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New Approaches to Drug Discovery

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New Approaches to Drug Discovery

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Preface

The last decade has seen many exciting new medicines being introduced to the market, such as novel oral anticoagulants, novel anti-diabetics, highly effective antiviral agents against hepatitis C, oral MS therapies, or targeted cancer therapies to name just a few. For the first time, diseases with orphan drug designations, e.g., cystic fibrosis or rare blood disorders, are treated with new chemical or biological entities. Biologics have now reached center stage especially for the treatment of immune disorders and oncologic indications, with the anti-TNFα agent adalimumab being the best-selling drug in 2014. These impressive successes notwithstanding, the so-called patent cliff, a conceived lack of productivity in the pharmaceutical industry, increasing expenses to discover and develop new therapeutic agents and reimbursement challenges have put pressure on the community to only target highly innovative approaches and to focus resources in selected areas of high expertise. With increased investments over the last years pharma companies continue to support large R&D efforts, while new venture capital backed biotech companies have surfaced, and universities attempt to translate their basic research into products through collaborations and the build-up of screening centers. Apart from this dynamic, the underlying process of drug discovery has not changed dramatically. It still starts with a solid disease hypothesis linked to a target which then needs precise validation (D. Sim, K. Kauser, B. Nicke) before the high-throughput screening is started for lead identification. As pointed out by J. Eder and P.L. Herrling, phenotypic screens have gained more attention recently, where a cell-based assay is used to first identify leads and later – hopefully – the corresponding target. Intractable targets, discarded as non-druggable a decade ago, are tackled today (S. Knapp), and new chemical matter intercepting protein–protein interactions (C. Ottmann), a revived interest in natural products (E.F. van Herwerden, R. Süssmuth), and powerful high-throughput synthesis (C. Rademacher, P.H. Seeberger) might help to dissect and address challenging pathways. Meanwhile classical medicinal chemistry can rely on improved predictive models (M.S. Lawless, M. Waldman, R. Fraczkiewicz, R.D. Clark) and strong in vitro assays (G. Langer) to identify and optimize leads. Understanding their pharmacokinetic properties (A. Reichel) is a prerequisite for lead refinement and candidate selection, before in vivo efficacy is demonstrated in relevant animal models (O.D. Slayden, H. Trübel, B. Albrecht, J. Hoffmann) and the potential

candidates are subjected to a thorough safety assessments (C. Stark) for final triaging. Early identification of biomarkers to either select patients susceptible to a certain therapy or as surrogate marker for efficacy (T. Krahn) and computational models to simulate drug effects (J. Lippert) have become essential tools when entering the clinical phase.

We hope the handbook conveys the excitement and progress made in drug discovery throughout the last decade. While the process has stayed the same, it has been enriched and reinvented along the value chain. Hence, Giuseppe di Lampedusa's *Se vogliamo che tutto rimanga come è, bisogna che tutto cambi* [If we want things to stay as they are, things will have to change, Il Gattopardo (1958)] might probably be an appropriate motto for this book.

Berlin, Germany

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Trends in Modern Drug Discovery

Jörg Eder and Paul L. Herrling

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Abstract

Drugs discovered by the pharmaceutical industry over the past 100 years have dramatically changed the practice of medicine and impacted on many aspects of our culture. For many years, drug discovery was a target- and mechanism-

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agnostic approach that was based on ethnobotanical knowledge often fueled by serendipity. With the advent of modern molecular biology methods and based on knowledge of the human genome, drug discovery has now largely changed into a hypothesis-driven target-based approach, a development which was paralleled by significant environmental changes in the pharmaceutical industry. Laboratories became increasingly computerized and automated, and geographically dispersed research sites are now more and more clustered into large centers to capture technological and biological synergies. Today, academia, the regulatory agencies, and the pharmaceutical industry all contribute to drug discovery, and, in order to translate the basic science into new medical treatments for unmet medical needs, pharmaceutical companies have to have a critical mass of excellent scientists working in many therapeutic fields, disciplines, and technologies. The imperative for the pharmaceutical industry to discover breakthrough medicines is matched by the increasing numbers of first-in-class drugs approved in recent years and reflects the impact of modern drug discovery approaches, technologies, and genomics.

Keywords
Pharmaceutical research · Pharmaceutical industry · R&D productivity · Target-based drug discovery · Phenotypic screening · Lead discovery · Target discovery

1 The Beginnings of Modern Drug Discovery

Modern drug discovery is one of the most complex scientific areas and involves many different scientific disciplines. It has its origins at the end of the nineteenth century in the experimental biological and medical research of Claude Bernard, Louis Pasteur, Robert Koch, Paul Ehrlich, and Joseph Lister, as well as in the great advances in organic chemistry at the same time, and has ever since dramatically changed the practice of medicine, our culture, and sociology. About 1,500 unique drugs are currently known which act through more than 350 different mechanisms (Overington et al. 2006). With these, many diseases are now curable or can at least be controlled at the symptomatic level including bacterial, parasitic and viral infections, rheumatoid arthritis, asthma, osteoporosis, thrombosis and other cardiovascular disorders, diabetes, psychiatric diseases, and various cancers. Moreover, drugs have enabled many surgical procedures of modern medicine and even made cell and solid organ transplantation possible.

The first 100 years of modern drug discovery were largely target and mechanism agnostic and primarily driven by chemocentric approaches, i.e., approaches based on a specific compound or compound class which served as starting point for further optimization. These chemotypes were either discovered through ethnobotanical knowledge or derived from natural ligands and substances. Serendipity, however, was also an important success factor in many instances. In the following we list a few examples to illustrate how drugs were discovered during this time period.

1.1 Aspirin

Extracts of the bark from the willow tree were used for thousands of years in Europe and North America for pain relief, treatment of inflammation, and fever. The active ingredient of the bark extract was first isolated by the German chemist Johann Andreas Buchner in 1828 and named salicin after the Latin name for the white willow (*Salix alba*). The glycoside can be converted to salicylic acid by hydrolysis and subsequent oxidation (Fig. 1). Felix Hoffman, a chemist at Bayer in Germany,

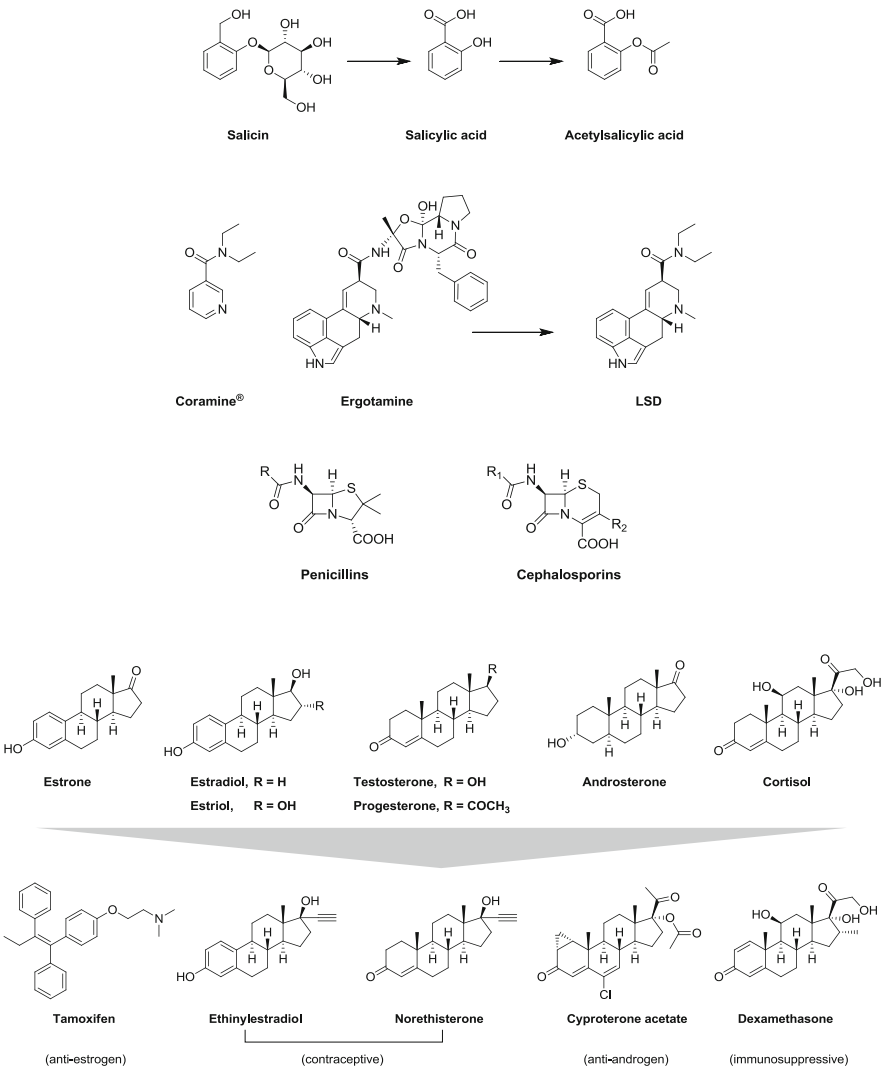

Fig. 1 Drugs discovered during the first 100 years of modern drug discovery were mainly based on ethnobotanical knowledge or derived from natural ligands and substances

systematically searched for derivatives of salicylic acid in 1897. His search was triggered by his father who suffered from rheumatoid arthritis and did not tolerate high doses of salicylic acid due to intestinal tract irritation and emesis. One of the first derivatives he synthesized was acetylsalicylic acid which is known since 1899 as Aspirin® (Schrör 2008). It took more than 75 years until it was discovered that the chemical derivatization also led to an advantageous change in the mechanism of action as it turns the drug into an irreversible inhibitor thereby preserving its therapeutic effect beyond compound exposure. The latter is the basis for the success of low-dose acetylsalicylic acid treatment as anticoagulant therapy used by millions of patients today.

1.2 Ergotamine

The fungus *Claviceps purpurea* and other clavicipitaleans form ergot sclerotia to produce their spores in oat, rye, wheat, and other grasses. These sclerotia contain more than 50 different indole alkaloids, referred to as ergot alkaloids. Many of these are highly toxic due to their vasoconstrictive properties leading to gangrenous loss of the limbs, hallucinations, and dementia. The first documented ergotism epidemic of human toxicity occurred in central Europe in 857 AD, and it took almost 1,000 years to realize the causal relationship and improve agricultural practices. In herbal medicine ergot was first mentioned in the late sixteenth century for use in obstetrics to induce uterine contractions and hasten childbirth, to reduce postpartum hemorrhage, and to induce abortion. When migraine was proposed to be caused by vasodilation by sympathetic deficit in the mid-nineteenth century, the British surgeon Edward Woakes recommended ergot as a vasoconstricting treatment in 1868 (Woakes 1868). The Swiss biochemist Arthur Stoll isolated ergotamine as the active ingredient of ergot sclerotia in 1918 at Sandoz, and the company started to market ergotamine (Gynergen®) in 1921 for the treatment of migraine. Twenty years later, Albert Hofmann, a chemist and coworker of Arthur Stoll who worked on the isolation and synthesis of active ergot constituents, wanted to engraft the respiratory and circulatory stimulating effect of nicotinic acid diethylamide (marketed under the trade name of Coramine®) onto the ergotamine structure. The result was the discovery of lysergic acid diethylamide, better known as LSD, a psychedelic drug (Fig. 1).

1.3 Penicillin

Alexander Fleming serendipitously discovered the antibiotic effect of the fungus *Penicillium rubens* in 1928 when working with staphylococci cultures (Fleming 1929). One such culture was contaminated with a fungus, and the colonies of staphylococci around the mold were destroyed, whereas other colonies farther away were unaffected. He grew the fungus in pure culture, established that it also killed other disease-causing bacteria, and named the unknown active ingredient

penicillin. Only 12 years later the pure substance was isolated and characterized and its chemical structure determined (Fig. 1). The success of penicillin and its derivatives triggered a search for additional antibiotics produced by other fungi and led, for example, to the discovery of cephalosporins (Fig. 1) which have the same mechanism of action but are less prone to hydrolysis by bacterial β-lactamases.

1.4 Steroid Hormones

The first steroid hormone was isolated from the urine of pregnant women by Adolf Butenandt in 1929 (estrone; see Fig. 1) (Butenandt 1931). To guide the isolation, he used a specific test system to detect the activity of the hormone. In the following years, he and others isolated and structurally characterized other female (estradiol, estriol) and male (testosterone, androsterone) sex hormones, progestogens (progesterone), and corticosteroids (e.g., cortisol). Their chemical optimization toward oral bioavailability and the search for more potent analogs led to a number of important drugs in the field of cancer (antiestrogens, antiandrogens; see Fig. 1) and immune diseases (e.g., dexamethasone). In addition, the idea to combine an estrogen and progestogen by Carl Djerassi and Gregory Pincus in the 1950s gave rise to the first oral contraceptive pill and revolutionized family planning in the industrialized world.

Until modern molecular biology techniques were established in the mid-1980s, the molecular basis of the pharmacology of most drugs was not known. Pharmacological receptors, a concept proposed by Langley (1905), were only a model inferred from dose-response curves derived from measuring the effect of pharmacological agents applied to whole animals or isolated tissues, such as the muscle, gut, and heart in organ bath apparatuses (Fig. 2). This was still the case in 1975 (Goodman and Gilman 1975). It was assumed already in 1880 by Langley (1880)

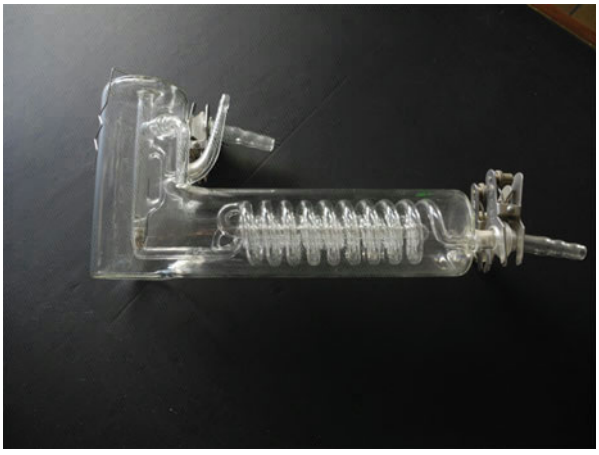

Fig. 2 Organ bath used in the author's laboratory (PH) in the 1980s

that actions of pharmaceutical drugs are governed by the law of mass action. This concept was further elaborated by Clark (1920). The receptor existed only as an abstract model, but its interactions with pharmacological drugs could be measured by constructing logarithmic dose-response curves as well as the interactions of agonists and antagonists at a particular receptor (Kenakin 1987; Arunlakshna and Schild 1959). The availability of radioactive selective receptor ligands and the development of receptor binding studies by Robert Lefkowitz et al. (1970) have greatly helped to localize receptors in different organs and tissues in particular in the brain (Cortes et al. 1987) as one example of many.

Meanwhile, due to advances in molecular biology, genetics, protein sequencing, and computing, many receptors and other drug targets are cloned, purified, and described atom by atom in spatial models allowing true target-based drug discovery, i.e., studying the interactions of targets with drug molecules in isolation and visualizing and calculating their interactions at atomic scale (Falchi et al. 2014; Chen et al. 2012).

2 Where Do Chemical Lead Structures Come from Today?

Target-based drug discovery has enabled a great expansion of chemotypes and pharmacophores available for the medicinal chemist during the past three decades. New techniques like high-throughput screening (HTS), fragment-based screening (FBS), crystallography in combination with molecular modeling, and combinatorial and parallel chemistry have created a considerable diversity of chemical lead structures well beyond the known natural products and ligands used as chemical starting points for drug discovery in the past. Moreover, this wealth of chemotypes can now be used as a source for tool compounds to study unexplored biological space and find new drug targets or for phenotypic screening using systems-based approaches to identify drug candidates in a target-agnostic manner (see below). Figure 3 shows examples of successful target-based drug discovery projects using the different methods available for identification of lead structures. These include high-throughput screening of diverse chemical libraries, fragment-based screening, rational drug design, the use of target family knowledge, and in silico drug discovery methods.

2.1 Origin of Libraries

Typically, the libraries are composed of the compounds synthesized over time by individual companies and influenced by a company's history, e.g., Novartis has a large number of ergot compounds in its library, and Roche would have many benzodiazepines. But as many companies work on similar targets or scaffolds, there must also be some overlap between the libraries. Nevertheless these libraries are a key component of the success of pharmaceutical companies, although they have once been in danger of getting lost. At the time combinatorial chemistry

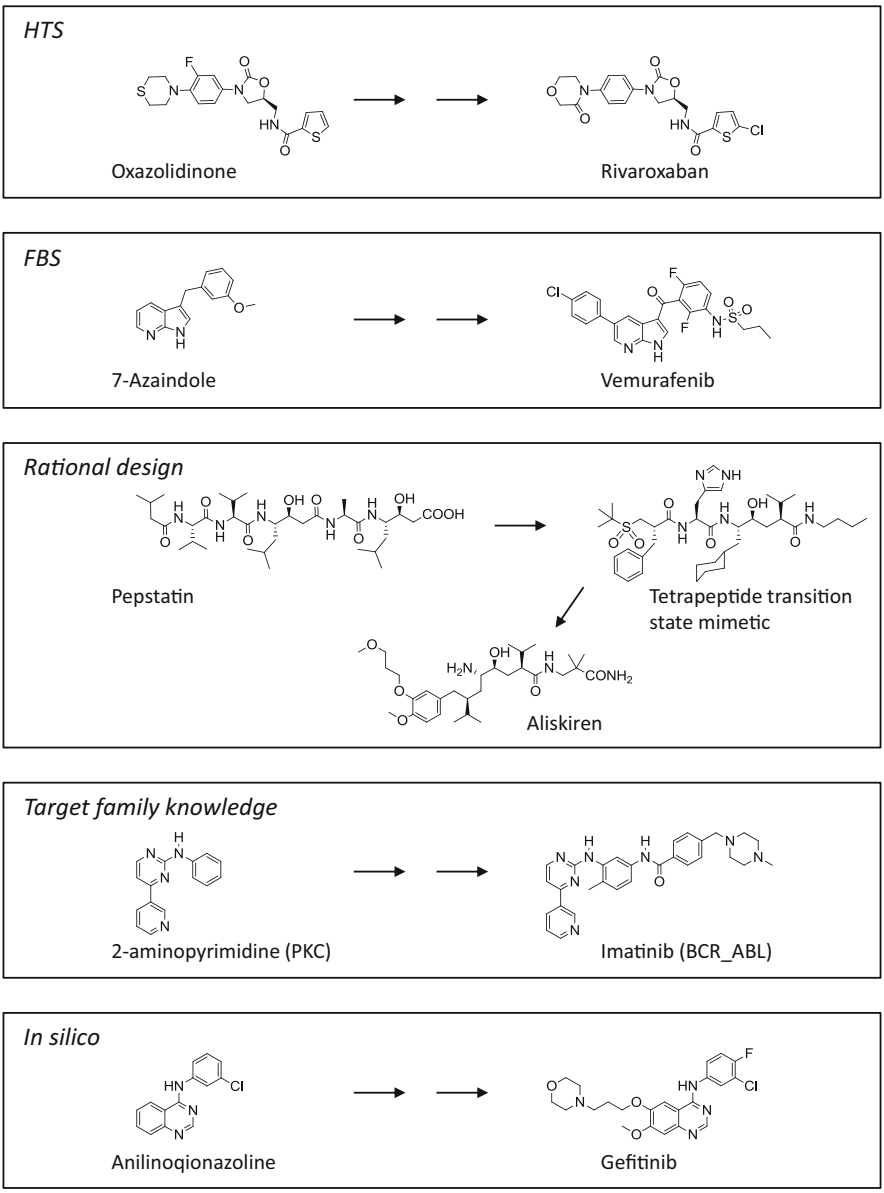

Fig. 3 Target-based drug discovery has enabled a great expansion of pharmacophores by using a variety of different methods

became possible in the 1980s eventually allowing the rapid synthesis of millions of compounds it was thought that all possible compounds could be made when needed by starting from individual scaffolds, and the historical libraries were neglected for

a while. However, it became apparent in HTS that the hit rate, when using these combinatorial libraries, was distinctly lower than with the historical libraries (Lahana 1999). One reason for this was that combinatorial libraries were strongly dependent on chemical parameters, such as the possibility to do chemistry with molecules attached on beads rather than on potential biological activity alone. This insight led to a revalorization of the collection of historical compounds that had been made for pharmacological activity. It also led some companies to maintain and expand their natural-compound libraries as these can be seen as compounds selected for biological activity for hundreds of millions of years. Medically useful compounds from natural substances are described above. Today the realization that even the millions of compounds available cover only a small part of the biologically active compound universe makes it important to continue the efforts to diversify our libraries as repeatedly few or no ligands are found in the existing libraries for some newly discovered targets.

2.2 HTS

Compound collections used for high-throughput screening are typically based on chemically diverse molecules as well as on chemotypes from previous projects and can reach a size of 1–2 million substances. The compounds are screened in biological test systems, and hits, once validated by independent biochemical or biophysical methods, are further optimized to drug candidates. An example is the discovery of the anticoagulant rivaroxaban, a factor Xa inhibitor approved by the FDA in 2011. The HTS hit selected for further optimization was an oxazolidinone derivative (Perzborn et al. 2011), a compound class previously worked on for inhibition of the 50S ribosomal subunit A site in bacteria.

2.3 FBS

A specific variant of HTS is fragment-based screening. It is based on the idea that smaller molecules (usually with molecular weights below 250 Da) are better suited to sample the chemical space because it is much less complex for small molecules than it is for bigger ones. Hits are generally more frequent but may only bind weakly to the biological target, which requires growing them or combining them to produce a lead with a high affinity. So far the only successful example of this relatively new technology is the BRAF V600E mutant kinase inhibitor vemurafenib. The underlying chemotype was discovered by FBS using a panel of recombinant kinases (Tsai et al. 2008). The 7-azaindole compound was subsequently optimized to the final inhibitor by conventional medicinal chemistry methods.

2.4 Rational Drug Design

The renin inhibitor aliskiren has been approved for treatment of hypertension in 2007. Renin is an aspartic protease which catalyzes the rate-limiting step in the renin-angiotensin system. Aliskiren is the product of rational drug design utilizing the inhibitory principle of pepstatin, a naturally occurring hexa-peptide which contains the unusual γ-amino acid statin. The statin-based inhibitory principle was grafted onto small peptide-like compounds derived from the natural renin substrate, and these compounds were further optimized to the final drug using structural information (Maibaum and Feldman 2009).

2.5 Target Family Knowledge

Leveraging target family knowledge is another way of generating chemical starting points for targets which are members of larger protein families such as kinases, proteases, E3 ligases, or G-protein-coupled receptors. The BCR-ABL kinase inhibitor imatinib, which revolutionized the treatment of chronic myelogenous leukemia (CML), was discovered based on an aminopyrimidine lead compound that was originally identified in a screen for inhibitors of protein kinase C (Capdeville et al. 2002). Chemical optimization toward BCR-ABL selectivity and oral bioavailability led to the final molecule.

2.6 In Silico Methods

The availability of three-dimensional structures and ever more sophisticated computer modeling programs also enables the in silico discovery of chemical starting points. An example is gefitinib, an epidermal growth factor receptor tyrosine kinase inhibitor for the treatment of lung and breast cancers. The proposed catalytic mechanism was used to define a query for structure-based searches which led to the discovery of anilinoquinazolines as potent inhibitors and suitable lead structure for this enzyme (Ward et al. 1994).

Today, in many cases, the X-ray crystal structure of a target is available early during a drug discovery project, and even the structures of membrane receptors can now be solved. With this structural information, it is often possible to combine the different lead-finding approaches into a broader, integrated lead-finding strategy. The structural information gained from each individual hit thereby adds to an overall understanding of how best to fill the binding pocket of a target and can be used to design new chemotypes based on a holistic understanding of the contributions of many diverse molecular substructures. Different to previous times, individual compound classes thereby no longer serve as separate and unconnected starting points for the medicinal chemist but contribute to an integrated strategy. Moreover, hit finding, in particular FBS, can be used to exhaustively map a target's binding site and provide the chemists and molecular modelers with

valuable ideas for the design of new chemotypes and, perhaps even more importantly, for the further optimization of lead structures. In this way, lead finding today may no longer be seen as a one-off activity at the beginning of a drug discovery project but rather as a continuing activity which accompanies compound optimization. Both, lead finding and lead optimization, can cross-fertilize each other, and the former may be run in iterative cycles with knowledge gained from previous cycles as well as lead optimization efforts feeding forward into the next cycle.

2.7 Biologics

Modern molecular biology techniques have also expanded the drug space beyond traditional synthetic small molecular weight compounds and have enabled the design, production, and development of biologic molecules as drugs. Of the 624 drugs approved by the FDA over the past 20 years, 84 were biologics (Mullard 2014). However, their impact for the pharmaceutical industry has been even bigger than these numbers suggest as seven of the ten biggest selling drugs in 2013 were biologics. So far these drugs were dominated by antibodies, soluble receptor constructs, immunoglobulin fusion proteins, and secreted naturally occurring proteins. The most prominent examples are tumor necrosis factor (TNF) alpha-blocking antibodies (infliximab, adalimumab) and the soluble TNF receptor fusion protein (etanercept) for the treatment of rheumatoid arthritis, the anti-CD20 antibody rituximab for non-Hodgkin's lymphoma, the anti-vascular endothelial growth factor A (VEGF-A) antibody bevacizumab for colorectal and other cancers, and the antihuman epidermal growth factor receptor 2 (HER2) antibody trastuzumab for the treatment of breast cancer. Beyond these "classical" drugs, the biologics space has grown over recent years, for example, by introduction of antibody-small molecular weight drug conjugates or bispecific antibodies, and is likely to continue to grow at a rapid pace over the coming years. The advantages of biologics are their high affinity for and specificity to their targets, but so far they are mostly limited to secreted or cell surface targets.

3 Where Do Targets Come from?

A minority of drug discovery projects prior to the mid-1980s were target based. One such case is the discovery of statins as HMG-CoA reductase inhibitors to lower cholesterol levels (Tobert 2003). Details of the cholesterol biosynthesis pathway were worked out in the 1950s and 1960s and HMG-CoA reductase established as the rate-limiting enzyme. The first potent inhibitor was found in the mid-1970s using an assay that involved radioactively labeled substrates in cell extracts. Today the establishment of targets for drug discovery is in many cases still based on advances in basic science over many decades and constituted by a series of important discoveries. For example, the capacity of tumor cells to stimulate angiogenesis was discovered in 1945 (Algire and Chalkley 1945) and the presence of soluble tumor-derived factors demonstrated in 1968 (Greenblatt and Shubi 1968).

This led to the formulation of the "antiangiogenesis" therapeutic concept for treatment of tumors (Folkman 1971). The subsequent purification of VEGF-A and its cloning in 1989 (Leung et al. 1989) facilitated the discovery of bevacizumab, the first anti-VEGF-A antibody (Presta et al. 1997). Another example is the discovery of imatinib for the treatment of chronic myelogenous leukemia (CML) (Capdeville et al. 2002). A chromosomal abnormality, the "Philadelphia chromosome," was discovered in 1960 in white blood cells of patients with CML. In 1973 the Philadelphia chromosome was shown to be a translocation between chromosomes 9 and 22. A series of subsequent discoveries resulted 1985 in the insight that the chromosomal translocation leads to the expression of the BCR-Abl fusion protein and the hypothesis that its tyrosine kinase activity drives malignant transformation (Shtivelman et al. 1985). Imatinib was subsequently developed as an inhibitor of the BCR-Abl kinase. The pace in the advancement of such fundamental science for the discovery of drug targets has dramatically increased with the sequencing of the human genome and the establishment of next-generation sequencing technologies. Many recently approved drugs, in particular in the oncology field, are targeting proteins that have been identified through human genetic information. This includes the discovery of ibrutinib, an inhibitor of Bruton's tyrosine kinase for the treatment of B-cell lymphomas (Honigberg et al. 2010); vemurafenib, an inhibitor of the activating mutant $BRAF^{V600E}$ protein for melanoma (Sala et al. 2008); and the Janus kinase 1 and 2 inhibitor ruxolitinib for myeloproliferative neoplasms (Quintás-Cardama et al. 2010).

Pharmaceutical or small molecular weight tool compounds have similarly helped to study complex biological systems and allowed the identification and characterization of novel drug targets. One of many examples is the discovery and validation of phosphodiesterase four isoenzymes for the treatment of lung diseases using nonspecific and isoenzyme-specific inhibitors (Torphy and Undem 1991). This ultimately led to the discovery of roflumilast for the treatment of chronic obstructive pulmonary disease. Over the past decades, these pharmacological tools were more and more complemented with biological tools, in particular antibodies, to study the functional roles of secreted proteins and receptors in vitro and in vivo. Many of these biological tools were directly developed as therapeutics once the target characterization and validation studies proofed promising. A showcase is cytotoxic T-lymphocyte-associated protein-4 (CTLA-4), a member of the immunoglobulin superfamily, which is expressed on the surface of T cells and transmits an inhibitory signal to these cells. The relevant scientific findings that define this target were made using specific monoclonal antibodies which block the binding to its ligands CD80 and CD86 on antigen-presenting cells thus leading to T-cell activation (Linsley et al. 1992) as well as with a CTLA4-IgG Fc fusion protein which binds to CD80 and CD86 and prevents T-cell activation (Linsley et al. 1991). The former has been developed as therapeutic for cancer immunotherapy (ipilimumab) and the latter for the treatment of rheumatoid arthritis (abatacept). Other important biological tools today are based on interference RNA (RNA_i) (Mohr et al. 2014) and CRISPR (clustered regularly interspaced short palindromic repeats) (Doudna and Charpentier 2014) technologies which allow specific gene expression silencing or even enable surgical genome editing, respectively.

Such tools can be used for both dedicated reverse genetic experiments and broad, even genome-wide, screens.

Today, the existence of large and diverse compound libraries in combination with great advances in cell and organoid culture technologies makes phenotypic screening also an interesting approach for target and drug discovery. The recently approved hepatitis C virus NS5A inhibitor ledipasvir is based on the discovery of the target as well as the chemical lead structure in a phenotypic screen using a viral replicon system in a human hepatocyte cell line (Gao et al. 2010). Such screens can be extended to whole organisms. The first-in-class antimalarial drug KAE609 currently in phase 2 clinical trials was discovered employing a *Plasmodium* whole-cell proliferation assay with cultured intraerythrocytic parasites (Rottmann et al. 2010). KAE609 is a spiroindolone that targets the P-type cation-transporter ATPase4, a membrane transporter protein regulating sodium homeostasis and thus the osmoregularity of the parasite. Like artemisinin, KAE609 targets all stages of the life cycle of malaria parasites which is important for fast parasite clearance. Interestingly, phenotypic screening appears to be particularly successful for antiparasitic drugs, and around 80% of new antimalarial drugs in preclinical or early clinical phases at the moment have come from phenotypic screens (Cully 2014). In addition to these more complex phenotypic screens, also screens interrogating biological pathways have been used successfully for drug and target discovery. An example is the porcupine inhibitor LGK974 which targets an acyltransferase in the Wnt signaling pathway and is currently in phase 2 clinical trials for Wnt-dependent cancers (Liu et al. 2013). The inhibitor was found in a screen for inhibitors of Wnt secretion using a coculture system of a Wnt-secreting and a Wnt-reporter gene cell line.

4 Changing Landscape of Academic and Pharmaceutical Research

During the period covered by this chapter, there also have been major environmental changes for drug discovery.

4.1 Laboratory Size

At the beginning of the period, the traditional small laboratory illustrated in Fig. 4 was still prevailing where a master (primary investigator) was working with few apprentices (PhDs, postdocs) in relative isolation conducting experiments by hand and with relatively simple apparatus. Today laboratories are extensively computerized and automated (Fig. 5). With the advent of target-based drug discovery, testing of drugs in whole organism was replaced increasingly by in vitro methods with binding studies probably reaching the bottom of complexity. Today

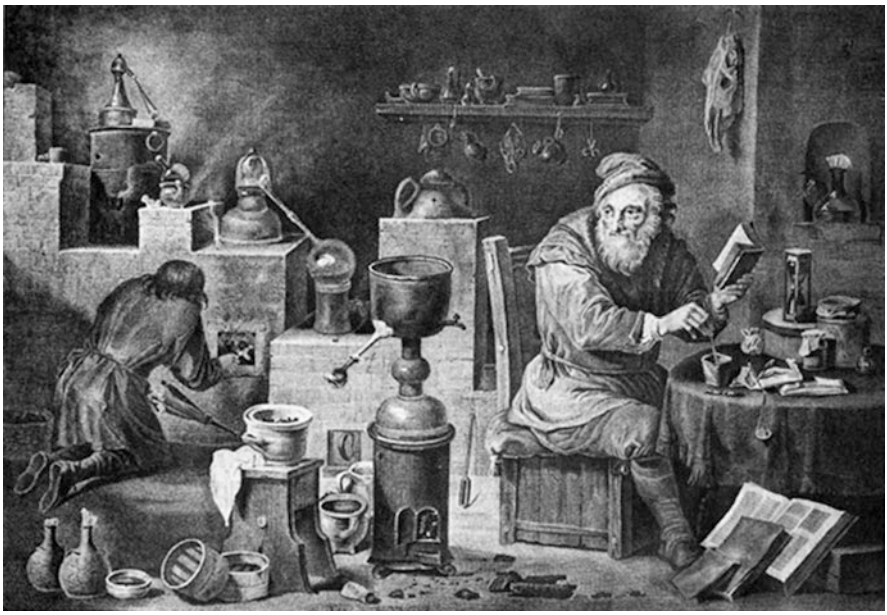

Fig. 4 "Der Alchemist" engraving by Pérée from a painting of David Teniers (1610–1690) illustrating the structure of small labs still retained today

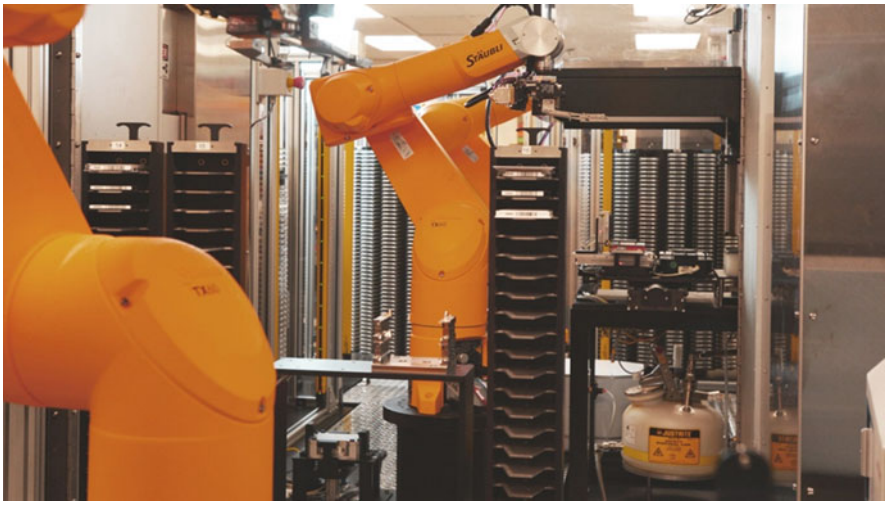

Fig. 5 Fully automated high-content high-throughput screening laboratory, Novartis Institute for Functional Genomics, La Jolla, USA

the complexity of the assay systems has increased again from high-throughput screens on purified proteins to high-content cellular screens allowing evaluation of the effects of new drugs on cells and within them on specific biological pathways.

4.2 Research Center Size and Distribution

In the 1990s it was fashionable to have scientists work in biotech like small groups that could be geographically disseminated under the assumption that their creativity would be better than in large research centers. More recently two factors have caused pharmaceutical research to be increasingly concentrated in large centers (campuses) with thousands of scientists working in walking distance from each other and as close to academic centers of excellence as possible (see below). The first factor is technological: some of the equipment needed to conduct modern drug discovery is very expensive and requires large infrastructures. Examples are robotized screening facilities, compound archives, state-of-the-art animal facilities, high-end microscopy, NMRs, and other analytical tools for chemistry. These tools are deployed in technological platforms that have become too onerous to be multiplied within the same organization at too many geographical sites. On the other hand, for a large pharmaceutical company, it is important to have access to top-quality talent in diverse cultures so that today they usually have Asian, European, and US hubs.

The second factor is biological. As our knowledge of biology grows, it becomes apparent that evolution has been reusing biological components and processes in different environments and in different organs and tissues. This applies prominently to proteins, signaling processes, and cellular pathways. Furthermore, diseases thought to be distinct because of different symptoms and occurrence in different organs turn out to have common molecular pathway malfunctions that simply cause different phenotypes but can be addressed by causal mechanism-specific interventions rather than by trying to correct the symptoms as was mostly the case in the past. An increasingly important consequence of this evolutionary conservatism at the molecular mechanism level is a trend to increasingly classify diseases not by symptoms and organs they occur in but by the causal mechanisms. This changed disease classification method is most advanced in the field of oncology but visible also in immunological disorders. This means that a successful research organization must have experts both in human disease and experts understanding molecular disease processes in all areas they want to be active in. As a drug affecting specifically a disease mechanism might be beneficial in diseases in different organs and with different symptoms, it is essential that scientists of different therapeutic areas interact closely to increase the probability that all potential applications of a drug are found thereby multiplying the clinical use and the returns on the investment. Drawbacks biotech companies must try to solve are that they often cannot afford all technologies needed for drug discovery and, as they

are often focused on one or few indications, that multiple applications of an innovative therapeutic mechanism they discovered will be found and exploited by others.

4.3 In-House and Outsourced Research, Academic Collaborations, and Consortia

Recently a trend has been seen in some large pharmaceutical companies to outsource increasing parts of their research activities under the assumption that they can buy the research products that they need for their commercial success. If the aim is a short-term improvement of the bottom line, then such a systematic outsourcing of research might be successful. If the longer survival of the company is the goal, then the outsourcing strategy might be a bad idea. Biomedical sciences and technologies are highly multidisciplinary scientific activities where the knowledge increases exponentially as was illustrated earlier in this chapter with the evolution of the knowledge about receptors, proteins, and pathways within the last 50 years. The same applies to causes of diseases. In order to understand and make use of the evolving knowledge for significant medical advances, there is only one way: participating in the science with your own scientists. People not participating cannot reach the level of understanding, and even if they tried to reconstruct it from the literature, they would have a significant time disadvantage in addition. Unless a company has a critical mass of scientists participating in all the areas it considers strategic, it will not be able to recognize where to "buy" in a timely and competitive way leading to a continued erosion of their pipeline.

A second essential reason why a critical mass of own scientific research is needed is that many or most of the technological and basic scientific breakthrough needed for pharmaceutical breakthrough relevant for patients occur at academic institutions. As mentioned the only way to recognize and understand the relevant science in a timely fashion the industrial scientists needs to be in close interaction and collaboration with academic scientists so that they will be able to translate the academic breakthroughs into medical breakthroughs which is the only goal of industrial biomedical scientists. Successfully achieving this goal is the only strategy ensuring the long-term success and survival of the company while delivering an essential service to patient and society.

Some scientific questions are too complex to be solved by individual scientists and small laboratories. Examples are systems biology or the quantitative description of cellular-, tissue-, organ-, or entire-organism processes to allow computer simulations that are adequate to predict how these systems will behave. Other examples are the Human Brain Project of the EU or The Cancer Genome Atlas of the USA. These projects are big science projects only possible if large numbers of scientists agree on complementary work programs, data standards, and common database formats. Such topics are sometimes addressed by consortia in public-private partnerships. One directly relevant to drug discovery is the "The Biomarkers Consortium" (Wholley 2014) where scientists from academia, the pharmaceutical

industry, and the US Food and Drug Administration (FDA) work together under the leadership of the Foundation for the NIH to explore and validate relevant biomarkers that could significantly improve the predictability and efficiency of clinical studies.

So in conclusion scientists from academia, the regulatory agencies, and the pharmaceutical industry all contribute to innovative drug discoveries, and, in order to translate the basic science findings into new medical treatments that fulfill unmet patient needs, the pharmaceutical industry needs a critical mass of excellent scientists working in many therapeutic fields, disciplines, and technologies.

4.4 Me-Too Drugs vs. Medical Breakthrough

In the previous section, medical breakthroughs are emphasized. Yet it is mostly easier to make me-too drugs or drugs that are copying an existing one without major additional medical advantage. (A second-generation drug with significant medical advantage is not a me-too but a breakthrough.)

The reason for the imperative to generate breakthrough medical pharmaceutical advances is due to a, to our minds, positive development in our societies. Firstly, pharmaceutical treatments are paid in one way or another by society. Secondly, pharmaceutical treatments work increasingly well, eliminating symptoms, improving the course of diseases, or prolonging life up to achieving complete cure. For these reasons society wants innovative drugs, but there is a limit to how much it can or wants to pay for them. While in the past it was possible to get approval and a market price for me-too drugs, today increasingly society and the payers of medicines are only prepared to pay for significant medical advantages or innovative drugs. Innovation in this context is defined only from one point of view: what needed medical advantage does a new medicine bring to a patient that was not available before. So innovation is not a new molecule, pathway, or target per se *unless* it delivers the medical advance required. A medically relevant innovation can be as "little" as an oral form of a medication in an indication where before only an intravenous injection or constant infusion was available or as "much" as life-prolonging therapies such as imatinib in chronic myelogenous leukemia. In the best case a new medicine is lifesaving as in the case of antibiotics overcoming emerging resistances of infective organisms. Other effective classes of medicines where innovation is urgently needed are the ones that can prevent the occurrence of diseases such as vaccines.

In conclusion, increasingly societies will be unwilling to pay a premium price for undifferentiated even though patented me-too drugs. Research-based pharmaceutical companies with a long-term strategy have a higher probability of success if they build the culture and expertise for biomedical breakthrough innovation addressing the many still unfulfilled medical needs.

4.5 Science Expertise and Culture at the Top

Another important cultural element in the pharmaceutical industry is the expertise of its top management. In the early days, pharmaceutical top management was predominantly composed of chemists, pharmacologists, and medical doctors along with commercial entrepreneurs. But in more recent times, it could be seen that the weight of the scientific disciplines in top management has declined in favor of very predominantly commercial/marketing and financial expertise to the point of being nearly nonexistent in some companies. This is a trend that is dangerous for research-based pharmaceutical companies that envisage a long-term strategy because the key strategic and pipeline decisions must be rooted in scientific, technological, and medical expertise. Both of these are essential to understand patient's needs and how to meet them with medicines that are highly sophisticated packages of scientific information incorporated into biologically active molecules. In view of the immense resources needed to discover and develop new medicines, it is of course important to include commercial expertise in top management but not to the exclusion of scientific expertise.

In conclusion, if a research-based pharmaceutical company wants to be sustainable for the long term, it is essential to have a strong and influential science, medical, and technological component in top management balanced by long-term visionary commercial expertise.

4.6 Productivity

Despite the many great successes of the pharmaceutical industry, there has been an apparent decline in R&D productivity during the past decades manifested by decreasing numbers of drugs approved per billion US dollar spent (Scannell et al. 2012). Paradoxically, a major reason for the apparent decline lies in these past and present achievements of the industry. The ever-growing number of successful drugs inherently increases the scientific, medical, safety, and regulatory hurdles that have to be overcome for new therapies. In addition, with a patent life of 20–25 years and an average preclinical/clinical drug development time of 10 or more years, a proprietary drug can be marketed by a company only for about 10–15 years. A large pharmaceutical company with annual sales of 10–30 billion dollars, therefore, strives to constantly invent a new drug portfolio of the same size (or ideally more) within a 10–15-year time frame just to maintain overall sales figures. To accomplish this, companies typically invest 10–20% of their revenues into R&D activities. And for these activities, the incentive to discover biomedical breakthrough drugs is high as outlined above. Moreover, according to a recent analysis, the first-, second-, and eventually third-in-class drugs will capture more than 90% of the market value in most therapeutic areas (Schulze and Ringel 2013), and thus the focus of many pharmaceutical companies today is to discover and develop first-in-class and best-in-class drugs. There was a widespread trend in the field during the late 1990s and early 2000s to industrialize drug discovery and to

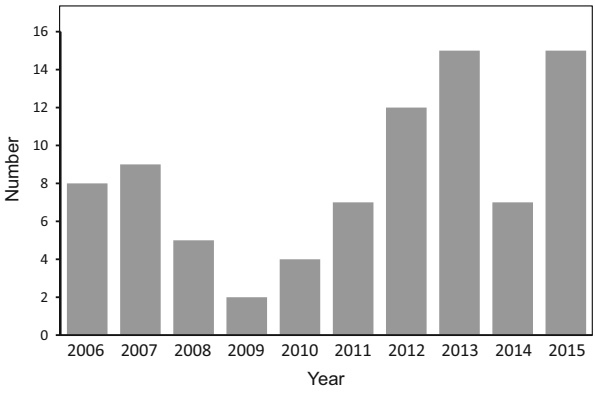

Fig. 6 Number of 1st-in-class drugs approved by the US Food and Drug Administration during the past 10 years

mold it into a linear process comprising a number of separate phases or process steps (target identification, tool production and assay development, hit finding and validation, hit-to-lead, lead optimization, preclinical development). Accepting a priori a highly increased attrition rate in the research phase, the assumption was that brute-force and ever larger numbers of projects and high-throughput experiments would drive productivity. Today, the pharmaceutical industry has largely taken a step back from this brute-force approach realizing that it rather hampered creativity, innovation, and ultimately productivity. Instead, the focus is now much more on science-driven approaches in areas of high unmet medical need where basic science has laid a good foundation for a sufficient mechanistic understanding to allow successful drug discovery. In addition, the industry has started to digest the recent revolutionary advances in technologies and genomics resulting in increased knowledge about complex biological systems and human pathophysiology. This is also reflected by the sharply rising numbers of approved breakthrough therapies by the US Food and Drug Administration (FDA) in 2013 and 2014. In addition, also the numbers of first-in-class drugs approved by the FDA have significantly increased in recent years (Fig. 6). Taking into account the long median time of about 20 years for drug discovery from target identification to regulatory approval (Eder et al. 2014), it appears that only now we are about to see the full impact of modern drug discovery approaches and of the information gained from sequencing the human genome on the productivity of the pharmaceutical industry. This will, no doubt, continue for many decades to come and further change the practice of medicine in significant and probably as yet unimaginable ways.

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Functional Genomics in Pharmaceutical Drug Discovery

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Abstract

Targeted therapies in personalized medicine require the knowledge about the molecular changes within the patient that cause the disease. With the beginning of the new century, a plethora of new technologies became available to detect these changes and use this information as starting point for drug development. Next-generation genome sequencing and sophisticated genome-wide functional genomics' methods have led to a significant increase in the identification of novel drug target candidates and understanding of the relevance of these genomic and molecular changes for the diseases. As functional genomic tool for target identification, high-throughput gene silencing through RNA interference screening has become the established method. RNAi is discussed with its advantages and challenges in this chapter. Furthermore the potential of CRISPR/Cas9, a gene-editing method that has recently been adapted for use as functional screening tool, will be briefly reviewed.

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