then shown to be a 275,000 dalton complex formed from multiple protein subunits.

Thus, 70 years after Ehrlich's time and nearly half a century from our own, the receptor theory was no longer dealing with the abstract but with real molecular entities.

1.2.3 Receptors, signals and enzymes

The nicotinic receptor highlighted earlier is now known to be one of some 300 ion channels, many of which are of major pharmaceutical interest. However, a significant number of current medicines act through a different system, the G-protein-coupled receptors (GPCRs). The prototypic GPCR is the visual transducer rhodopsin, first characterized in the 19th century and sequenced in the 1980s [7]. The protein sequence of bovine rhodopsin revealed a serpentine structure which traversed the cell membrane seven times. Work on rhodopsin signalling revealed the action of a GTPase, initially called transducin and later shown to be a heterotrimeric protein composed of α, β and γ subunits; thus, the term G-protein coupled or 7TM receptor entered the pharmaceutical lexicon. This signalling system is of course one of the many which have been exploited as drug targets or which have the potential to be so.

So far, this historical summary has focused on cell surface receptors for therapeutic ligands, but enzymes are also excellent drug targets. The true nature of enzymes as catalytic proteins was not known until Sumner's crystallization of urease in 1926 and Northrop's studies on pepsin in 1929. Nevertheless, enzyme inhibition was understood at this time and was exploited, for example, in the development in the 1930s of cholinesterase inhibitors that could be used in glaucoma treatment; unfortunately, they had more potential as nerve agents for military use. Later in the 1940s, the antibacterial sulphonamide drugs, by now being superseded by penicillin, provided a lead for novel diuretic drugs (thiazides) based on the

inhibition of carbonic anhydrase in the renal tubule of the kidney [8]. The list of enzyme targets discovered through the remainder of the 20th century includes those for highly successful drugs used in the treatment of millions of patients; these include angiotensin-converting enzyme for hypertension, cyclooxygenases as targets for anti-inflammatory drugs, HMGCoA reductase inhibitors for hypercholesterolaemia and tyrosine kinase inhibitors for oncology.

1.2.4 Recombinant DNA technology and target discovery

Results of the first molecular cloning experiment, in which ribosomal RNA from *Xenopus laevis* was transferred to *Escherichia coli* in a plasmid vector, were published in 1974 [9]. Ten years later, the first pharmacological receptor molecules were cloned (the α, β, γ and δ subunits of the nicotinic acetylcholine receptor from *Torpedo californica*; see Ref. [7]). This achievement was possible because sufficient amino acid sequence of the receptor protein was available to allow investigators to design degenerate oligonucleotides for screening cDNA libraries. This approach has been used many times since then to clone genes encoding a wide variety of human proteins, some of which are known drug targets. However, the only way to identify the full repertoire of human protein-encoding genes was to sequence the entire 3.5 gigabase genome, which of course is what happened between 1990 and 2003 [10]. At the time of publication (in 2001) of the first draft of the human genome sequence, there was some inevitable speculation about how this affected the search for drug targets [11]. In the intervening years to the present, the number of protein-coding genes has dropped from around 30,000 to around 19,000 as more proteomics data and improved bioinformatics analysis have become available [12]. This value of 19,000 genes is the one I have used to constrain the number of drug target proteins that could potentially exist. However, alternative splicing and the identification of micro and other non-coding RNAs have added a new dimension to the analysis of target numbers. A more detailed discussion about identifying drug targets from human genome and proteome data follows later in the book.

1.3 Drug and therapeutic targets in the biomedical literature

The explicit use of the phrase 'drug target' or 'therapeutic target' in the literature began around the time that molecular pharmacology was beginning to grow in the late 1970s. Figure 1.1 shows the number of papers containing each phrase taken from the PubMed database for every year from 1979 to 2014 (both phrases occurred in the same paper only 174 times). Prior to this date, there was only one use of the phrase 'drug target' (in 1975) and two for 'therapeutic target' (in 1954 and 1977). For the historical record, the 1954 paper was entitled 'The human mouth flora as a therapeutic target'.

This exercise has an important bearing on the way some of the data were gathered for this book; the results from these PubMed searches were used to annotate many of the compendium entries as described in the next chapter.

What is notable about this admittedly not very scientific analysis is that the explicit use of the phrases 'drug target' and 'therapeutic target' in the literature really only occurred in a significant way from the mid-1990s onwards. Incidentally, this exceeds the rate of growth in total PubMed citations over this period, which roughly trebled between 1979 and 2012. In a separate analysis, the Espacenet patent database was searched with the 'drug target' and 'therapeutic target' phrases, giving 548 and 1156 worldwide patent citations, respectively (at the time of writing).

1.4 How many drug targets are there?

The distinction between the number of targets for current drugs and the number of *potential* targets must be made at the outset; this book covers drug targets and not the drugs themselves; named drug molecules are listed in the entries listed from Chapter 3 onwards purely to highlight the fact that the target is of

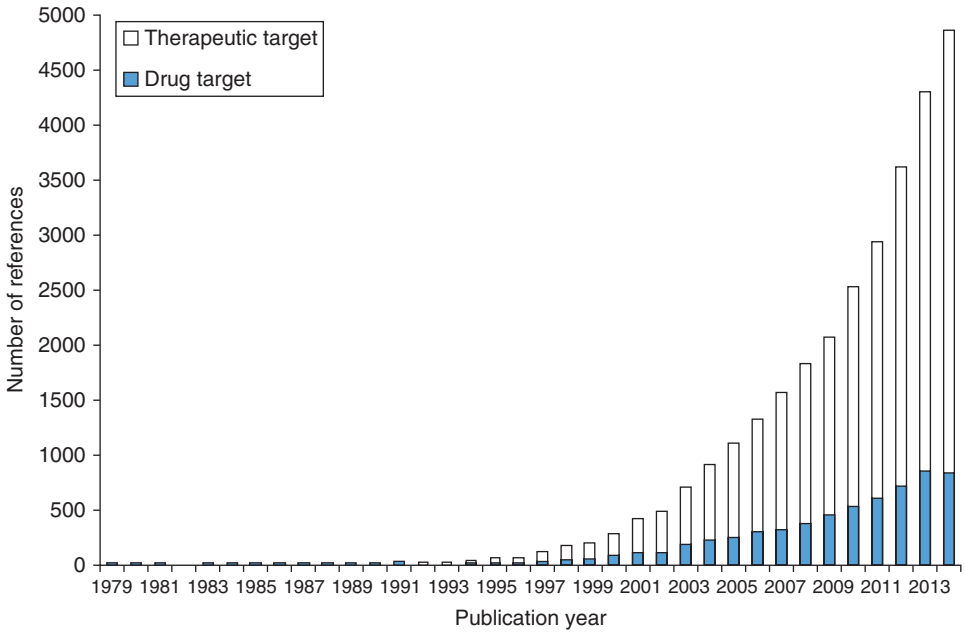

Figure 1.1 Occurrence of the phrase 'drug target' and 'therapeutic target' in papers published between 1979 and 2014. Data taken from a search of PubMed [30]

interest and has been subject to preclinical or clinical investigation. It should be noted that microbial targets are not covered since they are outside the scope of this book.

In 1997, Drews and Ryser [13] published an analysis of the number of targets for drugs listed in Goodman and Gilman's *The Pharmacological Basis of Therapeutics*. Their figure of 483 human and microbial targets was reduced to 324 by Overington *et al.* [14] and even further to 218 by Imming *et al.* [15], both in 2006. Rask-Andersen *et al.* [16] identified 435 human drug targets in the human genome which were affected by 989 unique drugs. These data were obtained by analysing the 2009 entries from DrugBank, a comprehensive database of drugs and targets curated at the University of Alberta in Canada [17]. Whatever the exact figure, the number of targets to FDA-approved drugs is of the order of hundreds rather than the thousands of targets that might be expected from the thousands of human protein-coding genes. While drug development has continued apace since 2009, there is clearly a large discrepancy between actual and potential target numbers, although how many of the latter would lead to useful therapeutics is presently just a matter of conjecture. Whatever the final size of the 'playing field', it is hardly surprising that industry and academia are investing much time and effort into the discovery and exploitation of new targets. Some of this activity is summarized in Section 1.4.1.

1.4.1 Systematic target discovery

The last decades of the 20th century saw the birth of genomics, a discipline which arose naturally on the back of DNA sequencing technology and bioinformatics. Further technological advances followed, in particular the introduction of DNA microarrays for high-throughput analysis of mRNA expression. Proteomics followed as a matter of course, based on sophisticated mass spectrometry for identifying multiple proteins in complex mixtures and affinity-based methods for purifying these proteins on defined ligands. Genomics and proteomics (along with metabolomics and other 'omics' technologies) have, for the first time, made it possible to consider pharmaceutical targets in a systematic way [18]. Given the commercial interest in finding novel targets, genome sequence became a commodity and was sold to pharmaceutical companies by companies such as Incyte and Celera. These commercial restrictions fell

away once the human genome sequence was made publically available at the beginning of the new century and posted online in databases such as GenBank [19] and Ensembl [20]; this has allowed a far wider group of scientists to scrutinize the sequence data than would otherwise have been possible. This ongoing analysis of sequence data is not only revealing new members of existing protein target families (e.g. the GPCRs) but also completely new molecules with important regulatory functions such as the microRNAs. Of course, sequence alone does not reveal the biological or pharmaceutical relevance of candidate proteins (or RNAs); a range of different technologies is required in order to achieve this. Examples of these include expression analysis of genes or proteins in normal or diseased tissues or *in vitro* cell culture systems. Many of the human genes referenced in this compendium have been highlighted as potential drug targets on this basis, often because aberrant protein expression was detected in diseased tissue using immunohistochemistry. Another approach is to create transgenic organisms with the gene of interest expressed or removed in order to determine its function *in vivo*. Amgen scientists generated transgenic mice expressing potentially interesting human genes and in doing so discovered osteoprotegerin (TNFRSF11B) which enhanced bone mineralization and led to the discovery of novel targets and treatments for osteoporosis [21]. Human genetics has been revitalized with the advent of next-generation sequencing technology to decrease the cost and increase the throughput of DNA sequencing. Many potential drug targets are being identified by sequencing samples of normal and diseased tissue; this is notably the case in cancer research, as many 'driver mutations' are being identified and assigned to particular tumour types. It is even possible to sequence DNA in individual tumour cells, thereby demonstrating tumour heterogeneity, something that needs to be considered in devising therapies [22].

Lastly, the powerful CRISPR–Cas system for selective gene editing has added to the existing antisense and small interfering RNA (siRNA) technologies for assessing the functions of potential drug targets and promises to broaden opportunities for novel drug development [23].

1.5 Screening for active molecules

This introductory chapter concludes with a brief discussion about how pharmaceutically active molecules are currently being discovered, either through exploiting drug targets that are initially defined or that have to be uncovered retrospectively.

New medicines (or the precursors to them) have been discovered in several ways: by accident, by screening for phenotypic changes or by screening defined molecular targets. Accidental discovery, like that of the cisplatin drugs [24], cannot be made to order, so it will be excluded from this discussion except to remind readers of Pasteur's dictum: 'in the field of observation, chance only favours the prepared mind'.

Current drug discovery practice involves the choice between screening test molecules on phenotypic targets or on defined molecular targets. For small-molecule screening, these activities have been described in terms of chemical genomics, with forward and reverse chemical genetics for phenotypic and target-based screening, respectively (for an overview of chemical genomics and proteomics, see Ref. [25]). There are benefits to each approach: phenotypic (or whole cell/organism) screening has the advantage of selecting molecules with desired biological actions without prior knowledge of the molecular target(s). In addition, only cell-permeant compounds will be selected if the drug target is intracellular. The disadvantage of course is that the target protein(s) has to be identified for compound optimization through a process of target deconvolution [26]. Defined molecular targets may be easier to screen and to obtain reliable SAR data for medicinal chemistry, but any hits must be extensively optimized to show *in vivo* activity. It is also (currently) impossible to predict in advance precisely which other cellular targets might be affected by the test compound, although chemical proteomics strategies for affinity purification of targets on drug ligands have proven successful (e.g. [27]).

It is worth examining the relative contributions of phenotypic and target-based screening to actual drug discovery. Two 2014 reviews have covered this topic; the first, by Eder *et al.* [28], presents an analysis of all 113 first-in-class drugs approved by the FDA between 1999 and 2013. The second review, by Moffatt *et al.* [29], describes a similar analysis but is restricted to oncology drugs. However, it is noteworthy that a very significant proportion of annotations in this compendium are related to oncology, the largest therapeutic

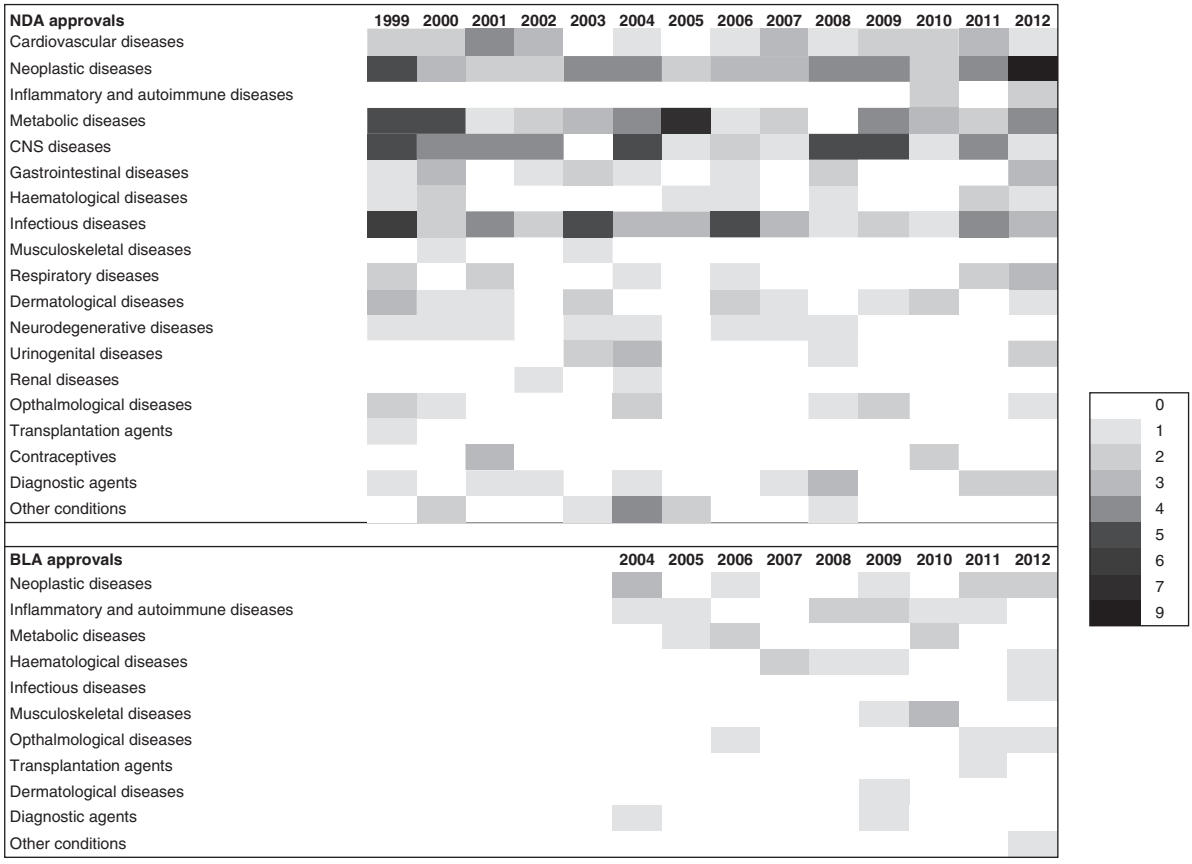

Figure 1.2 FDA approvals by therapeutic area. Data were taken from the FDA website [31]

Table 1.1 Number of approved first-in-class or oncology drugs described in Refs [25] and [26] according to means of discovery

Drug type	Target based	Chemocentric/ mechanism based	Phenotypic screen based
All small molecules from Eder *et al.* [25]	45	25	8
All biologicals from Eder *et al.* [25]	33	—	—
Oncology drugs from Moffat *et al.* [26]	31	7	10

area in 2013, with $10 billion more in sales than the runner up (pain) [1]. Figure 1.2 shows a table of all FDA approvals for small molecules and biologicals (for the periods 1999–2012 and 2004–2012, respectively), listed by therapeutic area. This again shows the importance of oncology targets in pharmaceutical development as well as other trends; for example, there has been a noticeable increase in the number of approvals for biological drugs for inflammatory and autoimmune diseases compared with their small-molecule counterparts.

The key data from Refs [27] and [28] are summarized in Table 1.1.

It can be seen that the majority of first-in-class approved drugs or oncology drugs (either on the market or in clinical development) were originally discovered using a target-centric approach. Despite this bias, there are clearly some advantages to phenotypic screening, as discussed extensively in both reviews, so it is reasonable to expect that this approach to drug discovery will continue to be used alongside target discovery

depending upon the individual features of the system under investigation. Whatever the type of drug screening undertaken, I hope that this book will prove useful in providing ideas for either selecting the target in the first place, or for assisting in the identification of the targets uncovered in phenotypic screens.

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