

Tutorial on Drug Development for Central Nervous System

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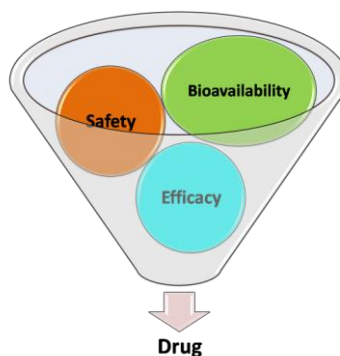
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SYNOPSIS

Many neurodegenerative diseases, such as Alzheimer's and Parkinson's disease, are devastating disorders that affect millions of people worldwide. However, the number of therapeutic options remains severely limited with only symptomatic management therapies available. With the better understanding of the pathogenesis of neurodegenerative diseases, discovery efforts for disease-modifying drugs have increased dramatically in recent years. However, the process of translating basic science discovery into novel therapies is still lagging behind for various reasons. The task of finding new effective drugs targeting central nervous system (CNS) has unique challenges due to blood-brain barrier (BBB). Furthermore, the relatively slow progress of neurodegenerative disorders create another level of difficulty, as clinical trials must be carried out for an extended period of time. This review is intended to provide molecular and cell biologists with working knowledge and resources on CNS drug discovery and development.



Keywords: drug development, blood-brain barrier, drug toxicity, drug safety, drug screening, neurodegenerative diseases

1. Overview of Target-Based Drug Discovery and Development

Historically, many drugs were identified and developed by **physiology/phenotype-based** drug discovery efforts. In physiology/phenotype-based drug development strategy, drugs were discovered by testing compounds in cells, animals, and sometimes even human at the first stage of drug development. This approach does not require initial target identification and validation and instead starts with the analyses of disease-relevant phenotypes and potential side-effects. Identification of drug target and the mechanism of action are not priorities in this case. In contrast, identification and validation of druggable-target are the first steps in **target-based** drug discovery and development. Although there have been some concerns over target-based drug discovery approach^{1,2}, target-based drug development has been the main strategy employed by pharmaceutical companies after the dawn of molecular biology and genomics. Typical steps required for target-based drug development are target discovery, target validation, assay development, lead compound identification and optimization, preclinical development, and clinical trials (Figure 1). Working definitions of commonly used terms throughout this review are summarized in Table 1.

Regardless of source of starting chemical material, medicinal chemical diversification is essential for the success of drug development. Ideally, chemicals must be readily synthesized from inexpensive raw materials to maintain the cost of drug affordable. Chemical diversification can yield either major changes in pharmacodynamics with minor chemical changes or small changes in pharmacodynamics while changing the pharmacokinetics.

Medicinal chemistry refinement is a recursive process with distinct goals that depend on the disease indication and the outcomes from preclinical screens. Two different approaches, **high-throughput screening** and **fragment-based approach**, are currently available (Figure 2).

Figure 1. Discovery and development of target-based drug. The fundamental premise of target-based drug discovery is to develop a drug that affects only one gene or pathogenic mechanism in order to selectively target a particular deficit causing the disease. In the target discovery phase, potential therapeutic target is identified based on the understanding of disease pathogenic mechanism. In the target validation phase, the therapeutic utility of the target is assessed in the specific experimental settings, i.e. cells, animals, or human patients. In the assay development step, high-throughput screenings are established by using in vitro, cell-based, and/or in vivo setting(s). In the lead identification step, compound libraries are screened to identify target-selective lead compounds by using high-throughput screening. In the lead optimization phase, lead chemicals are optimized for better efficacy and selectivity. The preclinical development step includes changes in chemical synthesis and purification strategies to improve reproducibility, efficiency and cost, as well as new formulations and stability testing to improve shelf life and delivery. Documentation of this phase completes the Chemistry, Manufacturing, and Control (CMC) section of the Investigational New Drug (IND) application. Clinical trials are organized into 3 phases, each with distinct primary endpoints. Clinical trial step accounts for 50-70 % of the drug discovery and development cost. ~90% of New Chemical Entities (NCEs) entering clinical trials have been failing. Phase 1 trial with healthy volunteers is designed to determine drug's safety and pharmacokinetics (PK), pharmacodynamics (PD), structure-activity relationships (SAR), mechanism of action (MOA) in human. Phase 2 trial with a randomized controlled setting is intended to get some preliminary data on the efficacy of the drug and short-term side effects. Phase 3 trial will provide safety and efficacy data required for drug approval.



Table 1. Working definitions of commonly used terms

Hits <ul style="list-style-type: none"> • Active compounds with selective binding behaviors, exceeding a certain threshold in given assays. 	Lead <ul style="list-style-type: none"> • A prototypical chemical structure with activity and selectivity in a relevant screening. • This is the basis for a more focused medicinal chemistry effort for lead optimization. 	SAR <ul style="list-style-type: none"> • Structure-Activity Relationship • The consistent correlation of structural features or groups with the biological activity of compounds in a given biological assay 	Pharmacophore <ul style="list-style-type: none"> • The spatial orientation of various chemical functional groups or features necessary for activity
Pharmacokinetics <ul style="list-style-type: none"> • What the "body" does to a "drug" • Determination of the fate of drug • Absorption, Distribution, Metabolism and Excretion (ADME) of a drug. • Compartmental versus noncompartmental analysis • Often studied using mass spectrometry to detect a drug or its metabolites 	Pharmacodynamics <ul style="list-style-type: none"> • What the "drug" does to the "body" • The study of the mechanism of drug action • Desired activity by stimulating normal physiological or inhibiting pathological processes • Undesirable side effects of a drug 	Therapeutic index <ul style="list-style-type: none"> • Also known as "Therapeutic ratio" • Difference between desired pharmacology outcomes over a dose range versus adversity observed with increasing dose. • The lethal dose of a drug for 50% of the population (LD50) divided by the minimum effective dose of 50% of the population (ED50) 	

1.1. High-throughput screening (HTS)

HTS aims to rapidly assess the activity of a large number of compounds on a given target. Therefore, the identification and validation of target are the most critical steps for the success of HTS-based drug discovery. If the chosen target turned out not to be a good target causally linked with disease pathogenesis, downstream efforts will be waste of resources. In HTS, large collections of drug-like molecules are screened for a modulatory activity in the disease-relevant assays. This initial step is important for the discovery of lead molecules. Typically, approximately a million compounds are tested at a primary screening step, in a parallel fashion using 96-, 384-, or 1536-wells in a matter of days. Full- or semi-automation of liquid handling, sample preparation, running of the actual assays, and data analysis are necessary for efficient HTS. Unlike low-throughput assay, HTS development requires careful considerations of reagent stability (i.e. oxidation), cost, environmental control (such as, temperature fluctuation and physical agitation) and many other potential artifacts. For example, some small molecules have their own fluorescent signals that can interfere with the fluorescent-based assay itself. It is important to keep in mind the possibility of false-positive hits due to a fluorescent quenching or increasing artifact. Time-resolved fluorescent method could be an effective solution. In a cell-based assay, proteins in the cell culture media could bind to testing chemicals and prevent the action of compounds. Molecules with the high potential of covalent attachment to protein need to be excluded from screening. Many drug candidates are insoluble in water and require dimethyl sulfoxide (DMSO) or other solvents to dissolve initially. Less than 0.1% of DMSO is acceptable for screening and the plate to plate variability should be kept below 10% coefficient of variation (CV). Although the use of primary cells derived from animals or patients may be ideal, the huge quantity of cells required for drug screening limits the use of primary cells. Advance of induced pluripotent stem cells (iPS) technology might provide a solution to this problem. If an assay relies on a kit from commercial providers, it is strongly recommended to test multiple kits from different companies before investing time and effort on screening ~ a million compounds. Some commercially available kits (i.e. calcium measurement kit and α -, β -, and γ -secretase activity kits) are notoriously not reliable and require another independent validation of assay. Given these complicating factors, while the actual screening may take only a few days, assay development itself usually involves weeks or months of engineering and fine-tuning to achieve sufficient throughput and robustness, as well as cost-effectiveness.

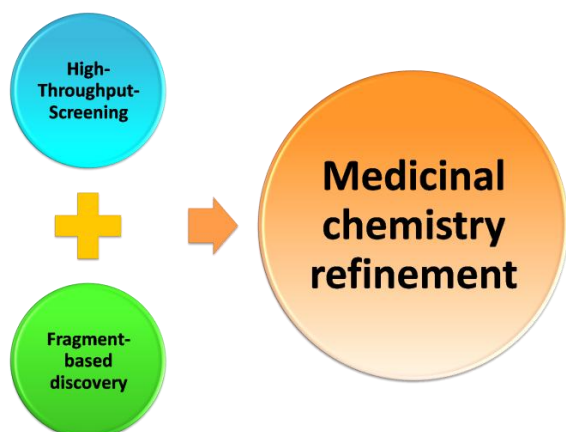


Figure 2. High-throughput screening (HTS) versus Fragment-based discovery. Primary screening of compounds can be performed by conventional HTS or emerging fragment-based discovery method. In contrast with traditional HTS of hits, the starting compounds in the fragment-based discovery have significantly lower molecular mass and often target structurally well-characterized identity.

1.2. Fragment-based approach

Unlike commonly used HTS, fragment-based approach starts with very small chemical motifs that have ability to bind to target protein. Following initial screening of core motif, other functional motifs are added to the core to make bigger and better drug candidates (Figure 3). Fragment-based approach is a complementary screening method to conventional HTS and started to attract much attention among pharmaceutical companies. In general, fragment-based lead discovery requires fewer compounds to be screened and synthesized at the initial step. In contrast to the commonly used “rules of five” (Table 2), the “rules of three” were proposed to identify an ideal drug candidate for fragment-based approach^{3,4}. The rules are as followings; 1) molecular weight is <300, 2) the number of hydrogen-bond donors is <3, 3) the number of hydrogen-bond acceptors is <3, and 4) LogP is <3.

2. Blood-Brain Barrier Penetration: a Unique Challenge with CNS Diseases

2.1. Blood-brain barrier (BBB)

The concept of BBB that segregates the blood and brain was proposed ~100 years ago, after the discovery that most peripheral organs could be stained by intravenous dye injection, with the exception of the brain and spinal cord. By definition, intravenous injection has 100% bioavailability for peripheral tissues. The relative impermeability of the BBB results from **tight junctions** between capillary endothelial cells. Tight junctions are mainly composed of occludin, claudin, junction adhesion molecule (JAM), and endothelial cell-selective adhesion molecule (ESAM) proteins⁵. Each of these transmembrane proteins is anchored into the endothelial cells by another protein complex that includes zonula occludens protein 1 (ZO-1), ZO-2 and associated proteins such as cingulin. In addition, endothelial cells express high levels of **active efflux transport proteins**, including P-glycoprotein (P-gp), Multidrug Resistance Protein- 1 (MRP-1), and Breast Cancer Resistance Protein (BCRP). P-gp is also known as ATP-binding cassette, sub-family B member 1 (ABCB1) or multidrug resistance (MDR1). It is a 170 kDa plasma membrane protein and functions as an energy-dependent drug efflux pump. Deletion of P-gp gene in mouse model causes a deficiency in the BBB and increased sensitivity to drugs⁶. **Verapamil**, a chemical inhibitor of P-gp, is sometimes used to increase the bioavailability of drug, therefore maximizing the effectiveness of drugs. Although P-gp plays a critical role in the pharmacokinetics of drugs that are P-gp substrates, the exact mechanism by which it extrudes substrates is not clear. Interestingly, synonymous single-nucleotide polymorphisms (SNPs)

in P-gp change the conformation of P-gp and lead to altered drug interactions⁷. As another approach to increase drug solubility and penetration into brain, complexing of drug with a chemical carrier, such as **cyclodextrin**, is also used in some cases⁸.

2.2. Designing small molecules with increased potential for CNS bioavailability

The magnitude of poor CNS bioavailability is exemplified by estimation that only 2% of small molecule drugs and virtually no proteins and nucleic acid therapeutics penetrate the blood-brain barrier (BBB)⁹. Bioavailability can significantly contribute to drug safety and efficacy. Therefore, establishing effective drug concentration in the brain is a major challenge in the development of CNS therapeutics. The biological processes underlying the *in vivo* fate of a small molecule drug are significantly influenced by the drug’s physical characters, termed “molecular properties”. Molecular properties represent the traits that help to make a chemical into a drug. Statistical analyses of molecular properties have been helpful in identifying general trends associated with oral bioavailability (Table 2). However, CNS targeting drug discovery requires a more stringent and different set of parameters and considerations due to BBB (Table 2).

The major mechanisms for delivery of compounds into the CNS are **transmembrane diffusion** and **saturable transporter**. Most CNS therapeutics are small, lipid soluble molecules that are likely to cross the BBB via transmembrane diffusion. Although some biopharmaceuticals, such as peptides and even small proteins, have a measurable transmembrane diffusion, saturable transporter are the most effective mechanism for delivering these molecules into the CNS. A chemical with low molecular weight and high lipid solubility favors crossing by transmembrane diffusion mechanism. However, increasing lipid solubility too much can also interfere with BBB penetrance, since a drug that is too lipophilic can be sequestered by the capillary bed and does not reach the cells behind BBB. The bioavailability of a drug in the brain is determined not only

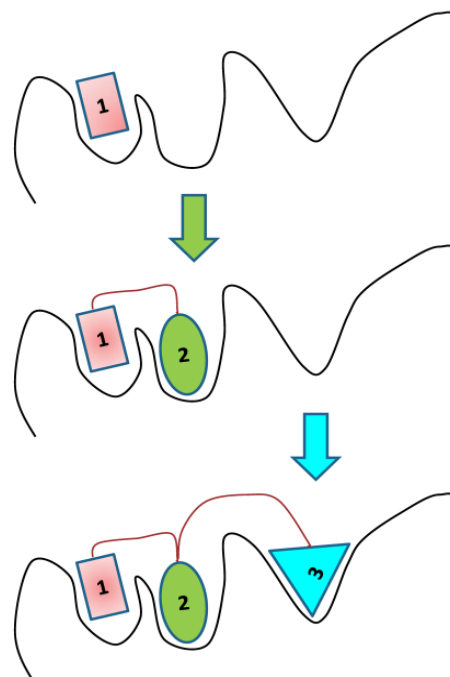


Figure 3. Fragment-based approach. After fragment 1 binds to one of the target sites, the compound is evolved to build a better lead molecule away from the starting fragment 1 by growing into a second pocket at an adjacent site. Fragments are joined together by a linking group that allows the lead molecule to span both sites. This reiterative process is repeated until more sites are targeted to make a bigger and better drug candidate.

Table 2. Molecular properties required to be a good drug candidate

Rules of 5 for oral drugs	Modified rules for CNS drugs
Less than 5 LogP (Lipophilicity)	Less than 4 LogP (Lipophilicity)
Less than 500 Daltons Molecular weight	Less than 400 Daltons Molecular weight
Less than 5 Hydrogen bond donors	60 - 100 Å ² Polar surface area
Less than 10 Hydrogen bond acceptors	

by the transport efficiency across the BBB but also by the amount of drug accessible to the brain. **Peripheral tissues** take up chemicals with higher lipophilicity, therefore limiting the amount of the drugs in the blood stream. In addition, increasing the lipophilicity of a molecule to improve transport can also result in making it a substrate for the efflux pump P-gp. Therefore, high lipid solubility could lower the amount of drug reaching to the BBB, although it will increase transport rate across the BBB. Taken together, increase of lipid solubility does not necessarily lead to better CNS bioavailability and its effect on decreased concentration in the blood should be taken into consideration.

Decreasing **polar surface area (PSA)** has been another strategy to increase BBB penetration but this approach also requires a careful implementation. In general, PSA discriminates CNS penetrating compounds better than the conventional **lipophilicity (LogP)**. Increasing logP and minimizing PSA are used to improve brain uptake of small molecules, but these modifications could also increase the likelihood that the small molecule will serve as a cytochrome P450 (CYP), especially CYP2D6, substrate. The CYP system in the liver is mainly responsible for the first phase in the metabolism and elimination of numerous endogenous molecules and exogenous chemicals. P450 enzymes catalyze the oxidation of chemicals and convert these substances into electrophilic intermediates. These intermediate chemicals are then further modified to hydrophilic derivatives that can be eventually excreted. Among the subtypes, cytochrome P450 2D6 (CYP2D6) is one of the most important enzymes involved in the metabolism of drugs. CYP2D6-mediated unwanted metabolism of drug will limit brain uptake by reducing systemic drug bioavailability and pharmacodynamics¹⁰. Therefore, when the PSA and LogP values of drug candidates are modified to improve brain uptake, there is a potentially undesired consequence of generating favorable CYP2D6 substrates. Optimization of a compound to improve brain uptake must be done carefully to minimize the probability of creating good CYP2D6 substrates.

3. Safety

3.1. Toxicity testing

In addition to the lack of efficacy, toxicity of drug candidate is one of the main scientific reasons for failure of drug discovery effort (Figure 4). In a toxicity assay, the degree to which a compound can harm humans or animals is evaluated. Toxicities are analyzed in both acute and chronic paradigms. Acute toxicity involves harmful effects on an organism through a single or short-term exposure over one or two weeks period. Chronic toxicity is the ability of a compound to cause deleterious effects over an extended period of time, usually by repeated or continuous exposure that could last for the entire life of the exposed organism. Screening processes include a P450 inhibition assay using either recombinant cytochrome P450 enzymes or liver microsome as well as MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) or other equivalent one, such as MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium))-based cytotoxicity assays. The effect of a candidate compound on cardiac human *Ether-à-go-go* Related Gene (hERG) channel (K_v11.1 potassium ion channel) is also investigated. Toxicity results from these relatively simple in vitro assays flag hits or lead compounds for further consideration which compounds can

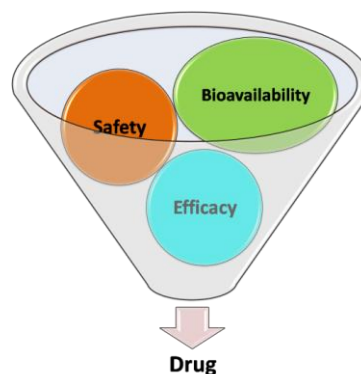


Figure 4. Main reasons for failure of drug in clinical trials. Previously, the main reasons for failure were poor bioavailability (40%), lack of efficacy (30%), and toxicity (12%). However, issues with bioavailability have been addressed at the earlier stages of drug development in recent year. Currently, lack of efficacy (27%) and toxicity (20%) are the main scientific reasons for failure. Commercial and market reasons account for 21%.

advances into the next preclinical studies. Along with in vitro assays, animal models are used for escalating dose studies aimed at determining a maximum tolerated dose. Body weight, food and water intake, blood chemistry and liver activity are monitored for any sign of toxicity. At the end of toxicity study, animals are sacrificed and subsequently analyzed for more in-death pathological analyses. It is important to design the in vivo toxicity experiments while considering whether a particular animal species is the best option for disease indication of interest. The metabolic and toxicity profiles of chemical could be widely different depending on which species were used. It is not easily predictable to extrapolate the toxicity between species. Such potential inter-species sensitivity needs to be considering before proceed to the costly next step of clinical trials. Since animal toxicology tests require relatively large amounts of compound, practical issues with mass production of chemical should be considered in advance. The purity of the compound needs to be very high in order to exclude potential toxicities of impurities.

3.2. Misconception regarding natural products and off-label use of approved drugs

Misconception of safety regarding “natural” product is a serious concern, since many public assumes that natural products are inherently safe. For example, caffeine is a natural product and has been a historical source of drugs for candidate compounds. It is also very safe. A fatal dose is more than 10 grams, which would require drinking 80-100 cups of coffee in rapid succession. Therefore accidental overdose is not an easy thing to do. However, natural products are not inherently safer than engineered or synthetic products. For example, arsenic is a natural product but it is very toxic with acute minimal lethal dose of 70-200 mg. Misconception about off-label use of approved medication is an even more serious problem. Drugs are approved by regulatory agencies for “a specific” disease indication. Therefore, the clinical use of an approved drugs for another disease or ignoring dosing recommendations is not necessarily safe. In terms of drug development, starting a new drug discovery with drugs already approved for another disease indication is not always inherently safer.

4. Future Direction

With rapid advance of genomics, proteomics, and metabolomics technologies, strategy for drug discovery and development will become more effective. Rather than an extreme reductionistic approach, holistic approach that incorporating these emerging systems biology technologies may complement the target-based

drug development effort. Biomarkers and personalized medicine will continue to be the major interests in the future drug development. Biomarkers are characteristics that are objectively measured and evaluated as indicators of underlying pathogenic processes, or pharmacologic responses of patients to therapeutic intervention. For example, high-density lipoprotein and low-density lipoprotein cholesterol are well-established biomarkers of cardiovascular diseases. Biomarkers can be used to identify patients at higher risk, differentially diagnose a disease, assess the severity and/or progression of disease, predict prognosis, and serve as surrogate marker of safety and efficacy. In drug development, biomarkers also help to identify and stratify patients who are most likely to respond well to a particular treatment or are least likely to suffer side-effects. Discovery of new biomarkers for measuring activity and toxicity of drug at an early stage will significantly improve the clinical trial study design and reduce attrition rates. Given the potential of biomarkers in the individualized treatment, biomarkers are gaining momentum in the personalized medicine field. For CNS disorders, biomarkers have another important application. Due to the relative inaccessibility of CNS, earlier detection of underlying pathogenic process in the brain has been one of the major hurdles in drug development for CNS diseases. Detection of ongoing disease processes during clinically silent period may provide a better treatment window and a customized therapeutic intervention based on disease heterogeneity. Since the rate-limiting factors for most biomarker discovery are the quality and depth of the clinical data and samples, strong collaboration between pharmaceutical industry and academic institution is essential for biomarker development.

5. Other Resources and Further Reading Materials

<http://www.sbsonline.org>
<http://ionchannels.org/>
<http://www.dcpvideronline.com/addf/>

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References

1. Lansbury, P.T., Jr. (2004). Back to the future: the 'old-fashioned' way to new medications for neurodegeneration. *Nat Med* 10 Suppl, S51-57.
2. Sams-Dodd, F. (2005). Target-based drug discovery: is something wrong? *Drug Discov Today* 10, 139-147.
3. Congreve, M., Carr, R., Murray, C., and Jhoti, H. (2003). A 'rule of three' for fragment-based lead discovery? *Drug Discov Today* 8, 876-877.
4. Rees, D.C., Congreve, M., Murray, C.W., and Carr, R. (2004). Fragment-based lead discovery. *Nat Rev Drug Discov* 3, 660-672.
5. Zlokovic, B.V. (2008). The blood-brain barrier in health and chronic neurodegenerative disorders. *Neuron* 57, 178-201.
6. Schinkel, A.H., Smit, J.J., van Tellingen, O., Beijnen, J.H., Wagenaar, E., van Deemter, L., Mol, C.A., van der Valk, M.A., Robanus-Maandag, E.C., te Riele, H.P., et al. (1994). Disruption of the mouse *mdr1a* P-glycoprotein gene leads to a deficiency in the blood-brain barrier and to increased sensitivity to drugs. *Cell* 77, 491-502.
7. Kimchi-Sarfaty, C., Oh, J.M., Kim, I.W., Sauna, Z.E., Calcagno, A.M., Ambudkar, S.V., and Gottesman, M.M. (2007). A "silent" polymorphism in the MDR1 gene changes substrate specificity. *Science* 315, 525-528.
8. Davis, M.E., and Brewster, M.E. (2004). Cyclodextrin-based pharmaceuticals: past, present and future. *Nat Rev Drug Discov* 3, 1023-1035.
9. Pardridge, W.M. (2006). Molecular Trojan horses for blood-brain barrier drug delivery. *Curr Opin Pharmacol* 6, 494-500.
10. Chico, L.K., Behanna, H.A., Hu, W., Zhong, G., Roy, S.M., and Watterson, D.M. (2009). Molecular properties and CYP2D6 substrates: central nervous system therapeutics case study and pattern analysis of a substrate database. *Drug Metab Dispos* 37, 2204-2211.