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New Trends in Drug Discovery

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1.1 Introduction

Productivity in drug discovery has been a prominent topic over the past years. The decline of number of new drug approvals and the parallel increase in research and development (R&D) costs have been a matter of concern [1]. It has raised questions on the overall strategy, the effectiveness of R&D, and the sustainability of the business model of pharma. The consequences of the productivity gap have been discussed extensively in many publications, and a plethora of proposals on how to overcome the issue have been made [2–5].

In 2014 and 2015, the number of new drug applications rose substantially, leading to a new 66-year high of Food and Drug Administration (FDA) drug approvals in 2015 [6].

1.1.1 Analysis of New Molecular Entities Approved in 2015

In 2015, 51 new molecular entities (NMEs) were approved by the FDA (see www.fda.gov/novel drug approvals CDER & CBER [7]), a number that has only been achieved in 1950. From these 51 approvals, 31 (61%) have been on new chemical entities (NCEs), while 20 were for new biological entities (NBEs). Over the past years, a considerable increase in the NBE share took place, rising to now 39% in 2015. Out of the 20 NBEs, 12 were “classical” antibodies and therapeutic proteins, and other approvals were on hematological supplement therapies, one on a vaccine and one on an oncolytic virus.

Linking the approvals to indications, it becomes apparent that the last decade’s research focus on oncological projects translated into 16 new cancer drug approvals (31% of all approvals). Rare diseases were the target of many approved drugs, with some examples given below, followed by hematological diseases and infectious diseases with five approvals each (10% each), cardiovascular and mental disorders with four approvals each (8% each), metabolic diseases (3 NMEs, 6%), and respiratory diseases (2 NMEs, 4%).

From these 51 new drug approvals, a remarkable number of 27 NMEs (53%) went through an accelerated FDA (CDER) approval process. These accelerated

approvals are indicators for an estimated therapeutic advance and are categorized into “fast track,” “therapeutic breakthrough,” and “accelerated approval.” 10 NMEs (20%) obtained the “therapeutic breakthrough” designation: five NCEs and five NBEs, as summarized in Table 1.1.

Thirty-five percent (18 NMEs) were “first-in-class” drugs: sugammadex (Bridion[®]) to reverse postsurgical neuromuscular blockade caused by certain kinds of anesthesia, palbociclib (Ibrance[®]) to treat advanced metastatic breast cancer, and idarucizumab (Praxbind[®]) to reverse adverse anticoagulant effects caused by the blood-thinner drug, dabigatran. Twenty-five NMEs were targeting rare diseases: Sebelipase α (Kanuma[®]) to treat lysosomal acid lipase deficiency, a rare disease that can lead to liver disease, cardiovascular disease, and life-threatening organ damage; asfotase α (Strensiq[®]), a long-term replacement therapy in patients with hypophosphatasia, a serious and sometimes fatal bone disease; dinutuximab (Unituxin[®]), a ganglioside GD2 inhibitor to treat pediatric patients with neuroblastoma; and uridine triacetate (Xuriden[®]), a new therapy to treat patients with hereditary orotic aciduria, which can lead to blood abnormalities, urinary tract obstruction, and developmental delays.

Noteworthy cancer treatments include daratumumab (Darzalex[®]), elotuzumab (Empliciti[®]), panobinostat (Farydak[®]), and ixazomib (Ninlaro[®]) (to treat patients with multiple myeloma), alectinib (Alecensa[®]) and osimertinib

Table 1.1 FDA 2015 approvals with therapeutic breakthrough designation.

Proprietary name	Established name	Applicant	Use
Alecensa	Alectinib	Hoffmann-La Roche Inc.	Non-small cell lung cancer (NSCLC) subpopulation
Kanuma	Sebelipase alfa	Synageva BioPharma Corp.	Lysosomal acid lipase deficiency
Empliciti	Elotuzumab	Bristol-Myers Squibb Company	Multiple myeloma subpopulation
Darzalex	Daratumumab	Janssen Biotech Inc.	Multiple myeloma subpopulation
Tagrisso	Osimertinib	AstraZeneca Pharmaceuticals LP	NSCLC subpopulation
Strensiq	Asfotase alfa	Alexion Pharmaceuticals Inc.	Hypophosphatasia
Praxbind	Idarucizumab	Boehringer Ingelheim Pharmaceuticals Inc.	Reversal of dabigatran-induced anticoagulation
Xuriden	Uridine triacetate	Wellstat Therapeutics Corp.	Hereditary orotic aciduria
Orkambi	Lumacaftor/Ivacaftor	Vertex Pharmaceuticals Inc.	Cystic fibrosis
Ibrance	Palbociclib	Pfizer Inc.	Breast cancer subpopulation

(Tagrisso[®]) (to treat certain patients with non-small cell lung cancer), cobimetinib (Cotellic[®]) (to treat certain patients with metastatic melanoma (skin cancer)), tipiracil (Lonsurf[®]) (to treat patients with metastatic colorectal cancer), and trabectedin (Yondelis[®]) (to treat soft tissue carcinoma). It is a reflection of the intense research and the success in the field of oncology that 5 out of the 10 FDA NMEs with “therapeutic breakthrough” designation are new cancer drugs (see Figure 1.1).

To summarize reflections on the year 2015 FDA approvals,

- 1) A new high of 51 approvals reflects the reemerging success in drug discovery.
- 2) NBEs have a share of nearly 40% of all approvals. These are mainly therapeutic antibodies and proteins, but vaccines and new modalities like oncolytic virus have also been approved. The share of NBEs increased considerably over the past years.
- 3) The majority of the approvals target oncology and rare diseases. It is interesting to note that the commercial viability of NMEs for rare diseases has been questioned in the past. With the new approvals, there will be a good opportunity to track reimbursement policies by healthcare systems.
- 4) Fourteen approvals were given to start-up companies and 37 to larger pharma companies (please note that the definition of “start-up” and “larger pharma” company is variable).

Despite the large number of new drug approvals in the past years, we need to acknowledge that the currently available therapeutic armamentarium is still insufficient in many aspects. In general, many diseases are still without satisfying therapy, and many widespread diseases lack therapies leading to significant improvements with respect to outcome. This leads to the conclusion that drug discovery needs to strongly align with medicine to clearly define target product profiles (TPPs) to precisely direct the search for new approaches targeting the

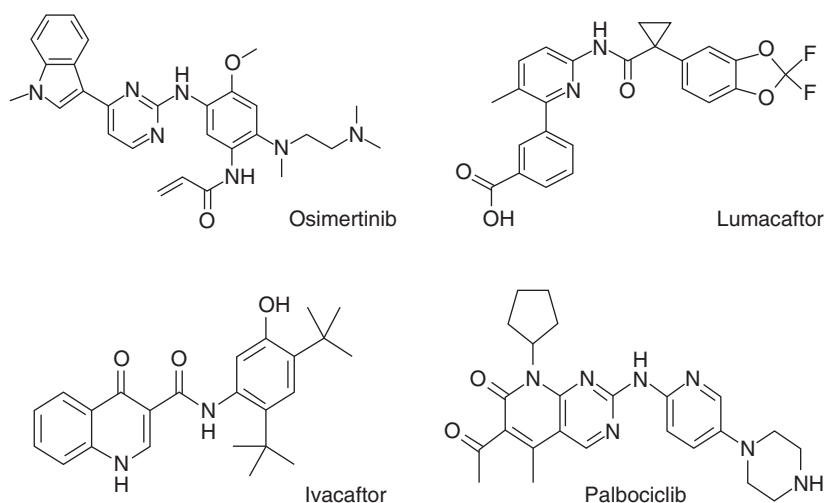


Figure 1.1 Structures of osimertinib, lumacaftor, ivacaftor and palbociclib.

therapeutic gaps. For illustration, a few examples of therapeutic gaps are given here. Many rare diseases are still without therapy though the consequences are fatal in many cases. Despite the large number of new cancer drug approvals, there are still cancer types and outcome aspects open for new drugs. The need for an effective pancreatic cancer therapy is still high, and with cancer types like non-small cell lung cancer to which new therapies have recently been introduced, we urgently need complementation by therapies targeting specific subtypes and mutations and targeting long-term survival. Also other broad diseases like cardiovascular diseases require new drugs to improve outcome and survival rates. In diabetes we need to better target diabetic complications like diabetic retinopathy and diabetic nephropathy. Moreover new diabetes drugs slowing or even stopping disease progression would address long-term outcome. In CNS drug discovery, we are still facing a significant therapeutic gap with respect to psychiatric diseases. Existing therapies either have a high rate of side effects or are only effective in part of the patient population. The newly introduced taxonomy of psychiatric diseases specifying symptom complexes may offer ways to more specifically target CNS diseases. Neurodegenerative diseases like Alzheimer's disease or Parkinson's disease lack disease-modifying therapies. Another therapeutic gap comes from the increased number of multidrug-resistant infections. Here we need more effective and MDR-overcoming anti-infectives to save lives.

In all these cases, we will have to identify new targets with a strong link to human disease. To establish this link to human disease will need considerable pre-investments into target characterization before proceeding to drug discovery. Many of these new targets will likely belong to preceded target classes like G-protein-coupled receptors (GPCR) with diverse functional impact like agonists (partial, inverse, full), antagonists, nuclear hormone receptors, enzymes with inhibitors or function/expression stimulation, kinases as enzyme subclass with ATP-competitive and noncompetitive inhibitors, ion channels with blockers or allosteric modulators (positive or negative), and newer target classes where science is currently collecting experience in drug discovery like protein-protein interactions and epigenetic targets.

At the beginning of drug discovery, a concept has to be defined. Depending on the type of target, the nature, and the characteristics of the binding site on the target, the compartment where the target is located and options for interaction need to be defined, be it for a small molecule, a therapeutic protein, or an antibody. In addition the intended functionality of the drug may suggest that additional features like antibody-dependent cell-mediated cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC) may synergistically enhance efficacy. Also new therapeutic modalities like cell-based or gene therapy add new opportunities to achieve the desired efficacy. In some cases, more than one therapeutic modality (NCE, antibody, therapeutic protein, cell-based/gene therapy) may be good options to follow. In these cases, it will be a decision on risk mitigation whether to follow both options in parallel or sequentially. The drug concept should take both the intended modulation of the target and the type of molecular entity to achieve efficacy into consideration.

In the following sections, some trends in NCE and NBE discovery will be highlighted, which should reflect the growing experience in both fields. Lessons

learned will guide drug discovery, provide new opportunities to scientists and the background to address critical issues early in a program's life span, and, therefore, lead to the selection of higher quality development candidates.

1.2 New Trends in NCE Discovery

NCEs have unique properties with respect to reaching almost all kinds of compartments in an organism. In this sense, membrane penetration is bound to specific molecular properties like molecular weight, overall polarity, and others [8], which can preferentially be obtained by small molecules. For this reason NCE discovery will continue to be the main choice for interacting with intracellular and CNS targets. Orally available NCEs will also continue to be advantageous in terms of convenience for patients and in general will be less costly and thus help to control healthcare costs.

These unique NCE properties differentiate small molecules from NBEs like antibodies, therapeutic proteins, and vaccines. NBEs can penetrate membranes only at a very low percentage, normally below the 1% range, and thus application of NBEs is restricted to extracellular targets and requires intravenous or subcutaneous administration. Recently new approaches in NBE discovery [9] aim to achieve membrane penetration by linking therapeutic proteins to active and passive transport systems. Some progress has been made [10], but there are no advanced clinical studies with such modified NBEs reported so far.

It is also interesting and important to compare the molecular space between classical NCEs and NBEs. One recent therapeutic approach has targeted endogenous peptide substitution, for example, GLP-1 analogues for the treatment of diabetes [6, 11, 12]. Analogues have been developed with improved efficacy and greatly improved half-lives for up to once weekly subcutaneous applications. These peptide analogues bear, for example, side chain modifications to tune half-life and utilize non-natural amino acids and therefore need to be synthesized by sophisticated peptide synthesis methods. From their properties, they lie between small molecules and proteins, their synthetic accessibility classifying them as NCEs.

The high proportion of NCEs in newly approved drugs in 2015 (61%) is clear evidence of a regain of efficiency and success rates in NCE discovery. Many factors are contributing to this trend. In this chapter we would like to focus on mainly two aspects that contribute substantially to increased success rates: (i) enhanced hit/lead generation strategies and (ii) enhanced characterization of development candidates reducing compound-related attrition rates.

1.3 Enhanced Lead Generation Strategies

At the beginning of every NCE drug discovery effort stands the question of how to identify a pharmacophoric model of interaction with a therapeutic target. The history of NCE discovery was dominated by the *analogue approach* [13, 14],

starting from a known and clinically investigated compound or from the active ingredient of a marketed product and exploring the structural space to yield specific interaction with a homologous target. The history of receptor agonists and antagonists and kinase inhibitors provides multiple examples of the success of this approach. A different approach is utilizing known drugs as starting points for nonhomologous target drug discovery in an agnostic and serendipity-driven approach termed repositioning. Alternatively, this approach can also be driven by new insight into additional modes of action of the known drug. The identification of new applications for known compounds has been greatly supported and optimized by the utilization of *high throughput screening* (HTS) [15, 16]. In this approach, compounds from previous drug discovery programs or, in general, compound collections of drug-like molecules are screened against a multitude of interesting targets with the aim to identify chemical starting points for further optimization. Utilizing robotic equipment, an automated process from sample handling up to testing and data collection has been established. Sophisticated software systems secure automated data evaluation. The throughput of efficient HTS systems can achieve the testing of hundreds of thousands of compounds in a few days. Both the “classical” analogue approach and the HTS approach have been optimized in several ways to improve efficiency and quality of hit/lead generation (see below).

These approaches have been complemented by several approaches that have proven greatly successful over the past years. Advances in molecular biology have provided access to protein drug targets like enzymes (proteases and kinases mainly) and GPCR, making them available for structure biology research. X-ray techniques have yielded substantial insight into the three-dimensional (3D) structure of enzymes and receptors as well as investigating ligand/protein complexes, binding modes, binding sites [17], and, with newer biophysical methods, even the kinetics of ligand/protein interactions [18]. These achievements have set the basis for an additional hit/lead discovery option based on *structure-based de novo ligand design*.

Experimental HTS has been complemented by a *virtual screening* (VS) approach, where virtual compound collections are screened *in silico* for target interaction. This approach can start either from known ligands in a ligand-based virtual screening (LBVS) method [19, 20] or from the 3D structure of a target protein by docking of virtual compounds into a target binding site, a process known as structure-based VS [21]. Recently the exploration of binding pockets by molecular dynamics simulations taking protein flexibility into consideration has complemented the generation of pharmacophore models for VS [22]. Another experimental approach was introduced in 1996 by Abbott scientists called *fragment-based drug discovery* [23]. The process starts by screening a library of fragments (typically low molar mass molecules with MW < 350) for low-affinity binding fragments that then, based on fragment/target interaction insight, can be further developed into leads by fragment growing, merging, and linking.

Phenotypic screening aims to detect desired functional effects in a cellular system in a target-agnostic approach. Leads are characterized by specifically yielding the desired phenotypic effect, a result that will then lead to investigation of the underlying target(s), the interaction(s), and the specific mode(s) of action.

Repositioning is defined as new application of approved drugs or development compounds for either new indications following the same mode of action or the discovery of new modes of action leading to application in new indications. The discovery of these new applications can either follow a rational approach or be driven by serendipity.

All these techniques and approaches have led to a greatly enhanced armamentarium to identify hits and leads that have enabled scientists in drug discovery to identify molecular entry structures for unprecedented target space. Advances within the approaches and examples of successful hit/lead generation will be given below in more detail and will provide evidence for the substantial impact of these techniques and approaches on recently increased NCE discovery success rates.

1.3.1 Analogue Approach

One example of how an approved drug can lead to a completely new application is given by the example of thalidomide. This drug was withdrawn as sedative after having shown teratogenic effects in pregnant women. Thalidomide was also found to exhibit immune modulatory and anti-angiogenic effects. In a phenotypic optimization of analogues, lenalidomide was identified as a differentiated thalidomide analogue that lacked sedative and teratogenic effects. The target of lenalidomide was found to be an ubiquitin ligase E3. Lenalidomide was eventually introduced as new treatment for multiple myeloma (see Figure 1.2) [24].

As a second example, the discovery of afatinib as a new EGFR kinase inhibitor for the treatment of EGFR-mutated non-small cell lung cancer is given. Starting from known anilinoquinazoline structures as ATP-competitive EGFR/Her2 kinase inhibitors, the side chain was modified by introducing a Michael acceptor function binding to Cys797 of EGFR and Cys805 of Her2 kinase. This resulted in irreversible binding to both kinases and led to an improved clinical efficacy profile in comparison with the known competitive inhibitors (see Figure 1.3) [25].

1.3.2 High Throughput Screening (HTS)

The power of HTS to discover new hits and leads has been demonstrated by numerous examples [26–29]. In the past, *natural products* have been a rich source for new drugs [30, 31]. However, the complexity of natural product

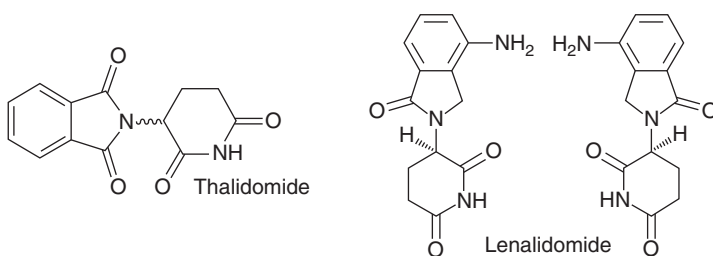


Figure 1.2 Structures of thalidomide and lenalidomide.

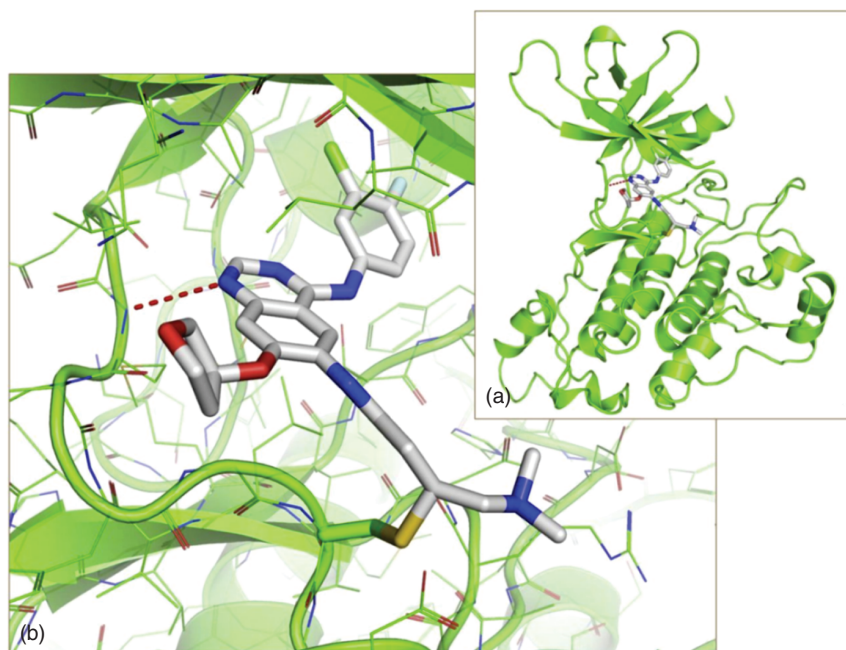


Figure 1.3 X-Ray structure of EGFR kinase (a) and afatinib in binding site of EGFR kinase (b).

screening is substantial. Two different aspects contribute to this complexity. Natural products are often screened as compound mixtures coming from extraction processes from plants/organisms or from fermentation broths. The extracts are often pre-fractionated but still are composed of complex mixtures of components. In case of being found active in an assay, these mixtures need further purification (a process known as “deconvolution”) to identify discrete active compounds, followed by structure elucidation. This process is tedious and needs larger amounts of samples, often requiring reacquisition of the original samples. Another complexity arises from the observation that many biological assays can be nonspecifically influenced by components of the complex compound mixture, leading to false positive data and thus compromising a deconvolution of the active ingredient. The complexity of tracing the active component of a natural product mixture slows down the identification of natural product hits in comparison with discrete synthetic compound screening and has led to a down-prioritization of natural product-based drug discovery in many companies.

In HTS of libraries of single discrete compounds, a large number of past examples have identified important factors that contribute to the quality of hits and their suitability for lead generation and optimization. These factors are divided into chemistry- and biology-related factors [32].

Building on past successful and unsuccessful experiences with turning hits into leads and development compounds, a variety of physicochemical and *in silico* parameters have been used to enhance the chances that hits will be “druggable.” Success factors that have been employed successfully include structural diversity,

defined, for example, by similarity scoring, physicochemical parameters like log P , and total polar surface area (TPSA) and structure-related parameters like molecular weight, fractional SP3, and quantitative estimate of drug likeness (QED) [18]. A consequent filtering of compound collections against these parameters leads to a significant improvement of hit quality.

Biology-related factors have been deduced from a comprehensive analysis of biological screening data. It became obvious that the so-called pan-assay interference compounds (PAINs, compounds that seem to show activity in a large number of assays they are tested in) [33] could be identified and should be excluded from regular screening. A careful analysis of structural elements of PAINs led to the identification of commonalities. Structural elements leading to covalent binding, redox reactions, and chelation are abundant in PAINs and should be avoided in screening collections. It also became obvious that the proper choice of the assay format and the binding detection method is of high impact on the quality of the screening results. The choices range from binding/inhibition of a defined and isolated target to whole cell screening of a defined cellular parameter to phenotypic screening in a cellular or even more complex physiological setting on a discrete pharmacologic effect. The proper selection of the assay depends on the purpose of the drug discovery project. A second aspect, the choice of the best detection method for activity within the selected assay, also contributes to the success of the HTS. Over the past years, a wide variety of detection systems [18] has been successfully introduced, and most of these techniques are readily amenable to automated robotic systems, online data collection, and evaluation.

Examples of approved drugs originating from screening include (a) cyclosporin A from natural product screening (immunosuppressant, Morbus Crohn, host versus graft); (b) nevirapine, a non-nucleoside reverse transcriptase inhibitor (HIV); (c) bosentan, an endothelin antagonist (pulmonary hypertension); and (d) fingolimod, a sphingosine-1-phosphate receptor-1 modulator (immunosuppressant, multiple sclerosis) (see Figure 1.4).

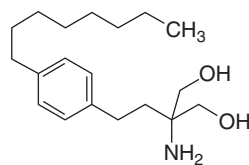


Figure 1.4 Structure of fingolimod.

1.3.3 Structure-Based Design

Advances in molecular biology have provided access to protein targets in quantities that allow structural elucidation and biophysical investigations. These analyses have yielded a large number of 3D structures of proteins. X-ray crystallography has been instrumental and a preferred approach for structure elucidation of single proteins, protein complexes, and ligand/protein complexes. These investigations have led to a deeper insight into ligand–target interactions and the conformational changes connected to binding in many cases and, thus, have yielded the basis for the rational design of ligands for new targets [17]. In many cases, X-ray crystallography provided not only the basis for design but also constant guidance during the optimization of initially designed hits. In this process, refined insight into binding modes as well as conformational changes in the target protein and the ligands have finally led to highly specific and potent

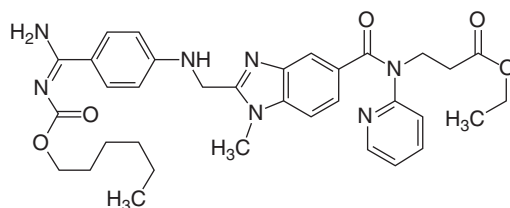


Figure 1.5 Structure of dabigatran.

compounds that have been investigated as clinical candidates. Some prominent examples are saquinavir (HIV protease inhibitor), oseltamivir (neuraminidase inhibitor, influenza A/B), dabigatran (factor IIa inhibitor, anticoagulant, secondary prevention of stroke) (Figure 1.5), boceprevir, and telaprevir (NS3 protease inhibitors, HCV).

1.3.4 Virtual Screening

VS utilizes different computational approaches for the selection of compounds from a database having the likelihood to bind to a target of interest. VS can start from either the structural knowledge of a target (structure-based virtual screening (SBVS) [21, 22]) or the knowledge of ligand structures active against the target of interest (LBVS [19, 20]).

LBVS does not require any information on the 3D structure of the target of interest. Starting from active structures machine learning tools like neural networks, Bayesian classifiers, decision trees, and others can predict novel structures with the likelihood to bind to the target [19]. Beyond these tools, cheminformatic-based VS, which builds on similarity searches, has been successfully applied. Molecular fingerprints have been broadly explored for similarity searches [21]. Whereas fingerprints define the absence or presence of specific structure elements, other approaches use physicochemical abstractions like 3D shape or electrostatic potential of substructures to identify similar compounds [34–36]. Success rates of VS can be increased by utilizing the various different approaches to define similarity subsequently or in parallel [37].

SBVS requires the knowledge of the 3D structure of the protein/target of interest and involves docking virtual structures into the putative binding site. A scoring process ranks compounds with respect to likelihood of binding [38]. Docking can either be based on a single conformation of the target protein, for example, from an X-ray structure, or can take conformational flexibility of the target protein both in backbone as well as side chain conformations into account, the so-called “ensemble” docking approach [39, 40]. Programs like FlexX [41], Gold, and Glide [42] also take water molecules and their replacement by ligand binding into consideration. Scoring is based on free binding energy calculations of the ligand with the target protein. Even if basic assumptions on the binding mode are predefined, these calculations are computationally very demanding.

For both LBVS and SBVS, it is obvious that the specific knowledge of active compounds, the target protein structure, and/or the binding site is a prerequisite for successful application. This limits the application of VS for new and unprecedented target classes.

In addition to confirming virtual results by assessing the activity of hits, in both LBVS and SBVS, an experimental follow-up by hit expansion is recommended. This process expands the knowledge on ligands and binding assumptions by showing consistency in structure–activity relationships for positive hits. It allows the researcher to refine the assumptions on binding modes and conformations, providing entry points for better defined VS campaigns in an iterative process.

One example of an approved drug derived from a VS hit is tirofiban, a GP IIa/IIIa antagonist for the prevention of myocardial infarction (see Figure 1.6).

1.3.5 Fragment-Based Lead Discovery

Fragment-based lead discovery (FBLD) is based on the screening of fragments of drug-like molecules with molecular weights below 300 Da. Fragments should bind to a functionally relevant pocket of the target protein. Binding fragments normally have low binding affinities that must be increased by an “analogueing” approach, which is guided by the knowledge of the binding mode of the fragment. The structure is rationally expanded into accessory binding site pockets. This fragment expansion is equivalent to a structure-based drug design approach. The advantage of FBLD comes from the low molar mass of the fragments. Structure expansion can be performed to give better binding molecules that still display the structural and physicochemical parameters of drug-like molecules [43, 44].

The most used binding detection methods for fragments are NMR spectroscopy, surface plasmon resonance (SPR), differential scanning fluorimetry (DSF), and microscale thermophoresis (MST). As the sensitivity and the throughput of these methods are very different and target dependent, the selection of the best detection method must be defined individually for each target. As a follow-up step, X-ray analysis should confirm binding and also generate knowledge on the specific binding mode of the fragment, thus allowing a rational hit expansion.

A large number of success stories of FBLD have been published [44], one of the more advanced examples being vemurafenib [45], a compound that recently reached the market for the treatment of malignant melanoma (see Figure 1.7).

Figure 1.6 Structure of tirofiban.

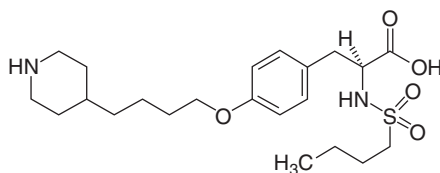


Figure 1.7 Structure of vemurafenib.

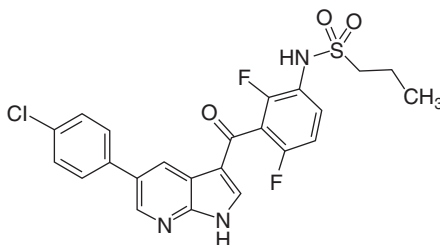


Table 1.2 Biophysical methods for analysis of protein–ligand interactions.

Method	Information gained	Strengths	Limitations
X-ray	Binding site target–ligand interaction	Visualization of structures	High quality crystals, no quantitative affinity info
NMR	Binding site target–ligand interaction	Determination of binding epitope, determination of K_D	Large amounts of protein–ligand isotopic labeling of protein
SPR	Time-resolved protein–ligand interaction under a variety of conditions	High sensitivity, high throughput, fragment-binding detection	Requires immobilization of protein keeping functionality
DSF	Conformational stability of a protein on ligand binding, T_m gain reflects stabilization and potentially K_D	Stable assay, low amounts of protein	May be compromised by fluorescent probe artifacts by quenching
MST	Detection of ligand-induced thermophoretic mobility changes K_D determination	Solution measurements, for example, soluble membranes, proteins	Fluorescent labeling required or intrinsic protein fluorescence

Fragment-based screening exemplifies how biophysical methods developed over the last years have stimulated and expanded hit/lead identification and, thus, contributed to increased success rates in NCE drug discovery. Table 1.2 summarizes broadly applied biophysical methods used in drug discovery, showing strengths and limitations of each method [18].

1.3.6 Repositioning

The repositioning example of thalidomide/lenalidomide has been recently complemented by nintedanib, which originally had been generated for anti-angiogenesis in solid tumors. Targeting the angiogenic factor FGF led to the hypothesis that by FGFR inhibition fibrosis development in idiopathic lung fibrosis should be decreased. The hypothesis was confirmed by both *in vivo* animal studies and later in clinical studies and led to the launch of nintedanib as Ofev for the treatment of IPF (see Figure 1.8) [46].

Biophysical methods have also helped researchers gain better insight into important parameters like binding affinities and kinetics (K_d , K_{on} , K_{off}). These parameters provide a better understanding of how compounds exert activity and thus impact drug discovery at every phase.

In Table 1.3 prominent examples of approved drugs are given where hit/lead generation was based on the different approaches discussed above.

In hit/lead discovery a common practice is to utilize combinations of the described approaches to maximize chances of success. The knowledge and nature of the target and ligands determine which approach can be applied and combined in a synergistic way [33]. Table 1.3 lists examples of successful

Figure 1.8 Structure of nintedanib.

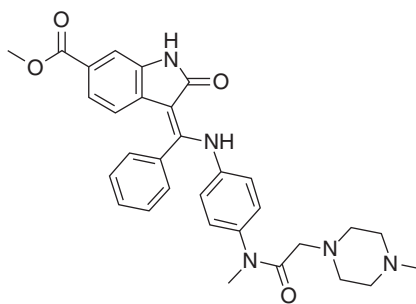


Table 1.3 Examples of successful hit/lead generation per strategy.

Hit/Lead generation strategy	Example	Therapeutic application	Remark
Analogue	Afatinib	NSCLC	First irreversible EGFR/Her2 inhibitor
HTS	Fingolimod	Multiple sclerosis	Sphingosine-1-phosphate receptor-1 modulator
<i>De novo</i> structure-based design	Boceprevir, telaprevir	HCV	NS3 protease inhibitors
Virtual screening	Aggrastat	Prevention of myocardial infarction	GP IIb/IIIa antagonist
Fragment-based lead discovery	Vemurafenib	Melanoma	B-raf inhibitor

hit/lead generation campaigns as well as the method(s) used to carry out those campaigns.

1.3.7 Additional New Trends in Hit/Lead Generation

Library sharing: Recently, initiatives have been started to share compound collections and information on compound properties among companies [47]. The aim is to increase chances to find molecular entry points into new targets by increasing structural diversity under strictly defined compound quality criteria.

Probe compounds: In many cases medicinal chemistry efforts lead to the identification of highly selective and active compounds toward new targets of unknown physiological relevance. These compounds have been recently made available to the scientific community as so-called probe compounds for testing in biochemical, cellular, or *in vivo* settings. This allows identifying an unprecedented therapeutic application for a new target. Follow-up activities can be started in a public/private partnership model. The differences between drugs and probes are summarized in Figure 1.9 [48].

The Structural Genomics Consortium (SGC) provides a good example of an entity that delivers powerful probes. Thirty chemical probes have been made available to the scientific community in an open-access mode [49].

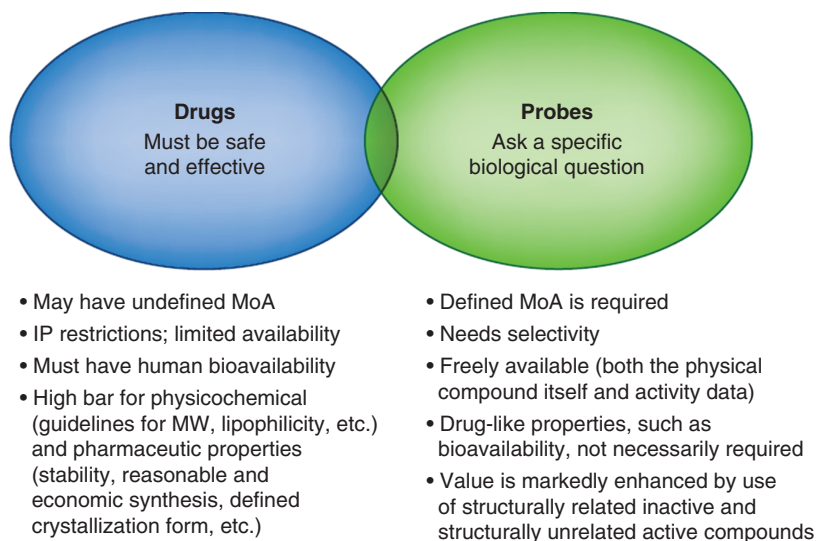


Figure 1.9 Different purposes and requirements for probes and drugs.

1.4 Early Assessment of Development Aspects during Drug Discovery

In the late 1980s, it became obvious that many small-molecule candidates failed due to the ability to develop aspects related to issues in pharmacokinetic (PK), tolerability, and physicochemical properties that compromised formulation development. It has been estimated that the number of new drugs approved by the FDA, per US\$ spent on R&D, has halved every 9 years since 1950 [1]. Possible causes of the pharmaceutical industry's productivity problems have been analyzed in depth, and contrasting suggestions for improvements have been proposed [2–5]. Recent attrition data show only 4.3% of drug discovery projects proceed successfully from the preclinical stage to a positive phase III outcome. The failure rate appears greatest at phase II, where lack of efficacy is cited [50] as the single major cause of attrition. The second most prevalent compound/candidate-associated root cause for attrition is related to tolerability issues, either detected by preclinical safety studies in animals or detected in early clinical phase I. Deficiencies in pharmacokinetic properties comprise the third most common cause for clinical candidate attrition. Therefore, major efforts were initiated in drug discovery to detect these liabilities early in lead optimization. First, approaches to increase the quality of molecular starting points have been incorporated into the lead generation process (see Section 1.3) by applying stringent quality criteria to libraries and compounds selected for follow-up activities. In the following section, a short overview on improvements in DMPK characterization, tolerability assessment, and physicochemical characterization of compounds during lead optimization with the goal of generating high quality candidates (in terms of efficacy, specificity, DMPK, tolerability, and

physicochemical properties) for progression into preclinical and clinical phases will be given.

1.4.1 DMPK

DMPK investigations can be categorized into *in vitro* models, investigations in subcellular fractions, whole cell systems, *in situ/ex vivo* models, and *in vivo* investigations [51]. A sequential filtering approach will test many compounds *in vitro*, triaging selected ones for in-depth *in vivo* characterization. A number of *in vivo* DMPK properties can be simulated *in vitro*.

In vitro: Cytochrome isoenzyme inhibition is measured in high throughput fashion to select compounds being devoid of drug–drug interaction (DDI) potential.

Subcellular fraction (liver, gut): S9 fraction (cytosol and microsomes) contains a nearly complete selection of metabolic enzymes and transporters. Investigation of compounds in these systems will yield predictive data on intrinsic clearance and the potential for DDI.

Whole cell systems: Hepatocyte investigations will give more comprehensive information on metabolic stability and transporter-mediated uptake. As hepatocytes can be obtained from many species, including man, further information on species-specific metabolization patterns can be derived. In addition acute cytotoxic effects can be observed.

Significant progress has been made in the use of hepatocytes [52]. Insight into the impact of culture conditions on hepatocyte function has been gained, for example, on the downregulation of transporter expression. The use of 3D cultures with extracellular matrix or self-assembled scaffold-free hepatospheres results in better polarized cell structure and, therefore, a better reflection of real *in vivo* liver function. 3D cultures or precision cut liver slices (PCLS) have been utilized for comprehensive metabolization and transporter studies. PCLS have also been shown to give hints on drug-induced liver injury (DILI) and reflect liver fibrosis development. Even early indicators for idiosyncratic DILI can be derived from PCLS. Better insight into liver carcinogenesis has been generated from investigating aryl-hydrocarbon receptor activation and PPAR α signaling pathways. Improved access to human hepatocytes has been achieved by cryopreserved human hepatocytes and induced pluripotent stem cells using embryonic, fetal, or adult stem cells as a source. These new insights have enabled the collection of a broader spectrum of DMPK parameters, given access to early indicators of liver damage and greatly improved the handling and access of human hepatocytes. These new developments will make a strong impact on the quality and degree of detail of *in vitro* DMPK and tolerability assessments in early drug discovery and the quality of candidates.

Cellular permeability: Caco-2 cell preparations inform on compound permeability and estimate the potential for oral absorption [8]. MDCK cells are versatile for exploring efflux and uptake transporters, specifically when both types are co-expressed [53, 54].

In situ/ex vivo models: An example is the liver perfusion model, which yields information on hepatic first pass effects, effects of protein binding, parent uptake

from the perfusate, metabolism, and parent and metabolite elimination. Toxicity signals from parent drug and metabolites, including chemically reactive metabolites, can be detected. *Ex vivo* investigations on liver changes after dosing of candidates can provide information on the DILI potential.

In vivo models: To enter into clinical trials, a drug candidate must be assessed in two separate species for safety and DMPK properties. The usual first species for *in vivo* models is rat. A non-rodent species will follow, preferentially dogs or mini-pigs. In the event that major differences in PK parameters between the two preclinical species are observed, a non-human primate *in vivo* investigation is recommended. A multitude of parameters can be measured in *in vivo* studies, including C_{\max} , T_{\max} , AUC, V_{ss} , CL, $T_{1/2}$, and bioavailability. This data will yield a good reflection of the PK characteristics of candidates in animals and also help select the best suited non-rodent species for subsequent toxicological investigations.

Allometric scaling of *in vitro* and *in vivo* data is used to estimate doses for clinical efficacy studies in man. Great progress has also been made by generating modeling techniques to better reflect the relationship between PK of drug concentrations versus time and the pharmacodynamic (PD) effects versus time [55, 56]. Very important in this relationship is the distribution of the drug from plasma to the target compartment. Mechanism-based pharmacokinetic–pharmacodynamic (PK–PD) models typically integrate the time course of drug concentrations (PK) including biophase distribution, the nature of drug–target interaction (pharmacology), and turnover processes reflecting the relevant physiology and disease. In many cases of clinical failures for efficacy reasons, it turned out retrospectively that the drug concentrations in the target compartment were not sufficient to exert pharmacological effects. Therefore PK/PD modeling needs to be integrated into the planning of *in vivo* efficacy studies in drug discovery.

1.4.2 Assessment of Physicochemical Parameters

Many physicochemical parameters are dependent on the salt form of a drug candidate. In a publication of Sanofi scientists in 2004 [57] and a follow-up in 2014 [58], a so-called 100 mg approach for salt form selection was published. In the first step, from a list of pharmaceutically acceptable acids and bases for a given candidate, those acids/bases are selected that have a 2 pK_a unit difference between candidate and counterion. In the second step, a preliminary salt screening is performed in microplate technique with 50–100 mg of the candidate. The formation of crystals is investigated by X-ray powder diffraction. Selected salts are further characterized by Raman spectroscopy, X-ray diffraction, and NMR for stability and stoichiometry. In the third step, an in-depth characterization of the top candidates is performed including hydrate/solvate detection by thermogravimetry, assessment of the chemical and physical stability, and investigation of solubility and polymorph formation after re-precipitation. Finally pH-dependent solubility, polymorphism, dissolution rates, and micronization feasibility are investigated.

In a publication by GSK scientists [59], the top 100 oral drugs prescribed in the period of 2011–2013 have been analyzed, and a risk categorization scheme has

been derived helping to find salt candidates with good prospects for formulation development. The property forecast index (PFI), a composite figure derived from the $\log D$ value from chromatography and the number of aromatic rings in the drug molecule, is established. In addition, Fasted-state simulated intestinal fluid (FaSSIF) solubility and absolute dose in milligrams is used to yield classifications ranging from high (PFI > 6, low FaSSIF solubility and high dose >100 mg), overly increased, and moderate risks to “desired” (PFI < 6, FaSSIF solubility above 100 mg ml⁻¹ and dose <100 mg). With the exception of oncology drugs and some anti-infective drugs, the majority of the top 100 oral drugs belong to categories “moderate risk” or “desirable.” This categorization also helps to select candidates with good prospects for a straightforward formulation development.

Beyond progress in salt form selection advances in pharmaceutical sciences with respect to nano-crystallization, lipid formulations and solid dispersions have helped to find developable formulations for drug candidates [60–63].

1.4.3 Tolerability Assessment

Initial data on potential tolerability issues can be derived from any cellular or *in vivo* investigation. Signs of cytotoxicity or tolerability issues in *in vivo* studies, be it for efficacy or DMPK, may set early alerts. In addition, hints on tolerability issues may come from general pharmacology studies. Experience with potential tolerability issues has also been collected from analysis of some target classes. The probability of tolerability issues has been especially high with, for example, kinases. Whenever hints for cytotoxicity or intolerabilities have been observed or the target belongs to a “high risk class,” it is highly advisable to plan for more detailed investigations. These investigations may be specific for the observed effect, for example, electrocardiogram (ECG) investigations in several species after detection of hERG channel liabilities or a more comprehensive investigation of general tolerability by an exploratory toxicology study, usually performed in rats. In such studies, compound candidates are normally administered in different doses covering multitudes of effective doses for two weeks. Both general observations during the in-life phase and a comprehensive histopathology can give a toxicology profile, allowing a first determination of the therapeutic window and therefore an assessment of the development risk from a tolerability perspective. Although the time for exploratory toxicology studies is in the range of 2–3 months, excluding compound supply, the chances for candidates having passed exploratory toxicology studies with a reasonable therapeutic window to progress into clinical phase I studies increase significantly.

1.5 New Biological Entities (NBEs)

In 2015, the highest number of FDA approvals of NBEs was achieved (see Table 1.4).

From the 20 approved NBEs, 9 were antibodies, 8 were recombinant proteins, one was a vaccine, one was an oncolytic virus, and one was a chelator. Five of these NBEs are introduced for the treatment of hematological disorders.

Table 1.4 NBE FDA approvals in 2015.

Proprietary name	INN	Applicant	Mechanism of action	Disease	Chemical name
Cosentyx	Secukinumab	Novartis Pharmaceuticals Corp.	Interleukin 17A antagonist	Psoriasis and other autoimmune diseases	Immunoglobulin G1
Natpara	Parathyroid hormone, recombinant	NPS Pharmaceuticals	Parathyroid hormone receptor 1 and 2 agonists	Osteoporosis	Parathormone
Unituxin	Dinutuximab	United Therapeutics Corp.	Ganglioside antigen GD2 antagonist	Cancer, neuroblastoma	Immunoglobulin G1
Praluent	Alirocumab	Sanofi-Aventis U.S. LLC	PCSK9 inhibitor	Hypercholesterolemia	Immunoglobulin G1 fusion protein
Repatha	Evolocumab	Amgen Inc.	PCSK9 inhibitor	Hypercholesterolemia	Immunoglobulin G1
Praxbind	Idarucizumab	Boehringer Ingelheim Pharmaceuticals Inc.	Reversal of dabigatran-induced anticoagulation	Hyperlipidemia	Immunoglobulin Fab
Strensiq	Asfotase alfa	Alexion Pharmaceuticals Inc.	Alkaline phosphatase stimulant	Hemorrhage, unspecified	G1-kappa, humanized monoclonal antibody
Nucala	Mepolizumab	GlaxoSmithKline LLC	Interleukin 5 antagonist	Hypophosphatasia Neurofibromatosis Asthma	Immunoglobulin G1-kappa, human monoclonal antibody
Darzalex	Daratumumab	Janssen Biotech Inc.	CD38 antagonist	Multiple myeloma	Immunoglobulin G1-kappa
Portrazza	Necitumumab	Eli Lilly and Company	EGFR antagonist	Cancer, non-small cell lung	Immunoglobulin G1-kappa
Empliciti	Elotuzumab	Bristol-Myers Squibb Company	SLAMF7 antagonist	Cancer, myeloma	Immunoglobulin G1
Kanuma	Sebelipase Alfa	Synageva BioPharma Corp.	Lysosomal acid lipase stimulant	Lysosomal acid lipase deficiency	Sebelipase alfa

Three were devoted to the treatment of hemophilia A and B by coagulation factor replacement, and one was for the treatment of Von Willebrand disease by enzyme supplementation. One antibody was introduced for reversal of coagulation in bleeding complications during dabigatran therapy, being the first example of a drug used for reversal of new oral anticoagulants.

Five NBEs were devoted to cancer therapy, two antibodies for the treatment of multiple myeloma (CD38 and SLAMF7), one EGFR antibody for lung cancer, one GD2 antibody for the treatment of neuroblastoma, and one oncolytic virus for melanoma treatment. The monoclonal CD38 antibody daratumumab has been launched as Darzalex®.

Four NBEs targeted metabolic diseases, namely, two PCSK9 antibodies, one new insulin analogue for diabetes treatment, and one enzyme replacement therapy for the treatment of hypophosphatasia.

Two NBES were approved for inflammatory indications, namely, an IL5 antibody targeting asthma and an IL17 antibody for treatment of psoriasis.

The vaccine targeted meningitides B, while the chelator targeted the reversal of neuromuscular blockade after anesthesia. Also approved was a therapeutic protein that aims for treatment of hypoparathyroidism.

In a 2006 review article, Paul Carter summarized the NBE approvals from mid-1990s to 2006 [64]. During this period 18 antibodies were approved by the FDA. This number of 18 from a period of 10 years compared with nine antibody approvals in 2015 demonstrates the increased importance of biologics in drug discovery. From the 18 antibodies approved from 1995 to 2006, the therapeutic applications included cancer, chronic inflammation, transplantation, and infectious diseases. Fourteen were unmodified IgG molecules, 2 were radio-immunoconjugates (RICs), one was an antibody–drug conjugate (ADC), and one was a Fab. In analyzing the period from 2010 to 2014 with respect to NBE approvals from CDER-FDA (see Table 1.5), a period of rather constant approval numbers from 2010 to 2013 was followed by a sharp rise in 2014, which continued in 2015, leading to a significant change in the NBE-to-NCE approval ratio.

Closer analysis shows that antibodies are the leading class of NBEs, followed by therapeutic proteins. As CBER reporting is performed differently, it is more challenging to analyze approval numbers of vaccines, blood factors, and other NBEs. But it is obvious that the third class, in terms of numbers of approved biologics, is vaccines followed by blood factors. An unprecedented addition to NBEs during this period was the approval of two cell-based therapies.

Within the class of antibodies, IgGs have a high share, with 13 approvals. Three of these possessed new formats (2 were scFv-Fc and one was (scFv)₂). Two others were ADCs. Within the class of therapeutic proteins, substitution of endogenous factors by seven enzymes (replacement therapy) dominated, with some of these enzymes being fused with PEG or Fc molecules to prolong half-life.

Within the 2010–2014 NBE FDA approvals, 15 drugs were approved for cancer treatment. Two of these were first approvals for immune oncology therapies (anti-PD1 antibodies). Six of the 2010–2014 approvals – all antibodies – were dedicated to the treatment of autoimmune diseases, and four biologics, including GLP-1 analogues, targeted metabolic disorders. Five new biologics were dedicated to the treatment of orphan diseases, mainly recombinant proteins as

Table 1.5 Examples of successful hit/lead generation per strategy.

	2010	2011	2012	2013	2014	Total
<i>CDER</i>						
# approvals NBEs	6	6	6	4	11	33
# ther. prob.	4	1	3	0	5	13
# Abs	2	5	3	4	6	20
# IgGs	2	2	2	3	5	14
# non-IgG class.	0	2	1	0	1	4
# Ab DC	0	1	0	1	0	2
<i>Indications</i>						
Cancer	0	3	4	2	6	15
Immunol.	1	2	0	2	1	6
Metab.	1	0	0	0	3	4
Orphan	4	0	0	0	1	5
Ophthalm.	0	1	1	0	0	2
Others	0	0	1	0	1	2
<i>CBER (?)</i>						
Vaccine	5	?	2	7	1	15
Call-based therapy	0	1	1	0	0	2
Blood factors	?	1	0	2	3	6
Others	?			1	3	4

replacement therapies for endogenous enzyme deficiency. Two antibodies were introduced for age-related macular degeneration treatment after intravitreal application.

Cumulative success rates were analyzed from first application in man to regulatory approval. They were well above 20% for chimeric antibodies and for humanized antibodies. Carter stated that these success rates compared favorably with that for small-molecule therapeutics at that time, being about 11% [64]. He predicted that, due to increasing experience with development of antibodies, the success rates would go up over time. Cumulative success rates from 1996 to 2014 in periods of 3–4 years have been investigated [65]. Their figures within this analysis differed slightly from Carter's but were consistent in terms of demonstrating a higher success rate for biologics than for NCEs (see Figure 1.10).

However, Carter's expectation of increasing cumulative success rates for NBEs over the coming years did not materialize. NBE approvals decreased until the period of 2008–2011 and only recovered slightly in the last period of 2012–2014. However, tremendous progress has been made with respect to new antibody formats, the optimization of antibody efficacy, a better understanding of causes for and possibilities to circumvent immunogenicity, and improved and more economic scale-up and manufacturing processes. This progress has significantly

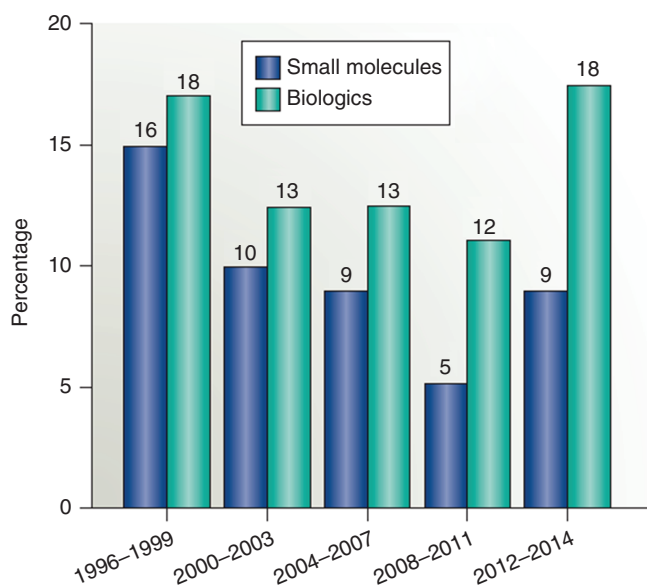


Figure 1.10 Cumulative success rate from phase I to launch.

contributed to higher success rates, increasing the share of NBEs within new drug approvals and broadening the scope of NBE applications to target diseases. The higher number of NBE approval in recent years and the increasing success rate are probably a reflection on how that progress is now yielding products.

1.5.1 Antibody Engineering to Reduce Immunogenicity

Chimerization and humanization of murine monoclonal antibodies have vastly improved their therapeutic application [66, 67]. Chimerization, the replacement of mouse constant regions by human sequences, and humanization, the additional replacement of variable framework regions, result in a significantly less immunogenic product. Fully human antibodies have been directly isolated from transgenic mice and phage display libraries [68, 69]. However, some humanized and even fully human sequence-derived antibody molecules still carry an immunogenicity risk [70].

The “immune response to antibodies” includes both a cellular arm (T cells) and a humoral arm (antidrug antibodies (ADA)), which may consist of IgM, IgG, IgE, and/or IgA isotypes. The risk of clinical impact of ADA ranges from alteration of the PK of the antibody to the neutralization of the antibody effect up to hypersensitivity ADAs and cross-reactive neutralizing ADAs. Numerous assays have been reported to screen for binding and neutralization of antibodies by ADAs. These assays include ELISA, radio-immunoprecipitation (RIP), SPR, and cellular assays detecting the functional neutralization of the drug antibody [71]. From collected experience it became obvious that many factors may contribute to the formation of immunogenicity [72]. The factors can be categorized into product-related factors, patient-related factors, and clinical trial design-related ones.

Product-related factors include changes to molecular structure/sequence, aggregates, fusion proteins, exposure to cryptic epitopes (e.g., by glycosylation changes), modified amino acids, and changes in glycosylation patterns in comparison with endogenous protein. Product-related factors can also come from production-specific attributes like host cell proteins or DNA and post-translational modifications like oxidation, deamidation, clipping, denaturation, and formulation [72].

Patient-specific factors are related to the individual immunocompetence; the genetic background, age and gender; the exposure to a pro-inflammatory environment; and the presence of preexisting antibodies due to prior antigen exposure or cross-reactive antibodies [72].

Trial design-specific attributes are the route of delivery of the NBE, dose, and frequency of administration and potential coadministration with other compounds. As an example, single administration in general induces an IgM response of limited magnitude. Two administrations may induce an isotype switching, leading to more pronounced immune response. Multiple exposures will also induce isotype switching and higher affinity of ADAs, potentially leading to severe effects. These insights into causes of immunogenicity of antibodies have and will continue to lead to higher safety in clinical studies and further improved success rates in NBE development.

1.5.2 Progress in Antibody Production and Engineering of Physicochemical Properties

In comparison with small molecules, production costs of antibodies and therapeutic proteins are high. These high production costs directly translate often into considerably higher medication costs compared with NCE costs. Monoclonal antibodies are very big molecules with complex structures and functions whose production costs partly rely on their production processes. Production processes and technologies are being improved constantly. Culture conditions and purification processes can evoke distinct product quality attributes such as differences in structure, posttranslational modifications, biological activity, and stability of the protein. All these factors will lead to distinct properties of individual antibodies independent of the format.

Many efforts have been directed to enhance production by optimizing and utilizing different expression systems [73]. Significant progress has been made with respect to achieving high expression titers, accelerating the turnaround times and optimizing the process economics.

The currently dominant expression system is still based on eukaryotic Chinese hamster ovary (CHO) cells, especially for full-length antibodies. For antibody fragments and therapeutic proteins, *Escherichia coli* expression has been utilized. The primary sequence of an antibody determines expression levels, propensity for aggregation, and protein stability, which are important factors for the ability to develop. Phage display techniques have been instrumental in optimizing protein sequences with respect to the aforementioned properties and thus facilitated production optimization and development success substantially. Examples of *E. coli*-produced NBEs are certolizumab (Cimzia[®]) (anti-TNF α

antibody for the treatment of Crohn's disease and rheumatoid arthritis) and ranibizumab (Lucentis[®]) (anti-VEGF antibody for the treatment of age-related macular degeneration). Advantages of the *E. coli* expression system include

- Ease of genetic manipulation
- Short process development times
- Simple and scalable fermentation
- Absence of viral contaminations

Full-length antibodies are usually expressed in eukaryotic cells, for example, CHO cells, or yeast. Eukaryotic cells possess the complex folding and secretory pathways, enabling the effective expression of heterologously expressed proteins. Furthermore, eukaryotic cells have the ability to posttranslationally modify proteins, for example, to glycosylate proteins. For antibody Fc regions, glycosylation is essential for effector functions like ADCC and CDC, but glycosylation also impacts upon antibody properties like PK, *in vivo* clearance, solubility, antigenicity, and cellular secretion. *N*-glycosylation within the variable domain may also impact efficacy, as seen with cetuximab (Erbix[®]) (anti-EGFR antibody for the treatment of colorectal and head and neck cancer). Normally, removal of glycosylation at the variable region is preferred to secure a homogenous antibody product.

Yeast as second eukaryotic expression systems has the advantage of simple and scalable fermentation combined with glycosylation capability. Yeast glycosylation, however, does not match mammalian glycosylation, leading to high mannose structures. Therefore efforts have been directed to engineer the yeast glycosylation enzymes to match the capabilities of the CHO system.

Every production process needs to be tracked by a comprehensive analytical evaluation to secure reproducibility and quality. First, a full physicochemical characterization of the mAb is performed by multiple orthogonal methods. Those results are subsequently used to describe the physicochemical characteristics of the mAbs. These characteristics include primary (amino acid) sequence, protein folding, truncations, posttranslational modifications (glycosylation), the amount of mAb protein, and the presence of degradation products and aggregates. The suitability of different excipients and primary packaging (e.g., vials) will be tested, and the presence of host cell impurities will be determined.

In summary, impressive progress has been made to secure both optimized CMC properties (allowing smooth development) and high yield expression levels (to control costs).

1.5.3 Engineering to Improve Efficacy

Efficacy of an antibody [64] is related to several factors:

- The antigen-binding affinity and the specificity
- The penetration ability toward the target cell/tissue
- The effector function depending on the therapeutic concept

For affinity maturation, phage display libraries as well as yeast- and ribosome-display libraries have been used very successfully to drive affinity into the

picomolar range. Both increases in dissociation rate and association rate are the consequences of affinity maturation. Which factor dominates is difficult to predict. Affinity maturation often translates into parallel increases in biological potency. In some cases, however, as exemplified in certain tumor types, lower affinity leads to more uniform tumor distribution and thus increased antitumor efficacy compared with higher affinity variants [74]. The ability to penetrate target tissue is also dependent on antibody formats. It has been shown that scFvs compare favorably with full-length antibodies in targeting tumor tissue.

The choice of the therapeutic concept decides whether Fc-mediated effector function is desirable or not. For example, in oncology, Fc-function selection will depend on whether destruction of tumor cells via ADCC and/or CDC should contribute to or support the desired effect of the antigen binding. A strong example for an Fc-mediated contribution to antibody efficacy comes from rituximab where efficacy in non-Hodgkin's lymphoma correlates with polymorphisms in the Fc γ receptors expressed on immune effector cells. Response rates of rituximab are highest in patients with the homozygous Fc γ IIIa (Val158) form and attenuated in patients with homozygous Fc γ RIIIa (Phe158) [75]. Fc-mediated ADCC and CDC may be modulated not only by Fc engineering of the primary sequence but also by engineering of the glycan structure of the Fc region [76–78]. Example of successful glycoengineering is the monoclonal CD20 antibody obinutuzumab approved as Gazyvaro[®] for the treatment of chronic lymphocytic leukemia (see Chapter 9).

On the other hand, Fc γ R binding may also lead to mitogenic effects via T cells and cytokine release. This can be prevented by the use of IgG2 and IgG4 isotypes or by IgG1 Fc engineering and has been exemplified by muromonab-CD3 for immunosuppression (graft vs. host rejection) [79].

It is very impressive how the versatility of antibodies has been and will continue to be utilized to design antibodies fit to the therapeutic purpose. Moreover, insight into specific mutations in patients will allow the design of targeted antibodies in a precision medicine approach.

1.5.4 New Formats

1.5.4.1 Antibody–Drug Conjugates

ADCs have been mainly applied in the therapy of cancer. Current clinical practice with conventional antibody therapeutics often shows limitations in efficacy. One promising approach to overcome efficacy limitations in cancer is the antibody-mediated delivery of immunoconjugates as highly potent effector molecules. These molecules are referred to as ADCs when a cytotoxic small molecule is attached to the antibody or as RICs when a radionuclide is attached [80].

Within the field of immunoconjugate development, there have been attempts to combine the specificity of mAb therapy with effector molecules that exert potent cytotoxic activity to tumor cells by inducing either direct or indirect cell death. Ideally the tissue specificity imparted by the antibody limits off-target toxicity to normal tissues. Coupling of the cytotoxic principle to the delivery vehicle is usually achieved via a linker or a chelator molecule, and stability of the linkage has a critical impact on clinical toxicology, pharmacology, and efficacy of the ADC.

Currently, four immunoconjugates are approved by the FDA. Ibritumomab tiuxetan (Zevalin[®], 2002) is a murine anti-CD20 IgG1 RIC targeting ⁹⁰Y for the treatment of low grade or follicular, relapsed, or refractory CD20-positive B-cell non-Hodgkin lymphoma. Tositumomab (Bexxar[®], 2003) is a second murine anti-CD20 IgG2a RIC targeting ¹³¹I for the treatment of CD20-positive non-Hodgkin lymphoma, with and without transformation, which is refractory to rituximab following chemotherapy. Brentuximab (Adcetris[®], 2011) is a humanized IgG1 anti-CD30 antibody/vedotin conjugate for the treatment of lymphoma and trastuzumab (Kadcyla[®], 2013) is an IgG1 anti-p185 neu receptor antibody/emtansine conjugate for the treatment of non-small cell lung, pancreatic, and bladder cancer.

Particular challenges in the design and synthesis of ADCs include (i) identification of appropriate antigens that are selectively overexpressed in tumor tissue and that are efficiently internalized after binding their mAb ligands; (ii) specificity of ADC binding to the target tumor antigen; (iii) development of an appropriate linker chemistry ensuring efficient linkage of cytotoxic moieties, high stability of ADCs during circulation, and specific cytotoxic drug release in tumor tissue; and (iv) physicochemical properties of ADCs and tissue penetration. To meet these challenges, ADCs are composed in a modular manner with three structural components (see Figure 1.11).

To achieve a beneficial impact on both the efficacy and tolerability of respective ADCs, the cytotoxic moiety and the particular linker have to meet several requirements. Due to the long half-life of IgGs and IgG-derived ADCs in circulation, a high stability of cytotoxic principle and linker has to be ensured. To specifically release the cytotoxic moiety solely at the site of action in tumor tissue, cytotoxic drug release is preferably achieved inside the tumor cells. Typically, antigen binding of ADCs is associated with an efficient internalization into the tumor cells, which ends up in the lysosomal compartments. Current ADCs are based on linker chemistries that release the cytotoxic principle within the lysosome. Thus, the propensity of an antigen-ADC complex on the cell surface to internalize into the cell is a driver of efficacy [81–83]. Three classes of internalization routes have been described: (i) rapid internalization via clathrin-coated

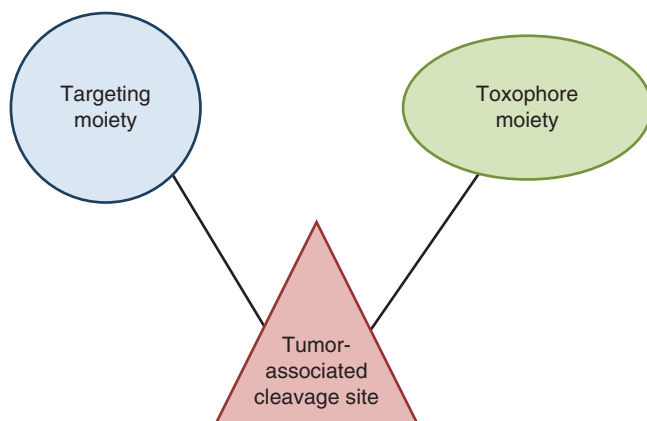


Figure 1.11 Modular composition of ADCs.

pit-mediated endocytosis; (ii) internalization via caveolae-mediated endocytosis; and (iii) internalization via pinocytosis [84, 85].

The concepts for tumor-associated cleavage of ADCs start with tumor cell recognition of the mAb component, internalization, and intracellular trafficking of the ADC–antigen complex. Appropriate cleavage mechanisms mediating the release of the cytotoxic principle include the reductive cleavage of disulfide bonds, a hydrolytic cleavage of hydrazones, acetals, and cis-aconitate-like amides in the acidic pH of the lysosomes, peptide cleavage by lysosomal enzymes (e.g., cathepsins), and release of the effector moieties following the complete mAb degradation in the lysosomes. ADCs and the linker chemistry [86] provide powerful examples of how medicinal chemistry contributes to the generation of effective new NBE concepts for cancer treatment.

In summary, great progress has been made in understanding factors, making ADCs safe and effective. This experience will guide future ADC drug discovery, and it can be expected from the insights into a growing ADC development pipeline that the number of new ADCs in cancer therapy will increase substantially.

1.5.4.2 Bispecific Antibodies

Clinical development of bispecific antibodies (bsAbs) is currently focusing on two main areas, namely, cancer therapy and inflammatory diseases. The major goal is to simultaneously address different targets involved in pathophysiological processes and therefore increase therapeutic efficacy [87, 88]. These two targets may be either addressed synergistically or targeted to break escape via redundant pathways.

BsAbs combine specificities of two antibodies and simultaneously address different antigens or epitopes. BsAbs with “two-target” functionality can interfere with multiple surface receptors or ligands associated, for example, with cancer, proliferation, or inflammatory processes. BsAbs can also place targets in close proximity, either to support protein complex formation on one cell or to trigger contacts between cells. Examples of “forced-connection” functionalities are bsAbs that support protein complexation in the clotting cascade or *tumor*-targeted immune cell recruiters and/or activators.

Two bsAbs are currently on the market. The anti-epithelial cell adhesion molecule (EpCAM)/anti-CD3 bsAb catumaxomab (Removab[®]) was the first bsAb to receive market approval [89]. It was developed as a trifunctional bsAb consisting of a tumor antigen and CD3-binding hybrid of murine IgG2a and rat IgG2b. It targets the tumor via EpCAM, recruits T effector cells via binding to CD3 of the T cell receptor complex, and also activates monocytes, macrophages, dendritic cells, and NK cells by Fc γ -receptor binding [90]. This induces the killing of tumor cells in patients with ovarian carcinoma and leads to the prevention of ascites.

The second bsAb receiving FDA approval is blinatumomab, a (bispecific T cell engager (BiTE) targeting CD19 on tumor cells and CD3 on T cells. Blinatumomab has been approved for the treatment of B cell acute lymphocytic leukemia (ALL). Unlike catumaxomab, the BiTE blinatumomab is a bsAb in the form of two scFvs connected via a peptide linker. It has a shorter serum half-life but functions as a pair of recruiter molecules and can undergo several rounds of target cell lysis.

There are currently more than 50 different bsAbs in clinical development. These cover very different formats. BsAbs can be divided into two classes, with and without an Fc region. Fc-containing bsAbs have the advantage of utilizing Fc-mediated effector function like ADCC and CDC, longer half-life, and improved solubility and stability. The efficacy of bsAbs without an Fc region depends fully on the functional impact of their dual antigen binding capacity. The introduction of polyethylene glycol (PEG) conjugation and albumin fusion moieties [91] has been used to prolong the half-life of bsAbs significantly.

From the many types of Fc region-containing BsAbs (summarized in Figure 1.12), the recent progress made by the “knob-in-holes” technology needs to be mentioned [92]. By introducing different mutations into two CH3 domains, heavy chain heterodimerization can be induced. Further progress could be made by introduction of the CrossMab technology to enable correct light chain pairing via exchange of one CH1 domain by the constant CL domain [92]. In addition, dual affinity re-targeting (DART) has been utilized to generate clinical candidates for T cell engagement. In DARTs the first variable region is linked to the variable light (VL) chain domain of the second binder and the variable heavy (VH) chain domain of the second variable region linked to the VL of the first. Then additional disulfide stabilization is introduced. In one clinical candidate, fusion to an Fc domain leads to half-life extension.

The broad range of formats for BsAbs as shown in Figure 1.12 allows the selection of the best option for given bispecific therapeutic concepts. This versatility is expected to stimulate bispecific drug discovery.

In summary, the discovery of NBEs realized substantial progress over the last decade. The field is dominated by antibodies, followed by therapeutic proteins

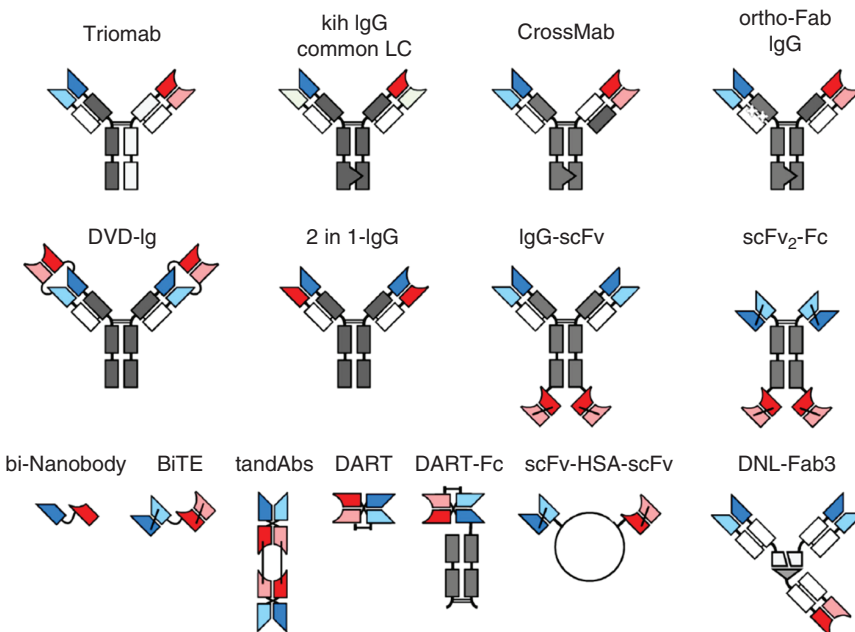


Figure 1.12 Bispecific antibody formats.

and vaccines. The increased number of NBE approvals is a reflection of major progress in the understanding of immunogenicity, contributing factors, and techniques to reduce immunogenicity. Significant advances have come from improved scale-up and production of NBEs by increasing expression titers and a better understanding and ways to overcome CMC issues. A third area of progress is related to the question of how to improve efficacy. Insight has been gained into how to find the right balance between affinity and tissue distribution and how and when to utilize effector function depending on the therapeutic concept. As a fourth factor, impressive progress has been made with respect to new formats, ranging from ADCs to bsAbs. This versatility allows application of tailor-made formats for new therapeutic concepts. These advances are not yet fully reflected in the FDA approvals of the last few years, but when analyzing the development/clinical pipeline, it becomes obvious that new formats will have good chances of contributing to NBE approvals. In addition, new therapeutic modalities will complement the NBE arena like cell-based therapies, gene therapy, and oncolytic viruses to only mention some of the innovative opportunities. It is also important to note that the clear differentiation between NBE and NCE discovery will fade. The impact of structural research, sophisticated shared assay systems, and medicinal chemistry on NBE discovery, for example, with linker chemistry in ADCs or gene switches in gene therapy, are all examples for a close collaboration between both disciplines.

1.6 General Challenges in Drug Discovery

When analyzing the root causes for attrition in clinical phases, it becomes obvious that dropout rates in phase II are very significantly linked to lack of efficacy [93]. Clinical efficacy is the main cause of failure (35%), followed by clinical safety (25%). This is a clear reflection of the fact that new therapeutic concepts defined in drug discovery are too often not translating into the desired efficacy in humans. It is obvious that both disease-related animal models and knockout animals do not always correlate with human disease. There are many ways to better establish links between a target and human disease. Two examples are (i) phenotypic screens with human primary cells or human stem cells and (ii) integrated omics analysis of human samples integrating genomic, transcriptomic, and metabolomic data. These data need to be correlated with clinical data of the individual patients. In this context the homogeneity of the patient population needs to be investigated. Many examples of failed clinical studies have been analyzed retrospectively, demonstrating that a defined patient subpopulation has shown improved response. One example is an IL13 antibody in asthma for high-periostin plasma levels [94].

For improving success rates in clinical efficacy studies, it is indispensable to better characterize new targets. The questions that should be addressed are

- 1) How strong is the link between a new target and the human disease?
- 2) Are there redundant mechanisms that may compromise efficacy when modulating the new target?

- 3) Are there hints that efficacy may be linked to a patient subpopulation?
- 4) Are there hints on potential tolerability issues when modulating the new target?
- 5) Can potential tolerability issues be tested early during drug discovery?

A second option for improvement is to generate early indicators of efficacy and tolerability issues. In this respect the systematic exploration and application of biomarkers can help guide preclinical and clinical studies. Biomarkers may guide decisions on continuation or termination of clinical and nonclinical development early before expensive advanced clinical studies are initiated. Since research target biomarkers are not available for many new targets, biomarker discovery and characterization activities need to be started early in drug discovery based on relevant cellular and *in vivo* models.

1.7 Summary

Future drug discovery and development is stimulated by increased success rates. It is obvious that this advance is linked to substantial enhancements in NCE and NBE discovery as outlined above. It can be expected that the more recent enhancements will contribute to make drug discovery even more successful. Examples from recent approvals also give evidence that the differences between NCE and NBE discoveries fade and that both disciplines need to join forces and learn from each other. It is also obvious that the collaboration with clinicians needs to start early in drug discovery. The definition of impactful new therapeutic concepts can only be achieved in close collaboration. The definition of therapeutic gaps needs to be translated into impactful research target profiles, and the definition of the target patient population and the generation of new therapeutic concepts, including efficacy and safety considerations, are integral parts of this collaboration.

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List of Abbreviations

ADA	antidrug antibody
ADC	antibody–drug conjugate
ADCC	antibody-dependent cellular cytotoxicity
ATP	adenosine triphosphate
BsAb	bispecific antibody
CBER	Center for Biologics Evaluation and Research

CDC	complement-dependent cytotoxicity
CDER	Center for Drug Evaluation and Research
CMC	chemical manufacturing control
CNS	central nervous system
COPD	chronic obstructive pulmonary disease
DILI	drug-induced liver toxicity
DMPK	drug metabolism and pharmacokinetic
EGFR	epidermal growth factor receptor
ELISA	enzyme-linked immunosorbent assay
FBLD	fragment-based lead discovery
Fc	fragment crystallizable
FDA	Food and Drug Administration
FGFR	fibroblast growth factor receptor
GLP-1	glucagon-like peptide 1
GP	glycoprotein
GPCR	G-protein coupled receptor
Her 2	human epidermal growth factor receptor 2
hERG	human ether-a-go-go-related gene
HTS	high throughput screening
IPF	idiopathic pulmonary fibrosis
LBVS	ligand-based virtual screening
MDR	multidrug resistance
NBE	new biological entity
NCE	new chemical entity
NME	new molecular entity
NMR	nuclear magnetic resonance
NK	natural killer (cells)
PAINs	pan-assay interference compounds
PCLS	precision cut liver slices
PCSK9	proprotein convertase subtilisin/kexin type 9
PDE	phosphodiesterase
PEG	polyethylene glycol
PK	pharmacokinetic
PPAR	peroxisome proliferator-activated receptor
R&D	research and development
SBVS	structure-based virtual screening
scFv	single-chain variable fragment
TNF	tumor necrosis factor
TPP	target product profile
VEGF	vascular endothelial growth factor
VS	virtual screening

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