# Part I Research and development

# 1 Discovery of new medicines

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## Introduction

Patients rely on discovery researchers to embrace innovation, make advances and deliver new therapies that will improve their lives. The discovery of drugs is a complex, costly and lengthy process involving several distinct stages on the path towards delivery of a marketable drug (Figure 1.1). It is becoming increasingly important in the ever competitive enterprise of drug discovery for researchers to develop innovative drug discovery strategies in order to fill their pipelines. This chapter is designed to highlight these modern approaches to drug discovery and the changing therapeutic landscape for the currently available drugs.

Progress in drug discovery relies on fundamental biological research in pharmaceutical and biotechnology companies as well as academia to identify new biological targets, to implement target validation strategies and to confirm target relevance in a disease state. Initially, drug discovery researchers select a target that can interact with a modulator, such as a protein or small molecule. After a target has been chosen, researchers must demonstrate that the target is relevant to a disease in both living cells and animal models. The promise of determining the whole genome sequence, new insights into molecular sources of disease, technological advances in both target and lead validation, and high-throughput screening (HTS) strategies all provide potentially novel opportunities for target validation in drug discovery. After such validation, the search begins for a 'hit' molecule that interacts with the desired target. These 'hits' may originate from nature, *de novo* design or HTS but, in most cases, require optimisation to 'leads' via cycles of altering the structure and properties of the molecules followed by iterative screening. During this process, lead compounds are further optimised for the desired *a*bsorption, *d*istribution, *m*etabolism, *e*xcretion and *t*oxicological (ADMET) properties. ADMET optimisation supplies the 'lead compound', which advances to later stages of drug development.

A case study around the investigation of phosphodiesterase (PDE) inhibitors illustrates the successful applications of the principles of contemporary drug discovery and development. Based on the discovery of an endothelium-derived relaxing factor and the interplay of nitric oxide, cyclic guanosine monophosphate (cGMP) and PDEs in vasodilation, researchers at Pfizer reasoned that a PDE inhibitor might be advanced for the treatment of angina [1]. Their comparison of the structure of cGMP, with consideration of how it may bind to PDEs, with that of the weak vasodilator Zaprinast, also a PDE inhibitor, further supported their hypothesis. The screening of existing compound collections as well as the rational design of analogues produced active molecules that targeted PDE-5, a cGMP-specific PDE located in coronary smooth muscle. Further optimisation of these compounds for the desired potency and ADMET properties led to a clinical candidate for angina; the compound was found to be ineffective, and its development as a cardiovascular drug was halted. During the clinical trials, however, some patients reported experiencing enhanced penile erections. Subsequently, PDE-5 was identified as the main cGMP-degrading enzyme in the corpus cavernosum. Thus, efforts were redirected toward proving the effectiveness of the lead compound as a treatment for

*The Textbook of Pharmaceutical Medicine*, Seventh Edition. John P. Griffin, John Posner, and Geoffrey R. Barker.

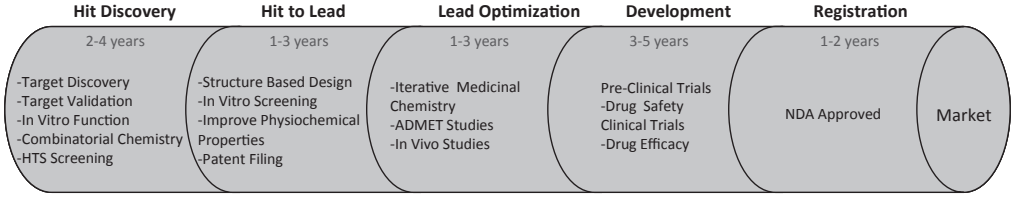


**Figure 1.1** Drug discovery pipeline.

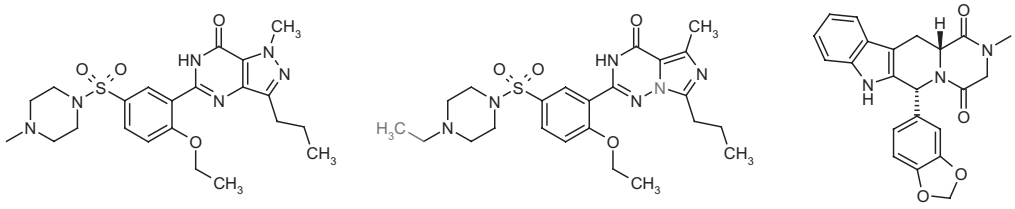


**Figure 1.2** Structures of PDE-5 inhibitors for erectile dysfunction.

erectile dysfunction and the eventual approval of sildenafil (Viagra) in 1998 as a prescription medicine for erectile dysfunction [2,3].

Important parts of drug discovery and development are intellectual property protection and the ability to navigate around prior art. Pfizer filed patent applications proactively around the lead compound/series, as well as its therapeutic use, to deter competitors from achieving success in the PDE arena in similar chemical space. Others interested in advancing compounds in this therapeutic area became faced with searching for gaps in the patent coverage or pursuing alternative structural classes. Implementation of a 'patent busting' strategy enabled the discovery of vardenafil (Levitra). Analogues outside of the Pfizer patents were identified, optimised and evaluated in clinical trials ahead of product launch in 2003. Conversely, Icos and Lilly investigated an unrelated molecule as a longer-acting PDE-5 inhibitor that led to the approval of tadalafil (Cialis), also in 2003. Figure 1.2 highlights the structural similarities, and differences, of these three medicines.

## Medicines marketed in the years 2008–2011

The pharmaceutical and biotechnology industries spend billions on cutting-edge research and development (R&D), clinical trials and marketing to introduce drugs to market. However, fewer than one in 50 drug discovery projects results in the delivery of a drug to market [4], and the average time from concept to market is 15 years, at a cost of nearly a billion dollars per drug [5]. Further, since 2008, new drug approvals have declined sharply despite an increase in R&D spending [6]. The observed high attrition rate is unsustainable and researchers must constantly reassess their tactics in order to translate discovery research into clinical success.

Despite the steady decline in overall new drug approvals there has been a steady increase of new products in the therapeutic areas of anti-infective, metabolic and orphan diseases, as well as a shift into specialty-care therapies (Tables 1.1, Table 1.2, Table 1.3 and Table 1.4) [7–9]. The majority of new molecular entities (NMEs) continue to be small molecules; however, vaccines and non-biological oligonucleotides employed as macromolecular therapeutics are directed at enzymes and receptors that have been classically modulated by small molecule drugs.

In response to the decline in new drug approvals, new approaches have been put in place:

1. drug combinations that target multiple pathways continue to increase in drug discovery to modulate the interplay of complex chemical pathways involved in diseases;

**Table 1.1** New molecular entities (NME) approved 2008. Reproduced from Hegde S, Schmidt M. To Market, To Market Annual Reports in Medicinal Chemistry 2009; 44: 577 with permission from Elsevier [8]

| Proprietary | Established | Applicant | Treatment/indication |
|---|---|---|---|
| Entereg | Alvimopan | GlaxoSmithKline/Adolor | Oral treatment of postoperative ileus following bowel resection surgery |
| Biomatrix | Biolimus drug-eluting stent | Biosensors | Antiresenotic |
| Lonasen | Blonanserin | Dainippon-sumitomo | Dual antagonist of dopamine D2 and serotonin 5-HT2 for schizophrenia |
| Zeftera | Ceftobiprole medocaril | Basilea/Johnson&Johnson | New injectable cephalosporin antibiotic |
| Cimzia | Certolizumab pegol | UCB | TNF-α blocker for Crohn's disease |
| Trilipix | Choline fenofibrate | Abbott/Solvay | PPARα dyslipidaemia |
| Cleviprex | Clevidipine | The Medicines Co. | IV treatement for hypertension |
| Pradaxa | Dabigatran | Boehringer Ingelheim | Oral administered anticoagulant |
| Pristiq | Desvenlafaxine | Wyeth | SNRI for antidepression |
| Intelence | Etravirine | Tibotec | NNRT antiviral for HIV |
| Toviaz | Fesoterodine | Pfizer | Orally active pro-drug from treatment of overactive bladder |
| Ivemend | Fosapreptiant dimeglumine | Merck | Anti-emetic |
| Firazyr | Icatibant | Jerini | Hereditary angiodema (HAE) |
| Vimpat | Lacosamide | Schwarz Pharma | Anticonvulsant |
| Relistor | Methylnaltrexone bromide | Wyeth/Progenics | Opiod-induced constipation |
| Pirespa | Pirfenidone | Shinogi | Idiopathic pulmonary fibrosis (IPF) |
| Arcalyst | Rilonacept | Regeneron | Recombinant fusion protein for symptoms of inherited auto-inflammatory syndrome |
| Xarelto | Rivaroxaban | Bayer/Ortho-McNeil | Anticoagulant |
| Nplate | Romiplostim | Amgen | Recombinant fusion protein for treatment of thrombocytopenia |
| Gracevit | Sitafloxacin hydrate | Daiichi Sankyo | Antibacterial |
| Bridion | Sugammadex | Schering-Plough | Reversal of neuromuscular blockade |
| Taflotan | Tafluprost | Santen/Asahi Glass | Antiglaucoma |
| Recothrom | Thrombin alfa | Zymogenetics | Recombinant human protein for haemostat |
| Recomodulin | Thombomodulin | Asahi Kasei Pharma | Recombinant human protein as an anticoagulant |

NNRT, non-nucleoside reverse transcriptase; PPAR, peroxisome proliferator-activated receptor; SNRI, serotonin–norepinephrine reuptake inhibitor; TNF, tumour necrosis factor.

**2.** drug repurposing has accounted for two-thirds of new drug applications. Increased focus on re-purposing existing drugs for orphan indications emanates from disease-focused philanthropic groups; and

**3.** collaboration strategies between pharmaceutical companies and academic research institutions have contributed to the drug discovery process [10,11]. While academic research is focused principally on the underlying mechanistic components of a disease and

**Table 1.2** NME approved 2009. Reproduced from Hegde S, Schmidt M. To Market, To Market Annual Reports in Medicinal Chemistry 2010; 45: 467 with permission from Elsevier [9]

| Proprietary name | Established name | Applicant | Treatment/indication |
|---|---|---|---|
| Nuvigil | Aromodafinil | Cephalon | Sleep disorder, α1-adrenoceptor agonist |
| Saphris | Asenapine | Merck/Schering-Plough | Schizophrenia and bipolar 1, dual antagonist dopamine D2 and serotonin 5-HT2 |
| Besivance | Besifloxacin | Baush & lomb | Antibacterial, ophthalmic use |
| Ilaris | Canakinumab | Novartis | Recombinant monoclonal antibody, anti-inflammatory |
| Removab | Catumaxomab | Trion | Trifunctional monoclonal antibody, anticancer |
| Priligy | Dapoxetine | Janssen-Cilag | Premature ejaculation |
| Firmagon | Degarelix acetate | Ferring Pharmaceutical | Antagonist of GNRH, anticancer |
| Kapidex | Dexlansoprazole | Takeda | Gastroesophogeal reflux disease |
| Multaq | Dronedarone | Sanofi-Aventis | Anti-arrhythmic |
| Promacta | Eltrombopag | GlaxoSmithKline | Antithrombocytopenic |
| Zebinx | Eslicarbazepine | Eisai | Anti-epileptic |
| Adenuric | Febuxostat | Takeda/Teijin/Ipsen | Antihyperuricaemic, selective xanthine oxidase inhibitor |
| Simponi | Golimumab | Centocor Ortho | Recombinant monoclonal antibody, anti-inflammatory |
| Onbrez breezhaler | Indacaterol | Novartis/Skye Pharma | Chronic obstructive pulmonary disease, inhaled β2 adrenoceptor agonist |
| Victoza | Liraglutide | Novo Nordisk | Antidiabetic |
| Recalbon, bonoteo | Minodronic acid | Ono/Astella Pharma | Osteoporisis |
| Remitch | Nalfurafine hydrochloride | Toray/Japan Tobacco | Pruritus (chronic itching) |
| Arzerra | Ofatumumab | Genmab/GlaxoSmithKline | Recombinant monoclonal antibody, anticancer |
| Votrient | Pasopanib | GlaxoSmithKline | VEGF, anticancer |
| Mozobil | Plerixafor | Genzyme | Haematological malignancies, autologous haemtopoietic stem cell transplantation |
| Folotyn | Pralatrexate | Allos | Injectable DHFR inhbitior, anticancer |
| Effient | Prasugrel | Daiichi Sankkyo/Eli Lilly | Antiplatelet therapy |
| Onglyza | Saxagliptin | Bristol-Myers-Squibb/ Astrazeneca | Antidiabetic |
| Nucynta | Tapentadol | Ortho-McNeil-Janssen | Analgesic, pain intervention |
| Arbelic, vibativ | Telavancin | Theravance/Astellas Pharma | Antibiotic |
| Samsca | Taolvaptan | Otsuka America | Hyponatraemia |
| Ellaone | Ulipristal acetate | HRA Pharma | Contraceptive, progesterone receptor antagonist |
| Stelara | Ustekinumab | Janssen-Ortho | Humanized IGG1K monoclonal antibody, antipsoriatic |

DHFR, dihydrofolate reductase; GNRH, gonadotrophin-releasing hormone; VEGF, vascular endothelial growth factor.

**Table 1.3** NME approved 2010. Reproduced from Bronson J, Dhar M, Ewing W, Lonberg N. To Market, To Market Annual Reports in Medicinal Chemistry 2011; 46: 433 with permission from Elsevier [7]

| Proprietary name | Established name | Applicant | Treatment/indication |
|---|---|---|---|
| Lastacaft | Alcaftadine | Vistakon Pharmaceuticals | Ophthalmologic, histamine antagonist |
| Nesina | Alogliptin | Takeda/Furiex Pharmaceuticals | Antidiabetic, DPP-4 |
| Bilaxten | Bilastine | Faes Farma, Menarini, Pierre Fabre, Merck-Serono | Antiallergy, histamine antagonist |
| Jevtana | Cabazitaxel | Sanofi-Aventis | Anticancer, tubulin inhibitor |
| Teflaro | Ceftaroline fosamil | Forest Laboratories | Antibacterial, bacterial cell wall synthesis inhibitor |
| Elonva | Corifollitropin | Merck | Infertility, FSH agonist |
| Ampyra | Dalfampridine | Acorda Therapeutics | Multiple sclerosis, potassium channel blocker |
| Prolia / xgeva | Denosumab | Amgen | Osteoporosis, recombinant monoclonal antibody |
| Diquas | Diquafosol | Santen | Ophthalmologic dry eye, P2Y2 purinergic receptor agonist |
| Kalbitor | Ecallantide | Dyax Corp | Angiodema, plasma kallikrein inhibitor |
| Halaven | Eribuline | Eisai | Anticancer, tubulin inhibitor |
| Gilenya | Fingolimod | Novartis | Multiple sclerosis, S1P receptor agonist |
| Inavir | Laninamivir | Daiichi-Sankyo | Antiviral, neuraminidase |
| Lurasidone | Lurasidone | Dainippon Sumitomo Pharma | Schizophrenia, dopamine 5-HT receptor antagonist |
| Junovan | Mifamurtide | Takeda | Anticancer |
| Rapiacta | Peramivir | Biocryst Pharmaceuticals | Antiviral, neuraminidase inhibitor |
| Daxas | Roflumilas | Nycomed | Chronic obstructive pulmonary disorder, PDE4 inhibitor |
| Istodax | Romidepsin | Celgene | Anticancer, histone deacetylase inhibitor |
| Provenge | Sipuleucel-t | Dendreon | Anticancer, therapeutic cancer vaccine |
| Egrifta | Tesamorelin | Theratechnologies | HIV lipodystrophy, growth hormone-releasing factor |
| Brilique | Ticagrelor | Astra-Zeneca | Antithrombotic, P2Y12 antagonist |
| Brinavess | Vernakalant | Merck/Cardiome Pharma | Anti-arrhythmic, atrial potassium channel blocker |
| Javlor | Vinflunine | Pierre Fabre | Anticancer, tubulin inhibitor |
| Civanex | Zucapsaicin | Winston | Analgesic, TRPV1 channel activator |

FSH, follicle stimulating hormone; PDE4, phosphodiesterease 4; TRPV1, transient receptor potential cation channel subfamily V member 1.

**Table 1.4** NME approved 2011. Reproduced with permission from U.S. Food and Drug Adminstration, 'How Drugs are Developed and Approved?', http://www.fda.gov/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/DrugandBiologicApprovalReports/ucm121136.htm. Last accessed 13 Aug 2012

| Proprietary name | Established name | Applicant | Treatment / indication |
|---|---|---|---|
| Zytiga | Abiraterone | J&J | Advanced prostate cancer |
| Eylea | Aflibercept | Regeneron/Bayer | Wet AMD |
| Edarbi | Azilsartan | Takeda | Hypertension angiotensin II receptor blocker |
| Nulojix | Belatacept | Bms | Renal transplant |
| Benlysta | Belimumab | HGSI, GSK | Lupus |
| Victrelis | Boceprevir | Merck | Hepatitis C |
| Adcetris | Brentuximab vedotin | Seattle Genetics | Hodgkin lymphoma, anaplastic large cell lymphoma |
| Teflaro | Caftaroline | Cerexa | Acute bacterial skin infections and pneumonia |
| Erwinaze | Crisantaspase | Eusa | ALL |
| Xalkori | Crizotinib | Pfizer | NSCLC |
| Promacta | Eltrombopag | GSK | Chronic immune (idiopathic) thrombocytopenic purpura |
| Potiga | Ezogabine | GSK, Valeant | Epilepsy |
| Corifact | Factor xiii | Behring | Prevention of bleeding with genetic defects in factor XIII |
| Horizant | Gabapentin enacarbil | GSK, Xenoport | Restless legs syndrome |
| Firazyr | Icatibant | Shire | Hereditary angioedema |
| Arcapta neohaler | Indacaterol | Novartis | COPD |
| Yervoy | Ipilimumab | Bms | Metastatic melanoma |
| Tradjenta | Linagliptin | Lilly, Boehringer Ingelheim | Type 2 diabetes |
| Edurant | Rilpivirine | J&J | HIV |
| Xarelto | Rivaroxaban | Bayer, J&J | Blood clot prevention |
| Daliresp | Roflumilast | Forest | COPD |
| Jakafi | Ruxolitinib | Incyte, Novartis | Myelofibrosis |
| Natroba | Sphinosad | Parapro | Head lice |
| Incivek | Telaprevir | Vertex, J&J, Mitsubishi Tanabe | Hepatitis c |
| Brilinta | Ticagrelor | Astra Zeneca | Blood clot prevention |
| Caprelsa | Vandetanib | Astra Zeneca | Medullary thyroid cancer |
| Zelboraf | Vemurafenib | Roche, Daiichi Sankyo | Melanoma |
| Zictifa | Vendetanib | Astra Zeneca | Thyroid cancer |
| Viibryd | Vilazodone | Forest, Merck KGAA | Major depressive disorder |

ALL, acute lymphocytic leukaemia; AMD, acute macular degeneration; COPD, chronic abstructive pulmonary disease; NSCLC, non-small cell lung cancer.

the pharmaceutical industry focuses on progressing discovery projects, a willingness to share expertise through these research alliances has resulted in advances in poorly funded rare diseases.

## Impact of high throughput screening in drug discovery

A judicious choice of therapeutic area and biological target, along with an acceleration of development time through scientific innovation, are critical to a successful R&D drug discovery programme. Pharmaceutical companies often engage in economic balancing when choosing therapeutic areas in which to begin research by weighing the probability of delivering a product against potential sales of this product. Additionally, companies must consider development costs and regulatory hurdles when choosing their research path. As an example, since 2000, the proportion of R&D projects from available corporate portfolios in the area of antineoplastic agents has increased by about 7% [12]. The average sales per year for an antineoplastic agent developed since 2000 was 92 million dollars, among the highest of the major therapeutic classes. However, the probability of success for reaching the market from the preclinical phase for an antineoplastic development project is quite low due to project attrition [12].

The drug discovery paradigm has evolved in recent times. In the simplest example, the mode of action of a compound (drug) centres on its binding to a receptor (target) that influences a biochemical pathway, which is relevant to a physiological process, and the sum of these events provides a therapeutic benefit to a disease state. In many cases the reality is not that simple, thus additional approaches have become necessary. Prior to 1990, the standard approach to small molecule drug discovery relied on iterative, low-throughput *in vivo* screening and optimisation of compounds to improve chemical or biochemical parameters (e.g. potency, selectivity or pharmacokinetic properties). Antihypertensive beta-blockers were developed through this process from adrenaline (epinephrine) [12] where analogues were synthesised individually and evaluated in concurrent assays (often *in vivo*) and optimised via medicinal chemistry to progress compounds to clinical trials. With the advent of HTS and the availability of large collections of compound libraries, this model was criticised for being slow, expensive and obsolete. Target selection has since become heavily influenced by the compatibility of that target with an HTS approach to enable rapid and cost-effective evaluation of hundreds of thousands of compounds from screening collections.

Much debate continues around the relative effectiveness of the two models outlined earlier. The premise that high-affinity binding to a single biological target that is associated with a disease state will afford a therapeutic benefit in humans [13,14] has been countered by opinions that pharmaceutical products developed pre-HTS in fact do *not* act on a single target, and are actually more promiscuous than previously thought, thereby exposing a deficiency in the HTS approach. Off-target binding could have an important role in the efficacy of a drug candidate that: (i) underscores not only the importance of target selection, but also the choice of the R&D strategic model; and, (ii) provides one plausible explanation for the success of the *in vivo* screening model in delivering NMEs.

## Impact of combinatorial chemistry on drug discovery

The paradigm of drug discovery experienced changes at the end of the last century. The acceptance of HTS methodologies reinforced opinions to prepare larger, diverse collections of test compounds, especially peptides and small molecules. Solid-phase chemistry [15] enabled the assembly of complex polypeptides on a polymer support, simultaneously providing access to previously unattainable molecules and foreshadowing the use of automation. The overarching features of this approach were the use of substrates covalently bound to a solid-support (polypropylene, polystyrene or other polymeric beads) and an excess of reagents to drive reactions to completion that could be washed away, thus eliminating the need for traditional purifications [16]. Means of accessing larger numbers of polypeptides as well as complex arrays of polypeptides naturally emerged [17,18]. Another methodology used in combination with automated parallel synthesis equipment enabled the synthesis of sets of compounds (libraries) containing literally millions of members. This 'split-and-mix' [19–21] methodology relied on a three-step regimen to build libraries of compounds on a solid support: performing the initial reactions on the beads, mixing the beads and then partitioning the beads for the next reaction. This process could be repeated many times, limited only by the targeted size of the final products.

As the number of final products increases by multiplication whereas the number of reagents and reactions used increases only by addition, this combinatorial approach could rapidly provide libraries of more complex compounds. Of course the inherent difficulty of assigning specific structures to the synthesised molecules became an issue, in terms of relating a chemical structure to a 'hit' from a biological assay. The use of inert chemical 'tags' (or labels) as an encoding strategy to describe the chemical reagents used for any member of a library offered a method to decipher structures associated with 'hits' [22].

Large investments in drug discovery, along with the advent of HTS methodologies and the ability to synthesise very large libraries led to a mistaken sense of exuberance in the field based on the founding (but flawed) principle that, 'given a sufficiently large and diverse set of compounds to test, the discovery of an ideal drug for any given disease state would be statistically unavoidable' [23]. Combinatorial libraries of peptides/nucleotides did not readily translate to functional and commercial drugs for a variety of reasons: (i) although millions of molecules were made, they represented only a minute fraction of the possible compounds that could be made [24]; (ii) the deconvolution of large libraries is not practical because the split-and-mix method requires the concomitant resynthesis (or partial resynthesis) to relate a 'hit' to a discrete chemical structure; and (iii) combinatorial chemistry produced libraries of compounds with physicochemical properties that deviated substantially from either drug-like or natural product-like compounds [25]. Despite these shortcomings, combinatorial chemistry efforts have produced one success story. The preparation and testing of approximately 200,000 compounds in a HTS assay for Raf1 kinase inhibition [26] identified a weak inhibitor of Raf1 kinase that was optimised to sorafenib, approved for the treatment of renal cell carcinoma after just 11 years of R&D.

Although the desire for drug discovery programmes to produce large [>10,000 new chemical entity (NCE)] libraries by the split-and-mix method eventually waned, medicinal chemists have developed a repertoire of tools for parallel synthesis. The advances in the use of parallel platforms have been employed to prepare smaller collections to enhance medicinal chemistry hit-to-lead programmes [27–32]. Furthermore, the skill sets developed by medicinal chemists have been adopted by the fields of agricultural chemistry, chemical biology, catalyst discovery, process chemistry and material science [23]. Also, the recognition of relationships between compound structure and biological activity has enabled enhancements towards library design based on *physicochemical* properties (properties that can be *measured* such as logD or solubility) and *constitutive* properties (features that can be derived, or calculated, from the arrangement of atoms in a compound) [33]. A popular example of the usage of constitutive properties was first described by Lipinski et al. [34,35] who developed a guide widely known as the 'Rule of Five' that states drug-like molecules should have: a molecular weight (MW) of ≤500; ≤5 hydrogen bond donors; ≤10 hydrogen bond acceptors and a logP of ≤5. The 'Rule of Five' has helped guide library design to bias a set of molecules towards having properties similar to known drugs.

The realisation that making large libraries alone would not necessarily provide chemical leads for drug discovery programmes was intimately associated with advances in computational methods. During this time, computational chemists were developing tools to assist with the design and selection of compounds for synthesis and screening. Chemists were equipped to engineer focused libraries with drug-like properties, and arguably a higher chance of success, as well as libraries to address specific questions or probe specific structure–activity relationships during a lead optimisation programme.

## Fragment-based design

Fragment-based drug discovery (FBDD) is a recent addition to the set of tools available to pharmaceutical scientists. In FBDD, collections of low molecular weight compounds, or 'fragments', are screened using biophysical methods to identify weak, as characterised by dissociation constants in the micromolar to millimolar range, but efficient binders to a target of interest, usually a purified protein. The fragments thus identified serve as starting points for further optimisation with the goal of producing potent drugs with favourable properties. Herein we discuss the key concepts of FBDD, the biophysical techniques involved, recent advances in the area and some FBDD successes.

Fragment criteria as well as the properties of collections are topics often debated in this field. A fragment in the context of FBDD is smaller and more polar than most drug molecules. Fragments are often filtered by a 'Rule of Three' [36] (analogous to

Lipinski's 'Rule of Five' [34,35]), to have MW <300, hydrogen bond donors or acceptors ≤3, the number of rotatable bonds ≤3 and ClogP ≤3 (there has been success, however, with a 'non-Rule of Three' compliant library [36]). Fragments bind to their targets weakly but very efficiently relative to their size. The concept of ligand efficiency stems from a better understanding of the free energy changes involved in the binding of a ligand to a protein. In fact, the free energy increases steadily as the number of non-hydrogen ligand atoms approaches 15, and plateaus as the number of non-hydrogen atoms in the ligand continues to rise [37].

Another often cited advantage of FBDD is the ability to sample diverse chemical space. The number of possible fragments has been estimated at around $10^8$ [38] as opposed to $10^{60}$ for discrete drug-like compounds with MW 500. Thus, a collection of even a few thousand fragments samples a relatively larger proportion of the available pool of entities, thereby increasing the chances of identifying hits.

Optimising a library toward a particular target is also feasible when dealing with only a few thousand fragments; for example, rigid fragments and those with aromatic rings may target protein–protein interactions [39]. Recent work demonstrates that additional key factors to consider when selecting fragments include solubility, reactivity and the tendency to aggregate in solution; these characteristics can confound data analysis and hinder the ability to identify successful binders correctly [40].

A range of biophysical techniques has been used to identify compounds that bind target proteins. The most popular techniques for FBDD are X-ray crystallography, nuclear magnetic resonance (NMR), surface plasmon resonance and isothermal calorimetry [40]. X-ray crystallography both identifies fragments that bind to the target and provides detailed structural information in a single experiment; however, expense precludes the use of X-ray crystallography as a primary screening tool. NMR can be utilised effectively to screen fragments against a target protein and has become a workhorse of FBDD. Saturation transfer difference [41] and WaterLOGSY [42] experiments can identify binding interactions between the fragment and protein. Isotopically labelled protein is not required, and these methods are more effective for larger protein targets. When the target protein is relatively small (<30,000 Da), techniques such as structure–activity relationships by NMR [43] can be used to acquire a 2D $^{1}H$-$^{15}N$ HSQC NMR spectrum of a $^{15}N$ labelled protein. A separate resonance is observed for every $^{1}H$-$^{15}N$ pair in the protein; chemical shift changes in the presence of fragments reveal the area of the protein involved in binding. Recent work describes the use of $^{19}F$-NMR in screening fragments [44,45]. Regardless of the techniques selected, more than one technique should be used to validate fragment binding, preferably in an orthogonal fashion [40,46] prior to further development of a FBDD programme.

Various strategies are employed subsequently to transform a selected fragment into a viable lead compound. The earliest approaches, fragment linking, advocated connecting multiple fragments that bind in different areas of the binding pocket. However, fragment linking has struggled to maintain the stringent distance and angular requirements between fragments while preserving their original binding modes. More common approaches have been described as fragment elaboration or fragment growth, where functional groups are added iteratively to assess potency and other properties [40]. Importantly, the growth of the fragment is guided by the structural information obtained from the biophysical techniques described previously.

The success of FBDD can be measured in several ways. Many companies have been founded on FBDD platforms or have incorporated fragment-based approaches into their discovery programmes. However, the real success of any drug discovery paradigm is the ability to deliver marketed drugs and improve patient outcomes. At last count, 17 compounds that originated from FBDD programmes had reached clinical trials, including seven that reached phase II and one, vemurafenib, that reached phase III [40]. Vemurafenib, a selective inhibitor of the B-Raf kinase for the treatment of malignant melanoma, was recently approved as Zelboraf by both the FDA and the European Commission and represents the first success for FBDD.

## Examples in drug discovery

### Hepatitis C virus

Hepatitis C virus (HCV) is a positive sense RNA virus of the family *Flaviviridae* which was first identified in 1989 [47] and causes an infection of the liver that is transmitted via blood and mother-to-child transmission. HCV does not kill the cells it infects, but it instead triggers an immune-mediated inflammatory response that either rapidly clears the virus or slowly destroys the liver. It is estimated that 130–170 million

people are chronically infected with HCV, with 3–4 million newly infected people each year, and that more than 350,000 people die from hepatitis C-related liver diseases each year [48]. The nucleotide sequence of HCV is variable and has been grouped into six main genotypes, each with multiple subtypes, based on sequence data [49]. HCV genotypes/subtypes and areas of prevalence are as follows:
North America – genotype 1a;
South America – genotype 1a, 1b, and 3;
Europe, Asia – genotype 1b;
Egypt, Zaire – genotype 4;
South Africa – genotype 5; and
Hong Kong – genotype 6 [50,51].

HCV infects a liver cell by first binding to surface receptors on the host cell membrane [50,51]. Receptor-mediated endocytosis promotes entry of the virion into the cell, where it is uncoated and releases its positive single-stranded RNA (ssRNA) genome. This ssRNA is then translated by host cell ribosomes via an internal ribosome entry site into a polypeptide that codes for 10 proteins including four structural proteins: capsid protein C (genome encapsidation), envelope proteins E1 and E2 (glycoproteins) and p7 (ion channel). In addition, six non-structural proteins are part of this replication: NS2 (cysteine protease); NS3 (serine protease, RNA helicase); NS4A (serine protease co-factor); NS4B (membrane altercations); NS5A (phosphoprotein); and NS5B (RNA-dependent RNA polymerase). The polypeptide is then cleaved into these 10 functional proteins. The NS5B protein then catalyses the replication of HCV RNA.

Unlike hepatitis A and B, there is currently no vaccine to prevent HCV infection [52]. Until 2011, the only FDA-approved treatment of HCV infection consisted of peg-interferon alpha (peg-IFN-α) and ribavirin for 48 weeks. IFN-α does not act directly on the virus or replication complex. Instead it induces IFN-stimulated genes, which establish a non-virus-specific antiviral state within the cell, mediated by IFN cell surface receptor subunits, JAK1, TYK2, STAT1 and STAT2, and IFN-regulatory factor 9 (IRF-9), which leads to the expression of multiple IFN-stimulated genes, many of which are related to antiviral activity [53]. Ribavirin is thought to inhibit HCV by four proposed mechanisms:
**1.** immunomodulation of T-helper-1 (Th1) over T-helper-2 (Th2) phenotype;
**2.** inhibition of inosine monophosphate dehydrogenase leading to guanosine diphosphate depletion;
**3.** direct inhibition of HCV RNA polymerase; and
**4.** mutagenesis resulting in reduced virion infectivity.
Despite the success of this therapy, less than 50% of the patient population exhibit sustained virological response (SVR).

Since 2008, the FDA has approved two drugs for treating HCV infection: boceprevir (2011) and telaprevir (2011), both of which are serine NS3/4A protease inhibitors. Boceprevir has been approved for treating HCV genotype 1 in combination with peg-IFN-α/ribavirin [54,55]. Boceprevir covalently, yet reversibly, binds to the active site of NS3 protease at a serine residue (S139) via an α-ketoamide, inhibiting the activity of HCV genotype 1a and 1b NS3/4A protease [56]. In clinical trials with genotype 1 HCV, boceprevir taken in combination with peg-IFN-α/ribavirin exhibited a SVR higher (63–66%) than those subjects taking peg-IFN-α/ribavirin alone (38%). Telaprevir has also been FDA-approved for treating HCV genotype 1 in combination with peg-IFN-α/ribavirin [57,58]. Telaprevir inhibits protease NS3/4A in the same manner as described for boceprevir. In clinical trials involving patients with genotype 1 HCV infection, telaprevir taken in combination with peg-IFN-α/ribavirin exhibited an SVR higher (79%) than those subjects taking peg-IFN-α/ribavirin alone (46%).

HCV drug discovery is relatively young, with the first enzyme-targeting drugs being approved by the FDA in 2011. While these new drugs provide alternatives to what has been the standard of the care, they both still require co-administration of peg-IFN-α/ribavirin and are limited to treating only those with genotype 1 HCV infection. Hence, there is still a great need for new therapeutics with different modes of action that, it is hoped, can treat all genotypes of HCV, either alone or in combination with one another, but in the absence of peg-IFN-α/ribavirin. Promising targets include NS5A and B inhibitors, entry/fusion inhibitors, nucleosides and inhibitors of host targets that are integrated in the HCV life cycle, such as cyclophilin.

## HIV

Human immunodeficiency virus (HIV) is a positive sense RNA retrovirus of the family *Retroviridae* which was first identified in 1981. It is transmitted via sexual contact, blood and mother-to-child transmission and primarily infects T-helper cells (CD4 Th cells) [59]. HIV kills T cells by directly killing infected cells, increasing the rate of apoptosis in infected cells and killing infected cells by cytotoxic CD8 TC cells

that recognise infected cells. When the CD4 Th cell number decreases below 200 cells/μL, the condition is categorised as acquired immunodeficiency syndrome (AIDS), during which cell-mediated immunity is lost, and the body becomes progressively more susceptible to opportunistic infections [60]. According to a 2009 UNAIDS report, 60 million people have contracted the HIV virus since its discovery in 1981, of whom 25 million have died from AIDS-related causes, and 33.3 million people are currently infected by HIV/AIDS [61]. There are two types of HIV: HIV-1 and HIV-2. HIV-1 is the more virulent and infective of the two, and is the predominant HIV virus globally, while HIV-2 is largely restricted to West Africa [62].

HIV infects a T cell by first binding to CD4 surface receptors on the host cell membrane [63]. Fusion between the HIV virion and the host is mediated by a fusogenic domain in gp41 and CXCR4, a G-protein-linked receptor in the target cell membrane. The nucleocapsid-containing viral genome and proteins then enter the host cell and are released following the removal of core proteins. Viral reverse transcriptase catalyses the reverse transcription of ssRNA, forming RNA-DNA hybrids. The original RNA template is then partially degraded, and the synthesis of a second DNA strand affords HIV double-stranded DNA. The viral double-stranded DNA is then translocated to the nucleus and integrated into the host cell DNA by the viral enzyme integrase.

Currently, there is no cure for HIV infection, nor is there any vaccine that protects individuals from HIV infection, thus the search continues for new therapies. Current treatment entails highly active retroviral therapy, which typically consists of combinations of drugs that inhibit different proteins in the HIV life cycle. These drugs are categorised as fusion/entry inhibitors, reverse transcriptase inhibitors, integrase inhibitors and protease inhibitors [64,65]. Since 2008, the FDA has approved four drugs for treating HIV [64]. Three of these are non-nucleoside reverse transcriptase inhibitors: etravirine (2008), nevirapine XR (2011) and rilpivirine (2011), while the fourth is a multi-class combination product Complera (a single tablet, fixed dose combination of emtricitabine/rilpivirine/tenofovir disoproxil fumarate) (2011). Etravirine [66–69] has been approved for treating HIV-1 in combination with other antiretroviral agents [70]. Likewise, rilpivirine [71,72] has been approved for treating HIV-1 in combination with other antiretroviral agents, and it has the same mechanism of action as etravirine [73]. Rilpivirine exhibited anti-HIV activity (83% virologic response) comparable to efavirenz (80% virologic response) and superior to etravirine (60% virologic response), but offers a more favourable safety and tolerability profile. The other two drugs, nevirapine XR (extended release) and Complera, are composed of previously approved drugs.

HIV drug discovery is a significantly mature field, with 35 FDA-approved drugs over 25 years that span six mechanisms of action, and many of these can be taken in combination with one another in order to combat resistance. Challenges that remain include the further development of drugs with high barriers to resistance such as second generation non-nucleoside reverse transcriptase inhibitors and, due to failure to cure, a strong safety profile that can be taken for a long period of time. Ultimately, the ability to achieve cure and not simply stasis of the disease is desired.

## Central nervous system therapeutic area

### Multiple sclerosis

Multiple sclerosis is an autoimmune disease affecting the brain and spinal cord of people typically between the ages of 20 and 40, although it can be diagnosed at any age. Caused by damage to the myelin sheath, it remains a significant unmet medical need and the subject of research at many pharmaceutical and biotechnology companies. The majority of patients (85%) suffer from the so-called relapsing-remitting form of MS in which acute flare-ups are followed by symptom-free periods, often progressing to the secondary progressive form.

The management of MS consists of two lines of therapy. In the first, symptoms associated with muscle spasms and acute flare-ups are treated. Use of muscle relaxants for spasms and high doses of steroids for vision loss are two of the common treatments. Drugs used to slow the progression of MS have seen varying degrees of success. Most of the currently used therapies involve modulation of the immune system. There are a number of different versions of IFN β-1b, which work by limiting the effects of anti-inflammatory cytokines. There is also evidence that they improve the integrity of the blood–brain barrier which is generally compromised in MS patients. Mitoxantrone is a known anticancer drug prescribed for MS based on its immunomodulatory effects. Another approved therapy is glatiramer acetate which is a random polymer of four amino acids found in myelin basic protein. The mechanism of action is unknown but several proposals have been put forth. One proposal has shown that glatiramer converts

pro-inflammatory Th1 cells into regulatory Th2 cells that are immunosuppressive [74]. Another proposal is that the resemblance to myelin basic protein causes glatiramer to decoy the autoimmune response to myelin in the myelin sheath [75]. Glatiramer acetate, unlike the other treatments, has shown no effectiveness in halting disability progression and thus has been approved only for reducing the frequency of relapses [76].

One of the more significant advances occurred in 2004 with the approval of natalizumab (Tysabri). Natalizumab is a humanised monoclonal antibody that acts against the cellular adhesion molecule α4-integrin to prevent penetration of the blood–brain barrier by inflammatory cells. It has shown efficacy in preventing relapse, vision loss and cognitive decline. Serious side effects are known, especially if co-dosed with interferon, and have muted enthusiasm for natalizumab. However, it has shown a significant improvement in quality of life for those MS patients who can tolerate it.

The success of these medications, and their limitations, continue to drive efforts to identify novel treatments that are efficacious and orally bioavailable. Two such drugs approved in 2010 are first in class molecules that extend the mechanism-based approach to MS treatments. Dalfampridine is the first drug approved by the FDA to improve walking in patients with MS. A voltage-gated potassium channel blocker, it acts by readily penetrating the blood–brain barrier and increasing the conduction and duration of action potential across nerve fibres. The result is improved functionality of the damaged myelinated fibres and improved locomotion [77].

The second new drug to be released in 2010 was fingolimod hydrochloride. A series of studies [78,79] showed that fingolimod is converted to fingolimod phosphate by sphingosine kinase-2 and this phosphate binds to multiple sphingosine-1-phosphate (S1P) receptors. Binding to the S1P1 receptor leads to internalisation of the receptor and sequestration of autoimmune reactive T cells in the lymph nodes. Because these T cells are believed to be responsible for the inflammation and myelin sheath damage observed in MS, their reduced circulation leads to improved symptom management and significant reduction in disease progression [80,81].

Despite these advances, MS remains a debilitating disease and an active area of research in both academia and industry. Indeed, several new drugs are in various stages of clinical trials and are utilising new concepts in the biology of MS. One such drug is ocrelizumab which targets the B-cell component of the immune system. The mechanism is currently unclear, but ocrelizumab appears to target a B-cell surface protein, CD20, which primes T cells for myelin attack [82]. Continued forward progress in the management of symptoms and disease progression will allow patients to optimise treatment on an individual basis. Drugs that actually repair damage to the myelin sheaths and permit some level of neurological restoration will be the next desirable addition to the MS pipeline.

## Alzheimer's disease

Alzheimer's disease (AD) is the most common form of dementia. Prevalence increases dramatically with age, with up to 50% of patients over the age of 85 having been diagnosed with AD. Onset is slow and insidious; progression is gradual with the average time from onset of symptoms to death being 8–10 years [83].

Although the exact pathophysiology is unknown, there are prominent findings that are characteristic of AD, including amyloid plaques, neurofibrillary tangles and neuronal degeneration [84]. Amyloid plaques are deposits of beta-amyloid and are believed to disrupt neuronal activity by increasing the production of free radicals, resulting in oxidative cell damage and eventual death of the affected cells. The second most prominent finding in the brains of patients with AD is the formation of neurofibrillary tangles. These tangles are primarily composed of tau protein which is essential for the growth and development of the axons of strong healthy neurones. Tau proteins can become hyperphosphorylated and form tangles, thus making it more difficult for neurones to maintain their normal formation of microtubules. It is interesting to note that the number of neurofibrillary tangles can be correlated with the severity of dementia (i.e. impaired cognition and memory loss) [83,84]. The third characteristic of neuronal degeneration (as well as subsequent synapse loss) can be directly correlated with the formation of neurofibrillary tangles. The death of cholinergic neurones ultimately leads to a loss of acetylcholine, necessary for synaptic transmission, which, in turn, leads to progressive memory decline.

The loss of cholinergic synapses along with diminished acetylcholine activity led to the initial development of acetylcholinesterase (AChE) inhibitors to treat patients with AD. AChE inhibitors help prevent the breakdown of the neurotransmitter acetylcholine (by the enzyme acetylcholinesterase) in the synapses of cholinergic neurones, thus improving cholinergic

transmission in the surviving neurones [83]. AChE inhibitors were the first agents to be approved by the FDA in the treatment of AD and include donepezil, rivastigmine, galantamine and tacrine. All of these are indicated for mild-to-moderate symptoms of AD. Donepezil is also indicated for moderate-to-severe symptoms and is available as an orally disintegrating tablet for patients who have trouble swallowing. A transdermal patch is an alternative to oral dosing in patients taking rivastigmine. Tacrine is rarely used in current therapeutic regimens due to its rigid dosing schedule and potential for causing hepatotoxicity [83,85].

*N*-methyl-D-aspartic acid (NMDA) antagonists have been the most recent agents to be approved for treating AD. The drug memantine is indicated in the treatment of moderate-to-severe symptoms of AD. It is surmised that by blocking the NMDA receptor, over-stimulation of the glutamatergic system is prevented. Because excess glutamate has been shown to lead to destruction of cholinergic neurones, the reduced levels of glutamate may prevent neurotoxicity without interfering with the role of glutamate in normal memory [83,85].

It is important to note that existing therapies do not reverse the progression of AD, but only slow the worsening of symptoms and improve quality of life. A principal thrust of current research seeks to investigate ways to prevent, slow or possibly cure AD.

Muscarinic agonists (especially M1 muscarinic receptor agonists) have been of interest in the fight against AD because they may prevent beta-amyloid formation via activation of alpha-secretase. If ultimately effective, these agents would have the potential to treat memory deficits as well as halt the disease process itself. Although none is currently marketed for AD, several muscarinic agonists have shown promise and are under investigation. Xanomeline, CDD-0102A, cevimeline and talsaclidine are M1 agonists currently under investigation. In 2009, the FDA allowed further clinical development of CDD-0102A to take place. At last report, the compound was in clinical trials and re-designated as MCD-386. Cevimeline has been used in the treatment of xerostomia (i.e. dry mouth) associated with Sjögren's syndrome, but its selectivity for muscarinic receptors is of great interest in AD. Interestingly, both cevimeline and talsaclidine have shown the ability to promote clearance of beta-amyloid from the cerebrospinal fluid of AD patients [86,87].

Beta-secretase (BACE) is an aspartic protease that cleaves the type I transmembrane amyloid precursor protein to form beta-amyloid peptides. It is believed that inhibition of this enzyme would decrease beta-amyloid production. Past research strategies have focused on selective inhibitors of gamma-secretase, but these have resulted in the accumulation of a potential neurotoxin; therefore, much of the focus has shifted to BACE. Some research findings involving BACE inhibitors have found that when covalently linked to a peptide that promoted transport into the brains of mice, levels of beta-amyloid in plasma and brain were significantly lower post-intraperitoneal administration. Such a result suggests that an orally active BACE inhibitor able to cross the blood–brain barrier would lower beta-amyloid levels in the brain. The key feature of the most active BACE inhibitors is the ability to mimic a transition state at the active site, thus conferring low nanomolar potency [83,88].

Glycogen synthase kinase-3 (GSK-3) is a proline-directed serine/threonine kinase that is involved in the phosphorylation of a number of substrates affecting numerous cellular functions. GSK-3 inhibition has become a subject of interest because of its role in the phosphorylation of tau protein which, in turn, is directly involved in beta-amyloid formation. Challenges involved in developing an effective GSK-3 inhibitor will be those of selectivity and the ability to cross the blood–brain barrier. Because GSK-3 is involved in many cellular processes, the issue of selectivity will be very important, especially to avoid toxicity in a clinical setting. Two classes of compounds that have been prominent in the literature surrounding GSK-3 inhibition are the indirubins and maleimides. While both classes have shown activity in kinase inhibition, finding a GSK-3 specific inhibitor has been challenging work. To date, neither class has a representative GSK-3 specific agent ready to enter the market. It is interesting to note that other established drugs may have the added mechanism of GSK-3 inhibition as part of their pharmacological activity. Donepezil, currently marketed as an AChE inhibitor, was shown to reduce tau phosphorylation in a study using primary cortical neurone cultures treated with donepezil and challenged with beta-amyloid. This result suggests GSK-3 inhibition as a potential mechanism. Olanzapine, an atypical antipsychotic agent, has demonstrated GSK-3 inhibition in the brains of mice and has since been shown to be a potent inhibitor of GSK-3 (beta isoform). Other existing drugs of interest as GSK-3 inhibitors include cimetidine, hydroxychloroquine and gemifloxacin [88,89].

To date, current therapies in the treatment of AD have only been able to address the symptoms of the

disease rather than its underlying aetiology. Continued research and emphasis on development of compounds that can combat the biological mechanisms seen in AD are of greatest importance. Specifically, research focused on medicinal agents that can prevent the formation of neuritic plaques or neurofibrillary tangles (or hasten their clearance) is key in the fight against AD.

### Cardiovascular disease

Two new medicines were approved for treatment of atrial fibrillation (AF) which accounts for approximately 35% of arrhythmia-related hospitalisations in the USA annually. In combination with electrical cardioversion, anti-arrhythmic drugs are used to maintain a normal sinus rhythm. The standard of care for AF is amiodarone, whose efficacy is believed to arise from a combination of effects. It has been shown to prolong certain cardiac action potentials, possess ion channel effects – both sodium and potassium – and bind to the nuclear thyroid receptor. Undoubtedly, these combinations of activities contribute to its usefulness, but also to its side effects. These include interstitial lung disease, pulmonary fibrosis and abnormal thyroid function, due to high iodine content. Dronedarone , an analogue of amiodarone, was introduced in 2009. It is a potassium ion channel blocker that has lower efficacy but fewer side effects than amiodarone [90]. The reductions in side effects are related to reduced lipophilicity, reduced half-life and the absence of iodine in dronedarone. Vernakalant is also a potassium channel blocker which was introduced for treatment of AF in Europe in 2010 [91–94]. Vernakalant has been used successfully to treat 51% of patients with short duration paroxysmal AF (up to 7 days) but is far less effective (8% of patients) in treatment of persistent AF.

In the field of lipidaemia there has been relatively little progress in terms of newly approved drugs. Choline fenofibrate was introduced in 2008 and is used in combination with a statin to increase levels of high-density lipoprotein cholesterol and lower concentrations of triglycerides in patients with mixed dyslipidaemia and coronary heart disease [95,96]. As a stand alone treatment it is indicated for patients with severe hypertriglyceridaemia. Choline fenofibrate is a salt form of fenofibric acid, an active metabolite of fenofibrate which was previously marketed for treatment of hypercholesterolaemia. As such, the mechanism of action of choline fenofibrate is consistent with fenofibrate.

Angiotensin converting enzyme (ACE) inhibitors and angiotensin II receptor blockers (ARBs) have long been prescribed for management of hypertension. The renin–angiotensin–aldosterone system regulates blood pressure; ACE inhibitors interrupt the conversion of angiotensin I to angiontesin II to minimise vasoconstriction and prevent high blood pressure. Aliskiren, introduced in 2007, represents a first-in-class treatment for its direct suppression of renin upstream of the ACE/ARBs [97,98]. Aliskiren is differentiated from existing treatments by its long duration of action (24-hour half-life) and reduction in the incidence of side effects (e.g. angioedema and coughing). However, high doses of aliskiren can result in diarrhoea. By contrast, clevipidene is a very short duration vasodilator used primarily for urgent treatment of hypertension during surgery [99,100]. Introduced is 2008, clevipidine is a calcium channel blocker administered intravenously and is so rapidly eliminated from the bloodstream that the dose can be titrated against the patient's blood pressure. In practice, the target blood pressure is reached within 30 minutes of administration. Common side effects include nausea, vomiting and headaches.

Hereditary angioedema is a rare but potentially life-threatening disorder when swelling occurs in the upper airway. Two new drugs for treatment of hereditary angioedema were introduced: ecallantide (2010, a 60 amino acid recombinant protein) [101,102] and icatabant (2008, a peptidomimetic) [103,104]. Both work to control bradykinin which, when present in elevated concentrations, leads to vasodilation and hypotension. Icatabant is a selective $\beta_2$ receptor antagonist while ecallantide inhibits plasma kallikrein, an immediate precursor of bradykinin, in the renin–angiotensin–aldosterone system system.

Three new treatments have recently been launched for the treatment of atherothrombotic events associated with acute coronary syndrome. Rivaroxaban (2008) is a highly potent inhibitor of factor Xa which is responsible for the conversion of prothrombin to thrombin during coagulation [105,106]. Prasugrel (2009) is employed for antiplatelet therapy and is a third generation thienopyridine following in the footsteps of clopidogrel [107,108]. Prasugrel inhibits platelet aggregation and is 10 times more efficacious than clopidogrel. Thienopyridine drugs irreversibly bind to the $P2Y_{12}$ receptor. Ticagrelor (2010) differs in that it is reversibly bound to the same receptor and as such the risk for uncontrolled bleeding is reduced [109,110].

## Chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) is a chronic inflammatory disease that is characterised by increased numbers of neutrophils, macrophages and T lymphocytes [111] present in the proximal airways, lung parenchyma and lung vasculature [112]. Repeated injury and repair leads to progressive structural changes that increase with disease severity [113]. COPD was ranked as the sixth leading cause of death worldwide in 1990 and is projected to be the fourth leading cause of death by 2030 because of an increase in smoking rates and demographic changes in many countries [114].

Bronchodilator medications are central to the symptomatic treatment of COPD [115–118]. They are given either on an as-needed basis for relief of persistent or worsening symptoms or on a regular basis to prevent or reduce symptoms. Acute application of inhaled glucocorticosteroids is appropriate for patients with severe COPD accompanied by repeated exacerbations [119–122]; however, chronic treatment is discouraged because of a plethora of potential side effects including neutrophilia, hyperglycaemia, immunodeficiency and steroid myopathy, which contributes to respiratory failure in advanced COPD cases [123–125]. Recently, two new molecular entities received regulatory approval from the FDA and European Medicines Agency (EMA). A new bronchodilator approved for COPD, indacaterol acts as an agonist on the $\beta_2$-adrenoceptor. This agonism causes smooth muscle relaxation resulting in dilation of bronchial passages [126,127]. Indacaterol is an ultra-long-acting agent with duration of action suitable for once-a-day dosing. It is supplied as the maleate salt in an aerosol formulation and is administered via a dry powder device [128]. Recently approved for COPD, the anti-inflammatory roflumilast is a selective, orally active PDE-4 inhibitor. The selectivity observed with roflumilast is in contrast to long-used theophylline, a non-selective PDE inhibitor, and is reflected in the improved side effect profile of roflumilast. Interestingly, the improvement observed with roflumilast may be brought about by an appropriate balance between binding at low- and high-affinity sites on PDE-4 and not simply isozyme specificity [111,129]. PDE-4 is expressed in several tissue types involved in diseases of the airway and is known to have a role in inflammation [130]. Inhibition of PDE-4 blocks the hydrolysis of cyclic adenosine monophosphate (cAMP), leading to elevated levels of cAMP, which in turn downregulates the inflammatory process. Roflumilast is indicated for maintenance treatment of severe COPD associated with chronic bronchitis in adult patients with a history of frequent exacerbations as an add on to bronchodilator treatment [131,132].

Pharmacological therapy is used to prevent and control symptoms, reduce the frequency and severity of exacerbations, improve health states and improve exercise tolerance in patients with COPD. While existing drugs do not modify the long-term decline in lung function or disease progression [133], these shortcomings should not preclude efforts to use medications to prevent or reduce symptoms.

## Diabetes

Diabetes mellitus is a group of metabolic diseases for which there is no true cure; however, all forms of the disease are treatable. The administration of insulin through external sources, the preferred treatment for type 1 diabetes, aids the body in processing blood glucose levels by providing a source of the hormone that is, in many cases, no longer secreted by the pancreas. Because of the structural nature of insulin and the related peptidic hormone amylin, oral administration of hormone-based drugs is ineffective: peptide-based drugs typically have poor pharmacokinetic and ADMET properties. Therefore, drugs in this class are chiefly injectables, although recent years have seen advances in formulations that offer hope for the development of oral insulin [134]. The vast majority of diabetes patients present with type 2 disease, where insulin levels may be insufficient to diminish the blood sugar concentration of patients. Those with type 2 diabetes are treated with a variety of pharmaceuticals developed specifically to combat the disease, which include combinations of newer medications with older therapeutics [135]. Attributes of non-related classes of therapeutics have positioned these entities for the treatment of type 2 diabetes as well. For example, colesevelam, a bile acid sequestrant, proved to lower blood sugar levels and was approved by the FDA in 2008; the orally administered drug is not absorbed by the body and therefore must function within the digestive tract [136]. Another such example, approved in 2009, is bromocriptine, a powerful D2 dopamine receptor agonist that lowers blood sugar levels in patients. [137].

Glucagon-like peptide 1 (GLP-1) is a digestive prohormone secreted by L cells found in the intestinal wall and metabolised into the active forms GLP-1-(7-37) and GLP-1-(7-36), all of which are under

investigation as therapeutics for type 2 diabetes. GLP-1 seems to retard apoptosis of pancreatic β-cells thereby increasing the secretion of insulin, as needed, during postprandial periods; unfortunately, the active forms of GLP-1 are very short-lived in the body with an estimated half-life of less than 2 minutes [138]. GLP-1 (and its active forms) not only upregulate insulin secretion, but they also slow 'gut emptying' which delays the digestion and uptake of carbohydrates after eating. This effect promotes satiety thus delaying the desire to eat and subsequent need for insulin production. In 2010, a GLP-1 mimic, liraglutide, was approved by the FDA for use in patients with type 2 diabetes in the USA; it had been previously approved for use in Europe in 2009. Liraglutide is a mimetic of human GLP-1-(7-37) that has been modified through the addition of a fatty acid residue to the peptidic backbone of the hormone. This change promotes binding with albumin in the bloodstream and subcutaneous tissues resulting in sequestration that extends the half-life of the drug to nearly 12 hours. Liraglutide is released from the albumin at a slow steady pace to maintain insulin concentration at efficacious levels [139].

Entities with modes of action related to human GLP-1 are also of interest. Exenatide is a synthetic form of exendin-4, a hormone found in the saliva of Gila monsters, which stimulates pancreatic activity. Exenatide has approximately 50% amino acid homology to human GLP-1 and displays a prolonged *in vivo* half-life. In 2005, exenatide was approved as an injectable medication for the treatment of type 2 diabetes; it was originally used in conjunction with other oral drugs for the control and treatment of elevated blood glucose concentration.

While increasing the amount of GLP-1 or mimicking its mode of action in the body achieves the desired affects for treatment of type 2 diabetes, another tactic that has gained momentum is to prevent the metabolism of GLP-1. It has been shown that dipeptidyl peptidase-4 (DPP-4) is critical in the degradation of GLP-1 and inhibitors of DPP-4 therefore prolong the half-life of active forms of GLP-1 in the bloodstream. Recently, small molecules that modulate DPP-4 have been developed to delay the breakdown of hormones crucial to the stimulation of pancreatic β-cells. These drugs not only increase serum GLP-1 levels, but they also can be administered orally [123–125]. The first of the DPP-4 inhibitors, sitagliptin, was released in 2006 [140], with saxagliptin following in 2009. While there is a risk that DPP-4 inhibitors do promote progression of some cancers, few side effects were noted during testing of saxagliptin [141]. Linagliptin, another DPP-4 inhibitor, emerged in 2011. Again, based upon studies of the toxicity of linagliptin, the beneficial affects outweighed the small risk of tumour development associated with the class of molecules [142].

### Osteoporosis

Osteoporosis is a disease characterised by low bone mass and structural deterioration of bone tissue that gradually weakens bones and often leads to painful and debilitating fractures. [143–148] Bone remodelling, the process through which bone tissue continuously renews and changes, is essential for adult bone homeostasis. The remodelling cycle consists of two distinct stages: bone resorption and bone formation. During bone resorption, surface osteoclasts dissolve bone tissue and create small cavities. During bone formation, osteoblasts then refill these cavities with new bone tissue. The failure or disruption of this process often leads to the development of osteoporosis [149]. Progress in understanding the bone remodelling process has been at the core of efforts leading to the discovery of drugs for the treatment of osteoporosis [150]. These drugs may stimulate bone formation, such as teriparatide, or may retard bone resorption, such as the selective oestrogen receptor modulator raloxifene and salmon calcitonin products.

Bisphosphonates have been the drugs of choice for preventing and treating osteoporosis over the last 20 years [151]. Studies indicate that these drugs are effective and safe for at least 10 years but note that bone loss does continue, albeit at a reduced rate. This continued loss is believed to result from the interruption of bone remodelling which ultimately impairs the bone formation portion of the process [152]. Complications have been reported with prolonged use of bisphosphonate drugs. The most serious, but rare, side effects are bone loss in the jaw (osteonecrosis) [152], a possible increase in the risk of unusual fractures of the thigh bones (femur) and atrial fibrillation [153,154]. In general, the benefits of bisphosphonates outweigh the risks they present. Nonetheless, the FDA continues to monitor the long-term effects of this important class of therapeutics.

Denosumab was approved by the FDA in 2010 for the treatment of osteoporosis. It has a novel mechanism of action, improved dosing convenience and provides an alternative to bisphosphonate drugs. Denosumab is the first human monoclonal antibody that inhibits the formation, function and survival of

osteoclasts. Elucidation of this new mode of action started with findings that tumour necrosis factor (TNF) increased the formation of osteoclasts [155]. Later, researchers identified a novel soluble TNF receptor, eventually named osteoprotegerin (OPG), which increased bone density by decreasing bone resorption [155]. Further studies showed that the TNF-like molecule receptor activator of nuclear factor-kB ligand (RANKL), existing in both transmembrane and soluble forms, was an OPG ligand. RANK–RANKL binding stimulates the differentiation, activity and survival of osteoclasts to amplify bone resorption. Denosumab binds RANKL thereby preventing the RANK–RANKL interaction, and encouraging bone formation through the OPG-mediated inhibition of bone resorption. Long-term studies are underway to address concerns regarding the effects of prolonged suppression of bone turnover as well as possible adverse effects on the immune system that might lead to increased risk of infection or malignancy.

### Gastrointestinal diseases

Traditionally, the human gastrointestinal tract is divided into two parts: the upper gastrointestinal tract stretches from the mouth to the duodenum and the lower gastrointestinal tract extends from the jejunum to the rectum [156]. Inflammation of the upper gastrointestinal tract, referred to as oesophagitis in the oesophagus and gastritis in the stomach or collectively as gastroesophogeal reflux disease, can result from a variety of reasons, the two most common being allergic reactions and reflux of stomach acid into the oesophagus [157]. One of the earliest treatments for upper gastrointestinal tract inflammation was the development of the H2-antagonists that bind to histamine-2 receptors in the parietal cells of the stomach – the cells that control the secretion of acid in the stomach. These compounds act as inverse agonists of the histamine receptor-2, which is a molecule that binds to the same receptor as the agonist, but induces a response opposite to that of the agonist, in this case, histamine [158]. The H2-receptor antagonists were replaced by the proton-pump inhibitors. These compounds bind to the hydrogen/potassium adenosine triphosphatase ($H^+/K^+$ ATPase, more commonly referred to as the 'proton pump') system in the parietal cells of the stomach's lining. The proton pump is at the end of the acid secretion step, and is therefore a logical target for lowering acid secretion. These compounds bind irreversibly to the enzyme, thus lowering acid production by up to 99%. Acid production recovers as the enzymes are destroyed and recycled through typical metabolism [159]. Dexlansoprazole, which is an enantiopure form of lansoprazole (Prevacid), was approved for use in the USA in 2009 [160].

Lower gastrointestinal tract inflammation is usually classified as inflammatory bowel diseases and is normally diagnosed as either ulcerative colitis or Crohn's disease. Ulcerative colitis is typically restricted to the colon and rectum and affects only the innermost layers of the organs. It differs from Crohn's disease as it is usually restricted to the colon and last parts of the gastrointestinal tract, whereas Crohn's disease can be found almost anywhere throughout the lower gastrointestinal tract [161].

Treatment of ulcerative colitis has traditionally been through the use of anti-inflammatory drugs or via immunosuppressive agents and, in some cases, through a combination of the two types of drugs. Corticosteroids, sulfasalazine and mesalamine are common anti-inflammatories used to treat the disease, with mesalamine being the preferred compound prescribed [162]. Immunosuppressants such as infliximab and ciclosporin are also used to treat the disease but, as they are systemically active, additional concerns accompany these treatments.

Crohn's disease is more widely distributed along the gastrointestinal tract and has significantly different pathology from ulcerative colitis; fevers, fistulae and weight loss are common with Crohn's disease. It also afflicts deeper layers of tissue in the gastrointestinal tractthan does ulcerative colitis. There is no cure for Crohn's disease, but it can be managed with several of the same drugs that are prescribed for ulcerative colitis. Anti-inflammatories have been used to combat some of the symptoms of Crohn's disease, but mesalamine is typically not effective for flare-ups outside of the large intestine. Fortunately, the disease seems to respond better to immunosuppressive medications, such as infliximab and ciclosporin [163, 164]. Recently, monoclonal antibody treatments have moved to the forefront of treatments for patients with Crohn's disease. These molecules, which bind to TNFα, have recently been approved for use in the USA, although the European market is still hesitant to move some of the antibodies to market because of safety concerns. By binding to TNFα these medications downregulate the inflammation response, which helps to relieve symptoms. Because TNFα is regulated by the body's immune system, these drugs are still immunosuppressive and their dosing regimens are typically short to prevent further damage to

the autoimmune system [163,164]. While they are not cures, remission of the disease has been reported. Certolizumab pegol is a pegylated antibody that was approved for use in the USA in 2008 and then for use in Europe in 2009 [165]. Adalimumab (Humira) was the first fully human monoclonal antibody treatment approved for use against inflammation diseases [166]; in 2007, it was approved for treatment against Crohn's disease [167].

## Oligonucleotide-based therapeutics

Non-biological oligonucleotides employed as macromolecular therapeutics can address targets beyond the proteins classically modulated by small molecule drugs. Three major mechanistic classes – antisense, small interfering RNA (siRNA) and steric-blocking oligonucleotides – target gene-specific pre-mRNA or messenger RNA (mRNA) carriers of genetic information from the chromosomes to the ribosomal protein production apparatus. Aptamers, folded oligonucleotides that bind to various molecular targets, comprise a fourth class.

The first three classes rely on base-pairing with the target mRNA, and are designed to have genetic complementarity to the target mRNA sequence. Antisense and siRNA oligonucleotides modulate protein production by inducing enzymatic degradation of the targeted mRNA, resulting in reduced production or removal of the encoded protein (e.g. an oncogene-encoded protein or a pro-inflammatory cytokine). Targeting of a specific RNA by a single-stranded antisense oligonucleotide was first described in 1978 [168]. siRNA oligonucleotides, double-stranded RNA fragments that are 21–22 nucleotides long, mediate degradation of the targeted mRNA by recruiting the multiprotein RNA-induced silencing complex, a process first described in nematodes and subsequently demonstrated in human cells [169, 170]. Steric-blocking oligonucleotides modulate protein production by preventing access of cellular enzymes to the target mRNA, resulting in altered processing of pre-mRNA to the mature species, reduced protein translation or other changes in RNA function [171,172]. Representatives of all three classes are being developed as therapeutic candidates, and one antisense drug, fomivirsen, has received FDA approval for treatment of cytomegalovirus-induced retinitis [173–175]. Aptamers, single-stranded oligonucleotides that fold in complex shapes, serve as scaffolds for intermolecular interactions and offer a means of selectively modulating protein targets of therapeutic interest. Oligonucleotides with the desired binding properties are iteratively selected and enzymatically amplified from a complex library (SELEX – Systematic Evolution of Ligands by EXponential enrichment [176,177]).

Aptamers, like monoclonal antibodies, can be selected for binding to multiple targets, but, unlike antibodies, can be synthesised chemically, thus simplifying manufacturing. One aptamer drug, pegaptanib, is currently marketed for the treatment of age-related macular degeneration, and a number of others are currently in clinical development [34,35,178].

Oligonucleotide-based therapeutics, like all drug classes, face challenges of *in vivo* stability, uptake, toxicity and delivery. Incorporation of chemically modified nucleotides can address stability and cellular uptake [175,178,179]. Such modifications are tolerated differently in the four classes. Antisense and siRNA oligonucleotides are the least tolerant of changes, as both base-pairing and interaction with cellular enzymes must be preserved. Steric-blocking oligonucleotides tolerate somewhat more chemical diversity, for while they must retain base-pairing properties, functional interactions with cellular enzymes do not need to be preserved. Aptamers accommodate the most chemical diversity, as long as the desired binding properties are maintained. Conjugation of polymers such as polyethylene glycol (PEG) is frequently used to reduce renal filtration rates and prolong systemic circulation times of oligonucleotides [179]. Off-target toxicities of oligonucleotides include anticoagulation, complement activation and innate immune stimulation, and may be addressed by strategies including chemical modification and dose limitations [179,180]. The two approved oligonucleotide-based drugs, fomivirsen and pegaptinib, are both delivered locally by intravitreal injection, and effective formulation for systemic delivery of oligonucleotides remains an active research area.

In summary, oligonucleotide-based drugs are poised to join protein biologics in the pantheon of macromolecular therapeutics. Their mechanistic versatility holds the potential of impacting previously undruggable targets, and thus of efficacy in heretofore untreatable diseases. Full realisation of this promise will depend upon continued innovation in translating mechanistic rationale to clinical reality.

## Orphan diseases

Diseases that affect a limited population are collectively termed 'orphan' or 'rare' diseases and, like the neglected tropical disease, have remained an attractive area for drug discovery research [181]. Since 2008, over 750 drugs have gained orphan drug designation with 78 receiving approval by the FDA [182] for treatment of orphan indications; in the same period, 22 drugs have received European marketing approval for treatment of orphan indications. Reasons for developing a new drug, or repurposing an existing drug, for an orphan indication vary, but it is widely accepted that the associated economic incentives, as established by the US Orphan Drug Act (1983) and adopted by the European Commission (2000), have stimulated efforts in this area [183,184]. In addition, the academic community has taken increasing interest in these diseases, in some cases via industrial partnerships, thus furthering the successes of research efforts [185,186]. Furthermore, the emergence of disease-focused philanthropic groups (e.g. the Cure Huntington's Disease Initiative, the Michael J. Fox Foundation for Parkinson Disease and ALS Foundation), academic consortia (e.g. Drugs for Neglected Diseases initiative – DNDi) and the establishment of the National Institutes of Health Therapeutics for Neglected and Rare Diseases (TRND) programme have contributed to a recent, increased focus on these diseases. As a testimony to this focus, TRND has solicited proposals for collaborations since its inception in 2009 and is currently pursuing programmes in sickle cell disease, acute myeloid leukemia, Duchenne muscular dystrophy and Niemann–Pick disease [187].

While the majority of drugs approved for orphan diseases have been repurposed from other indications, new drugs specifically targeted to an orphan disease are beginning to emerge. One such example is eltrombopag, a thrombopoietin receptor agonist which has been developed for treatment of chronic immune thrombocytopenic purpura (ITP) [188,189]. ITP is an acquired disease characterised by low platelet count and occurs at a rate of 2–6 per $10^5$ individuals annually [190]. An acute form of the disease in children is normally self-curative, but chronic ITP carries more significant long-term health challenges with surgical removal of the spleen as an alternative treatment in severe cases [191]. Eltrombopag was approved by the FDA for treatment of chronic ITP in 2008 [192] and by the EMA in 2010 [193], and may also be effective in treatment of thrombocytopenia in patients with HCV infection [194].

## Neglected tropical diseases

Neglected tropical diseases (NTDs) are infectious diseases such as malaria, tuberculosis, diarrhoeal diseases, filariasis, various helminthiases and those caused by kinetoplastids such as sleeping sickness, leishmaniasis and Chagas disease. NTDs affect over 1 billion people in developing regions of Africa, Asia and the Americas [195]. Because individuals in the regions affected are poor, their health systems have very limited resources and political capacity to influence global policy makers. There is also very little incentive for major pharmaceutical companies to develop basic treatments for these limited markets. For example, of the 1393 NCEs approved between 1975 and 1999, only 16 targeted NTDs [196]. Nine were targeted to the most neglected while the rest were developed for malaria and tuberculosis, which along with HIV account for 80% of all R&D spending in this field [197]. A more recent study has refined the number of new NTD approvals during this period to 46, with 85% of these being placed on the WHO Essential Drug List [198].

Investments in NTD drug development have increased dramatically in the past decade [199] and a significant proportion of these advancements are channelled through product development partnerships such as TB Alliance, DNDi for kinetoplastids and Medicines for Malaria Venture which bring together industry expertise and other partners with NTD experiences. A recent survey identified 173 drug products that are in various phases of development for treatment of NTDs [200]. However, the actual number of new drug approvals for NTDs from 2007 to 2012 is limited because of the relatively recent increase in R&D spending. Medicines for Malaria Venture has an impressive portfolio of new treatments launched in the past 4 years for uncomplicated malaria exemplified by various combinations such as Coartem, ASAQ, Eurartesim and Pyramax [201]. Tafenoquine for relapsing malaria, the synthetic peroxide (OZ439) and a spiroindolone (NITD609) represent novel classes of compounds that are in clinical development. With a robust portfolio of new malaria compounds and advancement in understanding of parasite biology, the goal has shifted from treatment to eradication [202]. TB Alliance is currently managing a portfolio of over 20 development products with three new drugs in late stage development [203]. TMC207 is a first-in-class diarylquinoline compound that inhibits bacterial ATP synthase. PA-824 is a pro-drug of the nitroimidazole class

requiring bioreductive activation of an aromatic nitro group to exert an antitubercular effect. The most advanced of the three comes from repurposing the fluoroquinoline moxifloxacin. New drugs or combinations are also emerging for treatment of NTDs caused by kinetoplastid protozoan parasites. Nifurnimox–eflornithine combination treatment was made available to patients in 2009 for the treatment of sleeping sickness to replace the often fatal melarsoprol regimen [204]. SCYX-7158, a novel boron containing compound, and fexinidazole, a nitroimidazole compound, are in clinical development as potentially safer alternatives for sleeping sickness [205,206]. For visceral leishmaniasis, a single dose ambisome and a three drug (ambisome, miltefosine and paromomycin) combination treatment were launched recently [207]. New antifungal azole derivative E-1224 and posaconazole inhibitors of parasite ergosterol biosynthesis are in clinical development for treatment of Chagas disease.

## Recent drug discovery strategies to improve success

### Natural products in drug discovery

Natural products are valuable as lead candidates in drug discovery research [208]. Well-established structural features of these chemical compounds, such as chirality and rigidity, are known to enhance specificity and efficacy. Because natural products are replete with these structural features, they are favoured as a starting point for lead discovery over entities obtained from combinatorial chemistry libraries. Approved NMEs are often derived from natural products with examples in multiple therapeutic areas such as infectious disease, oncology, hypertension and inflammation.

Despite the aforementioned advantages, natural products have not always been favoured as lead candidates for several reasons. Natural products are poorly suited for both HTS, the current standard method for lead identification, and combinatorial chemistry, a common medicinal chemistry synthetic technique used for generating large numbers of derivative molecules [209]. Further, drug discovery in microbial disease, traditionally an active area of natural product research, is itself in decline. Fortunately, there has been renewed interest in the use of natural products to treat illnesses because of the development of new, more powerful synthetic methodologies and improved structure and target elucidation techniques. Pharmaceutical companies are harnessing the potential of natural products by including research efforts based on traditional medicines of specific regions of the world [210]. In fact, a total of 19 natural product-based drugs were approved between 2005 and 2010.

The success of natural products as leads for approved drugs has been achieved in the therapeutic areas of oncology, antibacterials and pain management. A recent review on natural products in drug discovery highlights some of these drugs in more detail [211]. For example, everolimus, an mTOR inhibitor derived from sirolimus, a natural product immunosuppressant perhaps better known as rapamycin, has been approved as both an immunosuppressant and for use in treatment of advanced renal cell carcinoma. Telavancin, a synthetic derivative of the natural product vancomycin, has been developed to inhibit bacterial growth of Gram-positive methicillin-resistant *Staphylococcus aureus* (MRSA) and pneumonia. Additionally, romidepsin, a histone deacetylase inhibitor, was approved for treatment of T-cell lymphoma, and capsaicin, a vanilloid receptor inhibitor, was approved for treatment neuropathic pain and, more recently, for cystic fibrosis.

## Drug repositioning

Drug repositioning has grown into a mainstream strategy in academia, government, biotechnology and pharmaceutical companies as a cost-effective and lower risk approach to developing drugs on a faster timeline [212–214]. The process of drug repositioning or repurposing is the use of existing compounds or drugs for new indications. These efforts include repurposing: (i) clinical candidates removed from development for reasons other than safety; (ii) projects discontinued for commercial reasons; (iii) currently marketed drugs, particularly those facing patent expiration; and, (iv) drugs formerly marketed. Drug repurposing decreases the cost of bringing a drug to market by relying on a compound with known safety and pharmacokinetic profiles and established routes of manufacture and formulation. Repurposed drugs accounted for 30% of the 51 new medicines that reached their first markets in 2009, and approximately two-thirds of new drug applications currently involve repositioning strategies [215].

While drug repositioning is not new, its strategic use has been bolstered by several recent notable

success stories in the pharmaceutical industry. Cymbalta, a serotonin-norepinephrine reuptake inhibitor for major depressive disorder initially approved in 2004, was recently approved for treatment of fibromyalgia (2008) and chronic musculoskeletal pain (2010) [216,217]; significantly extending the profitability of the product line [218]. Other recent examples of drug repurposing include plerixafor, identified as an inhibitor of HIV infection but subsequently launched in 2009 for mobilisation of haematopoietic stem cells in the treatment of multiple myeloma, and milnacipran, a serotonin-norepinephrine reuptake inhibitor, initially developed as an antidepressant and approved by the FDA for the treatment of fibromyalgia in 2009 [219]. In 2010, doxepin, originally launched for anxiety and depression, was approved for insomnia [220, 221]. Aztreonam, a monocyclic beta-lactam antibiotic, was initially approved for the treatment of Gram-negative bacterial infections via intravenous injection, and was repositioned as Cayston, administered by inhalation, for the treatment of pulmonary *Pseudomonas aeruginosa* in patients with cystic fibrosis [222].

Research into new approaches for the identification of appropriate candidates for repurposing has recently exploded, and a large number of academic, public private partnerships, government, small biotech and pharmaceutical firms are engaged in these efforts [223–226]. Increasingly, repositioning strategies are also including consideration of intellectual property and regulatory matters in order to maintain exclusivity [227].

## The new role of academia in pharmaceuticals

Traditionally, academia has published results from basic research and the pharmaceutical industry has developed these findings into bona fide drug discovery projects. The academic drug discovery sector is emerging, fuelled in part by the restructuring of early discovery efforts in the pharmaceutical industry and the interest in innovative approaches to drug discovery and disease treatment [6,228,229]. Grants are still the main funding path for academic groups, where there has been a need for a practical justification for the planned programme, but the support for actual drug discovery efforts has been limited. In the USA, the Bayh–Dole Act gave universities and non-profit institutions the ability to own the intellectual property created from their federally funded research. This ability enabled technology transfers to fledgling companies who began to commercialise the work. What started as a boon to biologics, spurred small molecule drug discovery when the National Institutes of Health began their Molecular Libraries Program with a mission to provide mechanisms to develop new tools, which would encourage drug discovery. This programme would offer HTS and limited medicinal chemistry through a network of centres with the data generated available to both private and public sectors through the PubChem database. The original programme received mixed reviews [230], but it has since evolved and realised success; indeed, a project at Scripps Research Institute delivered the first phase I drug candidate for the programme. With access to the assays, medicinal chemistry and chemoinformatics of the Molecular Libraries Program, academic drug discovery flourished. Researchers at the University of North Carolina at Chapel Hill have found that the amount of academic drug discovery has doubled over the last 6 years [231]. They identified 78 academic drug discovery centres across the USA; 32 had a founding date between 2003 and 2008. This infrastructure allowed researchers to concentrate on understanding basic science behind diseases [232] with a strong emphasis on orphan and neglected diseases, non-commercial projects potentially more attractive to the private sector. Furthermore, with the recent restructuring of the pharmaceutical industry a large pool of experienced researchers is poised to join academic scientists as part of the next version of drug discovery.

Funding for the Molecular Libraries Program is ending in 2014, and the screening programmes it supports must find other ways to survive. Federal funding outside of grants, especially from private sources, can cover funding gaps. Public–private collaborations remain the most promising approach to bridge the funding gap. Companies are attracted to academic research efforts particularly because newer compounds are of higher quality than before because of the screening and medicinal chemistry resources at the universities. These compounds validate biological hypotheses and lead to pharmacological proof of concept, thus generating interest within pharmaceutical companies. Industry has been proactive in the effort as well. The Lilly Open Innovation in Drug Discovery programme was started specifically to make use of innovative work in academia to meet the challenges in drug discovery [232]. They have complementary sets of target-based and

phenotypic screening assays in strategic therapeutic areas available in exchange for negotiated access to interesting molecules. Other pharmaceutical companies have similar initiatives in place: GSK has created an Academic Discovery Performance Unit; Pfizer has Centers for Therapeutic Innovation; and Merck announced the founding of the California Institute for Biomedical Research. Whether all these institutions will be a success remains to be seen. The state of pharmaceutical pipelines and the drive for new ways to discover drugs has been an important catalyst for the increased interaction between academia and industry.

## Conclusions

Drug discovery, despite the inherent failures and complexities, remains a fascinating and rewarding pursuit. Researchers have evolved the traditional process to include new strategies to improve success rates and reduce the time and cost of bringing new drugs to the market. Combinatorial chemistry, fragment-based design and computational chemistry are just a few of the more recent additions to the drug hunters arsenal. Many unmet medical needs still exist as evidenced by the continued interest in CNS, cardiovascular, infectious diseases and metabolic dysfunction drug discovery. In addition, there has been a concerted effort by both pharmaceutical and biotech companies alike in addressing devastating orphan and neglected diseases. New entities are also emerging as alternatives to standard small molecule therapies. Oligonucleotides, antisense RNA and gene therapy have seen great advances, but still suffer from issues arising from poor pharmacokinetics and intravenous-only administration. Finally, along with new strategies in basic research, the industry has seen a new acceptance and reliance on academic partnerships as a means to improve target identification and validation. Along with this outsourcing of intellectual innovation, the industry has also witnessed a massive shift of resources to offshore chemistry houses in order to drive down costs associated with hit and lead finding endeavours. Initially focused on chemistry, outsourcing of biology and pharmacokinetics has also seen widespread growth. It is still too early to determine if the dismantling of traditional research methods in favour of offshore resources will result in increased drug approvals. The results of this experiment will warrant further discussion in future editions of this book.

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