Development of Therapeutic Agents Handbook

Edited by Shayne Cox Gad

WILEY
DEVELOPMENT OF THERAPEUTIC AGENTS HANDBOOK
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The Development of Therapeutic Agents Handbook represents a collective attempt to present the broad range of areas of therapeutic needs and classes of potential new therapeutic moieties, assembled in the context of the Wiley pharmaceutical development handbook series, which covers the entire process of pharmaceutical discovery and development. This volume, in fact, is the eighth in this series, which, in its entirety, is intended to be comprehensive in its coverage.

This volume is unique in that it seeks to cover the entire range of significant therapeutic areas and their underlying causes and then proceeds to overview specific classes of agents over a wide range of disease claims. The 54 chapters cover introductory and regulatory issues, disease mechanisms, and potential therapeutic targets, as well as new classes of therapy in development.

This book would not have occurred without the dedicated efforts of Wiley’s managing editors, Monika Laszkowska and Michael Leventhal. Their persistence in the recruitment of contributors and ensuring follow-through was essential.

While like all textbooks, this one presents the state of the practice and field at a specific period in time. I hope that it will become a frequently consulted friend.

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PART I

FUNDAMENTALS AND CONCEPTS
CURRENT NEEDS FOR NEW THERAPEUTIC AGENTS AND DISCOVERY STRATEGIES—A SYSTEMS PHARMACOLOGY APPROACH

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1.1 INTRODUCTION: A BRIEF HISTORY OF DRUG DISCOVERY

The use of remedies to treat or alleviate symptoms of a medical condition can be traced as far back as ancient Egypt. The Ebers papyrus, dating from 1555 BC, was found to contain 876 concoctions to treat a wide variety of disorders [1]. Early medicinal efforts were also used by the Greeks, most notably Hippocrates, and by several Asian cultures including the Chinese [2]. However, the identification of active ingredients and the development of the interdisciplinary science of pharmacology that bridged organic chemistry, zoology, and pharmacology did not emerge until the late 1800’s. These advances were made possible by progress in chemistry, including theories on acids and bases and on the structure of aromatic molecules such as dyes [3]. In the 1870’s, Paul Ehrlich proposed the existence of “chemoreceptors”, which differed between microorganisms and the host tissue, based on his studies of dyes in biological tissues [3]. He suggested these could be used therapeutically, which eventually gave rise to the development of a class of drug treatments known as chemotherapy.

At the beginning of the twentieth century, pharmacology progressed quickly. In 1905, J. N. Langley introduced the concept of a “receptive substance”, the modern basis for the study of receptor agonists and antagonists [4, 5]. In 1933, Meldrum and Roughton identified the enzyme carbonic anhydrase while studying the effects of sulfanilamide, the active metabolite of the antibiotic sulfamidochrysoidine [6]. This discovery led to the concept of enzymes as a good target for drug discovery and gave further importance to the biochemical characterization of cellular functions. Following Alexander Fleming’s discovery of penicillin as a product from the Penicillium mold that killed Staphylococcus bacteria [7], many drug companies invested in microbiology, resulting in the discovery of more antibiotic and other therapeutic agents [3].

Over the next decades, drug discovery progressed along with our understanding of the basic sciences that underlie the discipline of pharmacology. What is currently referred to as interdisciplinary and translational basic science became known as pharmacology and experimental therapeutics. The large explosion of chemical libraries in the 1980’s by the large scale implementation of combinatorial chemistry required the simultaneous development of high-throughput screening (HTS) methodologies. With these new technologies, many hundreds of thousands of compounds could be synthesized and then screened against a target of interest. However, this approach was often largely detached from a physiologically relevant screening signal. Another major breakthrough that changed the way society as
CURRENT NEEDS FOR NEW THERAPEUTIC AGENTS AND DISCOVERY STRATEGIES

4

ity or translation of a messenger RNA (mRNA) (such as fomivirsen (Vitravene, ISIS Pharmaceutical and Novartis), an antisense molecule designed to treat cytomegalovirus infections in the retina of immunocompromised patients). The decision to use a small molecule or a biotherapeutic agent is generally dictated by the desired target and, as discussed below, these two classes of therapeutic agents complement each other in terms of their respective capabilities and weaknesses.

Small Molecules and Natural Products  For drug discovery, small molecules are typically less than 500 molecular weight (MW) units, although this is not an absolute cut-off as molecules of higher molecular weight could be acceptable if sufficient bioavailability could be achieved. Ultimately, most small molecules are chemically synthesized, but their discovery may be the result of different strategies. Historically, most small molecules were initially isolated from natural products, which is now in disfavor due to the unfavorable logistics of developing commercially viable large-scale chemical syntheses of complex natural products. For example, the compound paclitaxel (Taxol, Bristol-Myers-Squib) was isolated from the bark of a North American yew in the 1960's (Fig 1.1) [9]. It was later shown to have novel anticancer properties by binding to tubulin, resulting in the stabilization of microtubules and disruption of mitosis [10]. However, the treatment of one cancer patient would have required the harvesting of six yew trees. To address this issue, a synthesis scheme was eventually developed using an analog of paclitaxel,

well as pharmacologists thought about therapeutics coincided with the development of the human genome project in 1986. The hope that a large number of drug targets would soon be identified and, in combination with the HTS technology, that a rapid increase in the discovery of therapeutic agents would result [8] effused through the pharmacology and biomedical sciences community as well as Wall Street aficionados. However, this original optimism met with a declining number of drugs approved by the U.S. Food and Drug Administration (FDA) since 1996, seeming to suggest that a target-based random screening approach was not the panacea originally envisaged for drug discovery.

In this chapter, we will provide a brief overview of some current therapeutic agents, including their weaknesses, and highlight selected opportunities for continued drug discovery efforts in certain disease areas. We will then focus our attention on different strategies used to identify new therapeutic agents, comparing target-based discovery (TBD) and what we refer to as systems-based discovery (SBD). Although pharmaceutical companies have favored TBD during the past thirty years, emerging evidence indicates that SBD may help in solving some of the issues that have plagued the modern approach to drug discovery. Finally, we will explore the challenges associated with the application of SBD to neurological disorders. This chapter is intended neither to be exhaustive or fair and balanced but presents a selective viewpoint. We apologize to those pioneers whose work we may have failed to cite and for simplifications we introduced in the interest of streamlining this chapter.

1.2 CURRENT STATE OF THERAPEUTIC AGENTS AND THE NEED FOR NEW AGENTS

1.2.1 Overview of the Current State of Therapeutic Agents

Therapeutic agents currently on the market can be placed into either one of four categories: small molecules, which are chemically synthesized compounds such as aspirin; natural products, which are small, naturally occurring molecules that are generally isolated from plants, fungi and mold (such as penicillin); biotherapeutics, which are macromolecules that occur naturally (such as insulin and tissue plasminogen activator) or are engineered based upon a biological template (such as trastuzumab (Herceptin, Genentech), a monoclonal antibody designed to treat HER2-positive breast cancer); and nucleic-acid-based therapeutics, which are deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) molecules designed to interfere with the stabil-

![Figure 1.1](image-url)  Chemical structure of (A) paclitaxel and (B) docetaxel.
In the final steps of the lead identification process, com-
modeling, bioinformatics, and computational chemistry.
Amino acids at the desired site of action using molecular
molecules may then be designed to interact with specific
magnetic resonance (NMR) spectroscopy. Candidate mole-
obtained using X-ray crystallography and nuclear mag-
of the active site and other binding pockets can be
emerged. Information required to deduce the structure
guided by the structure of the target recognition site(s)
concept that the design of small molecules could be
of the three-dimensional structure of proteins, the
variants of a known drug can give rise to novel, improved
of structure–activity relationship studies based on
schedule independent [14]. This example demonstrates
spectrum, docetaxel is also an improvement over pacli-
colon adenocarcinoma, in which paclitaxel was ineffec-
using in vivo xenograft models revealed that docetaxel
higher affinity for microtubules [13]. Further studies
using in vivo xenograft models revealed that docetaxel
was about twice as potent as paclitaxel. When compared to paclitaxel in different cell
line models of tumor, docetaxel was 1.3- to 12-fold more
potent and this was found to be due to docetaxel’s
higher affinity for microtubules [13]. Further studies
of the 1990’s but seemed to become incorporated with the plat-
form technology of medicinal chemistry rather than the
driving force behind new pharmacological discovery.
The Bcr-Abl kinase inhibitor imatinib mesylate
(Gleevec, Novartis) is considered to be the first drug
designed rationally [15, 16]. Chronic myeloid leukemia
is the result of a reciprocal translocation of chromo-
somes 9 and 22 which creates a gene encoding for the
Bcr-Abl kinase, a highly active tyrosine kinase [17, 18].
The design of a specific Bcr-Abl kinase inhibitor takes
advantage of the fact that, although several kinases
display sequence homology and similar active confor-
mations, the kinases differ in the conformation of their
inactive state [19]. Hence, a specific inhibitor could be
designed to stabilize the enzyme’s inactive confor-
mation, which is different from that of other kinases, and
act by preventing phosphorylation of the substrate(s).
The crystal structure of Bcr-Abl kinase bound to an
analog of imatinib mesylate appeared to reveal an
atomic basis for the specificity of interaction [19]. The
compound and Bcr-Abl kinase form a number of hydrogen
bonds and van der Waals interactions, which are not
possible with other kinases. Interestingly, imatinib
mesylate interacts specifically with the inactive confor-
mation of the Bcr-Abl kinase as activation causes a
conformational change in the kinase that prevents the
drug from binding.
During the development of small molecules thera-
peutics, optimization is geared toward obtaining a desir-
able set of physico-chemical properties. At the molecular
level, these generally include potency and efficacy and,
in most cases, specificity for a particular target. At the
level of the whole organism, low toxicity (at an effica-
cious dose) and adequate pharmacodynamic and phar-
macokinetic properties must be achieved. For most
therapeutic candidates, the ability of the compound to
cross the blood–brain barrier (BBB) and blood–nerve
fiber barrier (BNFB) must also be taken into consider-
ation. Nervous tissues are very effectively shielded from
endogenous and exogenous molecules, whether cen-
trally or peripherally, through the use of physical, bio-
chemical, and cell biological mechanisms (reviewed in
ref. [20]). The endothelial cells lining the intracerebral
capillary walls are unusual in that they are connected
by tight junctions forming patent zonula occludens
and lack fenestrations. In addition, these endothelial
cells possess metabolic enzymes and transmembrane
transporters that further decrease the penetration of
most drugs. Small molecules may cross the BBB with
both passive or active mechanisms, as well as vesicular
trancellular mechanisms. For passive transport, the size of the molecule, its lipophilicity and charge (pKₐ) are the main determinants. Drug candidates with specific functional groups may also be actively taken into the brain via transporters. In some cases, however, BBB integrity may be disrupted, facilitating the penetration of small and large molecules. This is seen most often following pathological conditions, such as stroke, trauma, infections, and neuroinflammatory diseases.

Over the course of the small-molecule development process, BBB permeability may be assessed at different stages by using several methods [20–22]. Computational models have been developed based on known drugs that act in the central nervous system (CNS) (called a “training set”). However, these models are limited by the small size of the training set and by the relatively narrow chemical space it covers [21]. In vivo assays can be conducted in both humans and animals using analysis of pure venous blood, microdialysis, and/or positron emission tomography (PET) imaging [20, 22]. However, these methods are generally low-throughput, labor intensive and costly. The use of in vitro assays, based on cell lines transfected with efflux transporters, was found to be a good complement to previously described methods [23]. It is also important to keep in mind that while BBB permeability is critical for drugs acting in the CNS, it is also an advantage to reduce the BBB permeability of drugs targeted outside of the nervous system to decrease potential CNS-mediated side effects. This is the case for loperamide, a therapeutic agent used to treat diarrhea [24]. Loperamide is a peripherally acting μ-opioid receptor agonist targeting receptors located in the large intestine. Because it does not cross the BBB, loperamide is devoid of analgesic properties usually observed with BBB-permeable μ-opioid agonists such as morphine.

Unfortunately, many drugs are marketed without providing doctors and patients information about BBB permeability and possible neurological side effects. An example of this situation is provided by the aromatase inhibitors (AIs), used to reduce the recurrence of estrogen-responsive breast cancer, either in conjunction with or following treatment with tamoxifen [25]. AIs work by inhibiting the aromatase (also called CYP19), the enzyme responsible for producing estrogen. Three AIs are currently on the market: the non-steroidal agents anastrozole (Arimidex, AstraZeneca) and letrozole (Femara, Novartis), and the steroidal compound exemestane (Aromasin, Pfizer). Aromatase is present in several tissues including adipose tissue, uterus, bones and brain. While a reduction of estrogen levels in adipose tissue is critical to prevent the resurgence of breast cancer, studies also suggest that estrogen may be important for maintaining cognitive functions [25]. Hence inhibiting estrogen production in the brain may lead to cognitive decline. Strikingly, no information regarding cognitive function following chronic AI treatment is provided to the physicians or patients, even though these drugs were approved over 8 years ago. Studies regarding the possible effects of AIs on cognition in breast cancer patients have only been recently initiated, and, although preliminary results are beginning to come out, the numbers are still too small to determine whether there is an effect (reviewed in ref. [26]). If one or more of the AIs were known to be BBB impermeable, doctors and patients may be able to make a better informed decision regarding the treatment choices and quality of life.

Small molecules may be active at intracellular, extracellular, or both locations of targets and, as such, have traditionally been the platform of choice to act on enzymes and receptors. Moreover, depending on their physico-chemical properties, small-molecule drugs have been designed to be orally bioavailable, which greatly facilitates administration and patient compliance.

Biotherapeutics A biotherapeutic has thus far been considered to be a protein that works by mimicking the action of a naturally occurring substance. In many cases, the therapeutic was no more than the endogenous protein that was produced by a different method, for example, insulin produced synthetically using recombinant DNA technology as compared with purified insulin. Historically, protein therapeutics were isolated from human or animal sources, and their use carried risks such as variable efficacy, contamination by resident infectious agents, and immunological reactions. Most biological therapeutics are now manufactured using recombinant DNA technologies, allowing for a reliable and consistent product [27]. Immunogenic reactions to biotherapeutics are still a concern, but the added level of safety that is derived from a more chemically defined preparation justifies the approval as adverse reactions can be managed in most cases [28].

Recombinant protein therapeutics developed on the heels of the revolution in the area of molecular biological technologies. In 1972, the first article describing the use of restriction enzymes to cut two viral DNAs was published [29], and, by 1978, Genentech, the first company founded to develop biotechnologies, had expressed human recombinant insulin in Escherichia coli (E. coli) and produced about 20 ng of purified protein [30]. This joint venture with the pharmaceutical giant Lilly resulted in clinical trials beginning in 1980, and in 1982 human insulin manufactured by fermentation of E. coli became the first recombinant biotherapeutic to be approved by the FDA.
Over the years, recombinant therapeutic design has evolved and most protein therapeutics that are on the market are not exact duplicates of their endogenous human counterpart. For example, amino acid substitutions are sometimes included to improve pharmacokinetic properties [28]. In other cases, mutations may be induced to change a protein’s function. Pegvisomant (Somavert, Pfizer) is a growth hormone receptor antagonist used to treat acromegaly (reviewed in ref. [31]). Substitution of 1 amino acid in the human growth hormone’s 191 amino-acid-sequence is enough to turn this agonist into an antagonist. To create pegvisomant, eight other residues were mutated, increasing binding affinity to the receptor, and polyethylene glycol molecules were added to promote stability of the antagonist. Interestingly, even with modifications recombinant protein therapeutics are generally well tolerated and, for the most part, do not trigger immunological complications [28].

The use of antibodies as therapeutic agents has also emerged as a new technology. The success of immunization as a method to protect against infectious agents such as the measles and rabies virus stimulated thought that polyclonal antibodies isolated from human or animal serum could be used to combat toxins and venoms as well as to protect against infections. When this approach was tried, hypersensitive reactions were often triggered because the immune system recognized these polyclonal antibodies as foreign agents that needed to be removed. The advent of monoclonal antibody (mAb) production techniques in the 1970’s, along with molecular biology, paved the way for the development and production of chimeric mAbs (murine Fv fragments linked to human IgG Fc fragment), humanized mAbs (human antibody except for the antigen-binding region, which is derived from mouse), and fully human mAbs (derived either from human B cells or from transgenic mice expressing human IgG). Although the immunogenicity of these mAbs is reduced, adverse reactions are still observed in some patients even with fully human antibodies, requiring coadministration of an immunosuppressive agent such as methotrexate (reviewed in ref. [32]). At the molecular level, antibodies may neutralize or antagonize the target, act as agonists, or deplete the target protein from the blood supply. The specific therapeutic action against a target is determined in part by the location of the epitope and by the constant region of the antibody. The ultimate pharmacological success of the therapeutic will require not only biological efficacy but a constant region that minimizes the induction of clearance, complement-dependent cytotoxicity, and antibody-dependent cellular cytotoxicity or apoptosis.

The development of a biotherapeutic for rheumatoid arthritis (RA) treatment provides a good example of the challenges associated with mAb therapeutics [32]. RA is a chronic disease caused by a complex pathology that involves the release of pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), and leads to the destruction of cartilage and bones of non-weight-bearing joints. A major difficulty associated with the use of mAbs is that animal models and ex vivo assays (such as whole-blood assays) offer limited predictability for human efficacy and adverse reactions. For example, at least eleven different antibodies against the T-cell surface antigen CD4 have been developed that are well tolerated and reverse autoimmunity in animal models. However, these same mAbs lacked clinical efficacy when tested in humans. The lack of equivalence between animal models, including non-human primates, and human subjects can sometimes have unexpectedly serious consequences. The activating, anti-CD28 mAb TGN1412 (CD28 SuperMAB, TeGenero Immuno) was shown to be extremely effective at reducing autoimmune reactions without apparent side-effects in both rodent and non-human primate models [33]. However, when TGN1412 was administered to healthy human subjects, all 6 volunteers developed multi-organ failure and extreme cytokine activation. It is unclear whether this reaction could have been otherwise predicted, but it emphasizes the differences between animals and humans when it comes to the pharmacological effects of antibody-based therapeutics.

In contrast to T-cell-directed agents, biological therapeutics targeting TNF-α have been very successful. FDA-approved agents include the anti-TNF-α chimeric mAb infliximab (Remicade, Centocor) and the humanized mAb adalimumab (Humira, Abbott). Both are well tolerated and produce immunogenic reactions in a very small fraction of patients, especially when taken with methotrexate. Another interesting agent is the fusion protein etanercept (Enbrel, Amgen/Wyeth), which is composed of the extracellular domain of the p75 TNF-α receptor attached to the hinge and constant domains 2 and 3 of the human IgG1. This biological therapeutic acts by binding TNF-α, reducing its concentration in blood and preventing its binding to the endogenous TNF-α receptors.

In addition to their traditional roles as immune modulators, antibodies can also be engineered to act as carriers designed to deliver a toxic “warhead” to specific target cells, such as in cancerous tissue. For this application, a specific mAb is conjugated to a toxin, small molecule, or radioisotope that is designed to selectively kill the targeted neoplastic cells [34]. This approach, in principle, allows the accumulation of a high local concentration of active toxic compounds while minimizing side effects. For example, 131I tositumomab (Bexxar, GlaxoSmithKline), which binds to CD20, is used for the
treatment of refractory non-Hodgkins lymphoma [35]. The CD20 antigen is present on normal and malignant B cells, including on 90% of B-cell non-Hodgkins lymphomas. Following a single infusion of $^{131}$I tositumomab, 68% of patients saw improvement in their pathological and clinical profiles, including 33% who showed a complete response (Bexxar product information, GlaxoSmithKline).

The large-scale development of biotherapeutics is hindered by the limited options for drug delivery. Due to their large size and susceptibility to degradation in the gut, these agents must be administered via injections or by implantable control release devices. It is also currently impossible to use biotherapeutics for the treatment of disorders of the nervous system due to the fact they lack BBB and BNFB permeability. However, this issue may be resolved as pre-clinical studies have been conducted using molecular Trojan horses; that is, fusion proteins between molecules that cross the BBB via receptor-mediated transport and BBB-impermeable agents (reviewed in ref. [36]). In those experiments, peptides such as the vasoactive intestinal peptide, and ribonucleic acid (RNA) interference (RNAi) against the epidermal growth factor (EGF) receptor were successfully delivered to the brain parenchyma. However, further studies will have to be conducted to determine the applicability of this method of biotherapeutic drug delivery to human nervous tissue.

**Nucleic-Acid-Based Therapeutics** The latest new category of therapeutic agents to emerge, in the 1990’s, was based on nucleic acids. The early drug candidates in this group took advantage of antisense sequences as a method to specifically inhibit translation of a given gene product. Although the exact mechanism of antisense inhibition is not fully understood, it is believed that hybridization of a short, single-stranded DNA or RNA sequence complementary to an mRNA targets that mRNA for degradation and/or prevents the ribosome from binding to the mRNA, resulting in decreased levels of protein product. Administration of antisense could be used to treat a variety of conditions such as viral infections, cancers and disorders caused by a mutant protein exhibiting a gain-of-function. However, there are also numerous challenges associated with these therapeutic agents including the short half-life of oligonucleotides in biological fluids, their cellular uptake, and the toxicity they may induce [37]. Chemically modified bases can be used to increase stability of the antisense oligonucleotides in vivo. The most common is the phosphorothioate deoxynucleotide, which increases serum half-life from 1 hour for standard oligonucleotide to 9–10 hours for phosphorothioate-containing oligonucleotides. Cellular uptake can be improved by using delivery systems composed of lipids, polymers or nanoparticles. An alternative strategy is to conjugate the antisense oligonucleotide to an antibody or ligand recognizing a receptor on the surface of the cell, achieving targeted delivery. Toxicity is often the result of an off-target effect(s) and needs to be assessed on a case-by-case basis [39].

The first and only FDA-approved antisense oligonucleotide to date is fomiviren (Vitravene, ISIS Pharmaceutical and Novartis). This therapeutic agent received approval in 1998 to treat cytomegalovirus-induced retinitis in AIDS (acquired immunodeficiency syndrome) patients (reviewed in ref. [38]). Fomiviren is composed of phosphorothioate deoxynucleotides and is administered intravitreally. However, although this medication answered a specific medical need, the small number of patients and low sales lead Novartis to discontinue its production. Several other antisense therapies have entered clinical trials, but none have demonstrated sufficient efficacy to obtain FDA approval [37].

RNA interference (RNAi) is a method endogenously used by a wide variety of organisms to target mRNA molecules for degradation [40–42]. Unlike antisense-mediated inhibition, the mechanism by which RNAi works is well understood, and several putative therapeutic agents at different stages of development make use of this technology [43, 44]. RNAi can be achieved using micro-RNA (miRNA), small interfering RNA (siRNA) or short hairpin RNA (shRNA). miRNAs are endogenously encoded single-stranded RNAs that interact with the 3′ untranslated region of mRNAs. Since they do not require perfect sequence homology, miRNAs can interact with 100–200 genes, some of which may be involved in the same signaling pathway [43]. Inhibition of a given mRNA by miRNAs is not complete, but additive effects may be observed if several transcripts in the same pathway are targeted by one miRNA. Endogenous miRNAs, such as miR-146, miR-155 and miR181a, are involved in the development and regulation of the immune system and are altered in chronic inflammatory diseases [45]. In addition, miRNA changes have been reported to correlate with the progression and prognosis of certain cancers [43]. These observations suggest that miRNAs may be an interesting therapeutic target.

The other two types of RNAi tools, siRNAs and shRNAs, are exogenously applied to modify gene expression [46]. siRNAs are short (19–23 nucleotide) double stranded RNAs with a perfect complementarity to their target mRNA. Once they enter the cell, they are taken-up by the RNA-induced silencing complex (RISC) which unwinds the siRNA and degrades the sense strand. The antisense strand is then used as a template to identify the target mRNA, which is then degraded by Argononute-2. A good siRNA can mediate >90% inhibition of its target. A number of siRNAs are
currently in clinical trials [43]. While naked siRNAs may be appropriate for targeting easily accessible tissues such as the eye or the lung, they are not stable enough to be delivered via systemic circulation. For deeper tissues such as the kidney, chemical modifications and/or delivery systems are used [43]. The most advanced siRNA, currently in phase III clinical trials, is bevasiranib (Opko) against vascular endothelial growth factor. It is a naked siRNA administered by intravitreal injection for wet age-related macular degeneration. AKi-5 (Quark/Silence) is a chemically modified siRNA that targets p53 and that is delivered intravenously. It recently began phase I trials for acute renal failure.

As their name indicates, shRNAs are short, single stranded RNA sequences that form a hairpin. In the cytoplasm, this hairpin is cleaved by Dicer, leading to an siRNA that can interact with the RISC as described above. The advantage of shRNAs is that they can be encoded on a viral vector, which can be engineered for tissue-specific and/or controlled delivery (reviewed in ref. [47]). A variety of vectors have been used successfully in animal models, including adeno-viruses, adeno-associated viruses (AAVs), and lentiviruses. Two shRNAs recently entered clinical trials, targeting hepatitis B infections (Nucleonics/Novosom) and AIDS-related lymphoma (Benitec).

RNAi methods are not without risks and challenges. The most common issue observed in animal models has been the risk of saturation of the endogenous RNAi machinery. For example, in mice receiving large doses of one of 49 different AAVs encoding for shRNAs against 6 different genes, 36 constructs led to liver toxicity, including 23 which resulted in death [40]. This was shown to be due to saturation of Exportin-5, a transporter for precursors of siRNAs and miRNAs. This serious problem can be avoided by using naked siRNAs (mature siRNA enter the RNAi machinery downstream from Exportin-5) or by using a lower titer of virus. Viruses engineered to offer controlled expression of their transcript may also be useful, representing a significant opportunity for the development of new therapeutics.

Using RNAi may also cause off-target effects and/or trigger an interferon-mediated response. Both of these can be reduced by chemically modifying the siRNA and by avoiding specific sequences, which are known to be immunogenic. Finally, resistance to a particular RNAi may develop as a single base-pair mutation is enough to disrupt the interaction between an siRNA and its target. This may be avoided by engineering a vector with a few different shRNAs against the same target.

**Portrait of Drug Approval in Recent Years** Most new drugs authorized by the United-States Food and Drug Administration (FDA) are classified as small molecules, whereas biotherapeutics account for 5–29% of the yearly approvals (Fig 1.2). However, despite technical advances and increased spending in research and development by pharmaceutical companies [48], the number of drugs approved has been declining since 1996. This effect is particularly noticeable for small molecule
therapeutics. There are many reasons for this trend, including increased requirements for safety, reduced side-effects, and a litigious system of law [39, 49]. Given the current risks and elevated cost of developing a new drug, pharmaceutical and biotech companies often turn to repurposing, the identification of new indications for existing drugs, or to reformulation, such as developing continuous release or combination therapies, to increase their market share [49, 50].

If the outlook for new drug discovery at large is declining, the glacier trail leading to breakthrough neurotherapeutics is even more daunting and treacherous. According to the World Health Organization [51], 40 million people worldwide are affected by epilepsy, 24 million suffer from Alzheimer’s disease or other forms of dementia, 62 million are diagnosed with cerebrovascular diseases, and 326 million suffer from migraine. However, despite the high prevalence of these disorders, only a few drugs with neurological indications are approved each year (Fig. 1.3). Clearly, therapeutic agents targeting neurological conditions must cross the BBB, but it is estimated that 98% of putative neurotherapeutics fail due to lack of BBB permeability [52]. In addition, candidates often fail during clinical trials due to poor efficacy or safety concerns. As expected, CNS-directed agents tend to cause CNS-mediated side effects such as seizures, dizziness and nausea. Moreover, the lack of validated biomarkers makes it difficult to assess whether the drug reaches concentrations that are sufficiently high at the target to be efficacious [53]. In 2006 alone, 11 drug programs targeting 7 neurological disorders were halted during clinical testing [54]. In the current situation, there is a dire need for new therapeutics directed towards even the most common of neurological conditions. It is sobering to note that with all of its undesirable side effects and adverse reactions mor-

![Figure 1.3](image1.png)

**Figure 1.3** Number of new drugs approved by the FDA from 2003 to 2007 with a primary indication for a neurological disorder or neuropathic pain. Small molecules are indicated by gray bars and biotherapeutics by black bars. The only biotherapeutic recently approved is natalizumab (Tysabri, Biogen/Elan), an anti-α4 integrin monoclonal antibody used to treat relapsing multiple sclerosis (Data source: www.fda.org.)

phine, and its derivative opiate receptor agonists, still provide the mainstay of analgesics.

### 1.2.2 Need for New Therapeutic Agents

Over the last century, there have been major advances in our understanding of pharmacology, biology of disease, and in methods for the discovery of therapeutic agents toward a wide range of maladies. Indeed, the discovery of the “wonder drugs”, the tricyclic antidepressants, phenothiazine antipsychotics, and benzodiazepine anxiolytics, completely changed the way society thought about mentally ill: from the notion of insanity and incarceration to the concept of neurological disorder and outpatient treatment. However, there is a great need for continued research and development to improve existing therapeutics, develop new classes of pharmacotherapies for diseases where the current treatments may not work in some or even most patients, and to establish new treatments for conditions where none are available. Given the decrease in new drug approvals in the last several years, it is important to emphasize that there is a crucial need for more new therapeutics to be developed and submitted for review. In order to improve its productivity, not only must the pharmaceutical industry rethink its drug discovery strategies but the federal government and international world health agencies must work to provide an optimal setting for the high risk high stakes business of drug discovery and development to thrive.

**Improving on Currently Existing Therapeutics** It is easy to imagine the properties of the ideal drug: orally bioavailable, once a day treatment, with no side effects, and completely effective in all patients. Improving on existing therapeutics is an important and valuable method to generate new therapeutics. This strategy mitigates the risks associated with first-in-class therapeutic agents as the chemical genres and target are often well-validated, and certain aspects associated with modulation of the target in humans have been explored. Among the improvements that are generally feasible are enhancements in efficacy, and/or potency, and/or specificity, all aimed at enhancing desired as compared with undesired pharmacological effects via altered pharmacodynamic and/or pharmacokinetic properties. Incremental improvements on the method or frequency of delivery can also provide significant benefits to patients and thus justify the allocation of limited resources to such objectives.

However, this is not a safe haven for the pharmacologist as any modification can cause deleterious side effects even if the discovery effort focused on the creation of a new therapeutic agent acting on the same