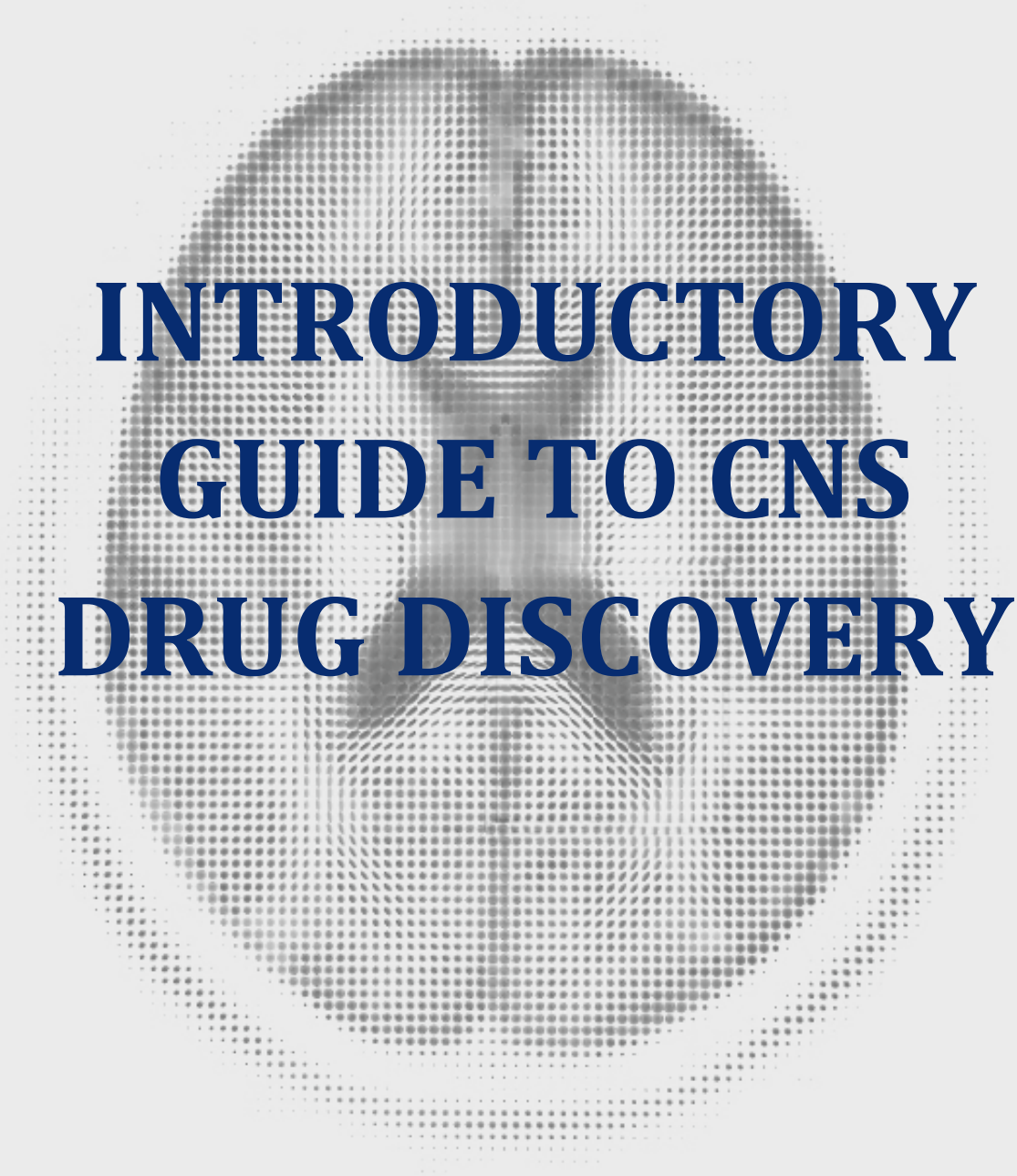
Alzheimer's
Drug Discovery
Foundation



INTRODUCTORY GUIDE TO CNS DRUG DISCOVERY

Alzheimer's Drug Discovery Foundation

ADDF ACCESS

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SUMMARY

From initial target discovery to approved use in the clinic, it takes an average of 10-15 years and up to $2 billion US dollars to develop an effective drug that can safely be used by patients. Drug discovery and development is a highly integrated and iterative multi-step process. Along the pipeline, teams of biologists, chemists, pharmacologists, toxicologists, physicians, and other experts are required to guide drug discovery efforts to the finish line. These teams will need specialized equipment and facilities, access to compounds and animal disease models, and study design expertise to meet stringent Food and Drug Administration (FDA) requirements for approval in humans. Many of these resources and services can be found at contract research organizations (CROs), if not readily available in-house. The following is an introduction to the major stages and key terms involved in the drug discovery and development process, with particular emphasis on diseases of the central nervous system (CNS). This guide briefly outlines the steps involved in developing new chemical entities (NCE), which include both small molecules and biologics. Sections 1-6 primarily focus on small molecules and Section 7 provides additional information about biologics.

More detailed information on each stage of discovery and development, as well as additional resources to accelerate your drug discovery program, can be found at the ADDF ACCESS Resource Center at www.AlzDiscovery.org/ACCESS.

OVERVIEW

1. Target Identification and Validation

Novel molecular targets should be associated with defined pathophysiology. While potential targets may have interesting disease-related biology, they may not be considered "**druggable**" or suitable to being modulated by a drug in a selective manner. Structural or computational data can help to determine druggability by predicting whether a target has potential drug binding sites that can alter its activity. After identifying potential targets, genetic overexpression, knockdown, or knockout studies in cell and animal models and/or pharmacological manipulation studies should be conducted to validate their link to the disease. Human genetic associations or altered mRNA or protein expression in human disease specimen will strengthen target validation data.

2. Assay Development and Screening

Once a target or molecular mechanism has been identified, libraries of chemical compounds are screened for desired disease-related activity in biochemical or cellular assays. Compounds that produce responses above a set threshold are called **actives**. Actives that produce confirmed activity in a repeated screen with fresh compound sample and have confirmed structural identity and purity are called **hits**.

A hit serves only as a structural foundation for an extensive medicinal chemistry campaign during lead optimization to improve activity and drug-like properties. Careful design, optimization, and validation of novel assays are critical. Assays should be scalable, cost-effective, and produce robust, reproducible results. Secondary assays should be performed to rule out false positives.

2.1 High Throughput Screening (HTS)

HTS is a rapid, iterative, and automated screening approach to test the activity of compounds on biological targets or pathways in biochemical or cellular assays. A screen is generally considered high throughput for >10,000 assays per day. The platform (e.g. ligand binding, ion transport, signal transduction, cell viability) and detection (e.g. absorbance, radioactivity, luminescence, and fluorescence) formats selected should be able to be miniaturized to a high-throughput format.

2.2 High Content Screening (HCS)

HCS provides functional readouts in live cells, and in some cases, tissue slices or whole organisms (e.g. *Drosophila* and *C. elegans*), using automated microscopy and quantitative image analysis. Although HCS is lower throughput than HTS, it can

provide more biologically relevant phenotypic profiles and can be used to screen compounds for disease-related effects. Examples include neurite extension, vesicular trafficking, protein aggregation, and autophagic flux.

2.3 *In Silico* Screening

***In silico* screening**, or virtual screening, predicts the efficacy and safety of potential ligands based on their drug-like properties. *In silico* screening results can influence library design and library selection in high-throughput assays or can be used in post-hoc analyses of HTS results. This method provides a cost-effective starting point for investigating new uses for existing drugs or drug analogs.

2.4 Compound Libraries

Compound libraries are large collections of small molecules. Targeted or focused libraries are structurally diverse and can be designed to have particular features, like biological activity and membrane permeability. Many are commercially available and include:

2.41 CNS Libraries

Libraries of small molecules intended for CNS targets that are designed to cross the blood-brain barrier.

2.42 FDA Approved

Compounds that have been approved for use in humans can be used to identify new targets for old drugs. Since bioactivity and safety have already been confirmed in humans, these compounds can potentially be developed for clinical use in another indication more rapidly.

2.43 Fragment Libraries

Compound libraries assembled by medicinal chemists for **fragment based drug discovery** composed of small and relatively simple fragments that bind their biological targets with low affinity. These fragments are subsequently built out in a step-wise fashion to increase the affinity for the target and improve drug-like properties.

2.44 Natural Product Libraries

Natural products (NP), derived from microbes, plants, and other organisms, have long been a source for the development of new drugs. NPs can serve as templates for combinatorial chemistry to generate NP analog libraries, which may have more drug-like properties.

3. Hit to Lead and Lead Optimization

In **the hit-to-lead process**, the "**druggability**" or drug-likeness of hits is determined by assessing physiochemical properties, solubility, selectivity, cytotoxicity, and other parameters to identify **lead compounds**. In **lead optimization**, lead molecules undergo extensive medicinal chemical refinement and testing with the goal of enhancing activity, safety, and **ADME** (absorption, distribution, metabolism, and excretion) properties.

3.1 Compound Physiochemical Properties

Physiochemical properties, which include molecular weight, hydrogen-bonding, and lipophilicity (log*P*), determine how well compounds are absorbed, distributed, metabolized, and excreted by the body and how they interact with their molecular target. Modifying one of these inter-related properties can have a significant impact on others.

3.2 Medicinal Chemistry

Medicinal chemistry uses several approaches to design, modify and synthesize chemical compounds. Medicinal chemistry refinement is an iterative process where potency, selectivity, ADME, and toxicity profiles are continuously improved by synthesizing new compounds with slight modifications. Compounds are then tested in a series of assays that screen for various drug-like properties. These results inform the next round of medicinal chemistry refinement. Even small modifications to chemical structure can have a significant impact on compound physiochemical and ADME properties.

3.21 Synthetic Chemistry

Combinatorial chemistry is used to synthesize large numbers of compounds using simple techniques that can be automated. This method is used to generate compound libraries that share a common scaffold or backbone or are built around a common **pharmacophore**, which is the minimal portion of a structure required to achieve biological activity.

3.22 Fragment Chemistry

Small and relatively rigid fragments of potential ligands are screened for binding efficiency and chemically modified to enhance their drug-like properties. Fragment chemistry builds upon these low-molecular weight core fragments to add functionality and improve ADME properties.

3.23 Natural Products Chemistry

While natural products (NP) have a long history of producing drug leads, their extraction and synthesis can be slow and expensive, and their larger size and complex structures make medicinal chemistry refinements complicated. However, NP's can serve as templates for combinatorial chemistry to

generate NP analog libraries, which may have better drug-like properties. Additionally, NP's have been used to identify novel disease targets.

3.24 Computational Chemistry

Computational chemistry integrates structural biology of known protein targets or ligands with *in silico* applications to identify target binding sites, predict ligand docking, or develop *de novo* compounds. The discovery of new drug compounds has been enhanced by quantitative **structure-activity relationship** (QSAR) models, which codes physiochemical and structural properties into molecular descriptors in order to predict compound activity at a target. Computational methods also guide lead optimization by comparing to large datasets of compounds with known ADME properties.

3.25 Synthesis

Most small molecule drug discovery programs require an iterative cycle of custom synthesis and biological testing that often involves library synthesis and compound purification. Many projects will require the construction of novel chemical synthetic pathways and the synthesis of drug candidates.

3.3 PK/PD

Pharmacokinetics (PK) – what the body does to a drug – refers to the movement of a compound through the body over a period of time. PK assesses **bioavailability** (how much drug reaches the systemic circulation), the rate and extent of tissue distribution, and the mechanism and extent of metabolism and clearance. **Pharmacodynamics** (PD) – what the drug does to the body – refers to the mechanism of drug action at the target organ or molecular target, and determines efficacy and potency. *In silico* PK/PD modeling throughout the discovery process can identify optimal drug candidates or predict optimal dose ranging in preclinical animal studies or later clinical trials.

3.31 Permeability and BBB Penetrance

There are a variety of *in vitro* assays used to test drug permeability and predict whether a compound is suitable for oral administration and brain penetration. The **Caco-2** cell line, which is derived from human colon carcinoma and resembles the intestinal epithelia, is used to assess intestinal permeability for orally administered drugs. **MDR1-MDCK** cells are derived from canine kidney and express a single efflux transporter, P-glycoprotein (**P-gp**), which is expressed in brain as well as other organs. This *in vitro* assay can be used to predict brain penetrance.

Finding or synthesizing drug compounds that cross the **blood-brain barrier** (BBB) represents a significant hurdle in CNS drug development. *In vivo*

methods for assessing BBB permeability include measuring the ratio of drug concentration in plasma vs. brain or by monitoring radiolabeled compounds in tissue or fluorescently-tagged compounds by two-photon microscopy.

3.32 Metabolic Stability and Metabolite Identification

Drug metabolic stability is one of the main factors used to determine dosing frequency. Metabolic stability and intrinsic clearance rate can be tested *in vitro* in both **hepatocytes** and **liver microsomes**, which are subcellular fractions that contain membrane-bound metabolizing enzymes. It is also essential to identify compound metabolites because they can alter drug efficacy or produce toxic effects. While a rapidly metabolized drug may require multiple daily doses or a continuous infusion, drugs with high metabolic stability can be toxic.

3.33 Selectivity Panels

Determining compound specificity is critical in reducing the chances of off-target side effects. Selectivity panels test whether compounds are biologically active at off-target sites. Panels include collections of related target classes, like kinases, ion channels, or G-coupled protein receptors with similar structures or activity.

3.34 Toxicity

Cardio- and hepato-toxicity are the leading causes for drug withdrawal from the market, and therefore, *in vitro* toxicity should be tested early in the development of **new chemical entities** (NCE). The **hERG** (human ether-a-go-go related gene) ion channel binding assay is an indicator of cardiotoxicity and predicts cardiac channel blockade through automated patch clamp. The **cytochrome P450** (CYP450) superfamily includes the six major isoenzymes involved in the metabolism of most drugs. Some drugs are poor substrates for CYP450 but can still bind their active sites, leading to CYP450 induction or inhibition. This can affect the metabolism of other drugs, leading to potentially dangerous drug-drug interactions.

In vivo tolerability testing should also be considered once a lead candidate has been identified and has been assessed in *in vitro* toxicity assays. Tolerability studies in rodents are used to define dosing, **maximum tolerated dose** (MTD), **lowest observable effect level** (LOEL) and **lowest observable adverse effect level** (LOAEL).

4. *In Vivo* Efficacy Studies

In vivo efficacy studies assess the ability of a compound to modify disease-related phenotypes in an animal model. These studies should include multiple pathological, biochemical, and behavioral readouts that parallel the human disease. Species and

model selection, route of administration, dosing regimen, vehicle (solutions versus suspension, concentration), formulation, bioanalytical measurements, immunological response (for biologics), and treatment duration should be determined before initiating these studies. Ideally, *in vivo* efficacy studies should include a biomarker of target engagement that can be translated to the clinical setting.

4.1 Animal Models

Transgenic models are commonly used for efficacy studies because they carry known human genetic mutations that partially recapitulate neuropathology and behavioral impairments associated with neurodegenerative diseases. Other animal models of disease are induced through surgical, chemical, or biological interventions. Animal models should be carefully selected based on the specific hypothesis being tested, i.e. if your drug targets a particular protein oligomer, the animal model should display that pathology. Animal models should also be extensively characterized by the lab conducting the studies before testing the effects of your drug candidate. Ultimately, rigorous preclinical study design is critical to improve the quality of efficacy studies. For full guidelines, see Shineman, et al and Snyder, et al.

4.2 Functional Outcome Measures

These include measures of target engagement and biological responses to the drug compound. The analyses listed below can be used to further characterize existing CNS disease models and to assess efficacy and the site of action for drug candidates at the subcellular, cellular, and network levels.

4.21 Biochemical Analysis

Assays measuring biochemical changes in brain, cerebral spinal fluid (CSF), and plasma include, but are not limited to, levels of pathogenic proteins in soluble and insoluble forms, pathogenic-specific phospho-epitopes, and neuroinflammatory markers like cytokines and chemokines.

4.22 Histopathology and Microscopy

A postmortem analysis examines disease-specific neuropathology at various time-points. These include disease-specific protein deposits and changes in inflammation and oxidative stress markers. Abnormal dendritic spine morphology and synapse loss are prominent neuropathological features in neurodegenerative diseases. 3D electron microscopy can be used to evaluate alterations in spine density, morphology, and maturity, as well as myelin thickness and mitochondrial morphology. Two-photon platforms can be used to analyze a number of functional parameters including neuronal and glial morphology, vasculature and blood flow, autophagic flux, and calcium signaling.

4.23 Electrophysiology

Electrophysiological properties can be evaluated in cell lines or, more commonly, in slice preparations from rodent CNS tissue. Electrophysiology can be used to validate the action of a compound on the activity of a single ion channel or properties relevant to cognition, such as LTP or synaptic strength.

4.24 Behavior

Animals are subjected to one or more behavioral tests to determine the effects of modulating a target or administering a compound. Automation, standardization of behavioral paradigms, blinding, and randomization will reduce the occurrence of false-positives. Behavioral studies should also be well-powered and include larger numbers of animals (determined by a power analysis) due to variability in strains with the same genotype and inter-lab variability in mouse breeding, testing equipment, personnel, animal housing, and experimental conditions (i.e. time of day).

5. IND Enabling Studies

In order to advance a lead compound into the clinic, an extensive drug discovery and development package is required by the FDA as part of the **Investigational New Drug** (IND) application. The pre-IND process and regulatory guidance will depend on the specific class of candidate compound (small molecule, biologic, gene therapy, or reformulated/repurposed drugs), indication to be treated, route of administration, and the design of the proposed clinical trials, as there is no one-size-fits-all approach. However, all IND applications must include adequate safety and toxicity studies, as well as providing a plan for the production of consistent batches of drugs suitable for use in humans.

5.1 Safety and Toxicity

Safety and toxicity studies generally fall into two categories: (1) exploratory studies, which include rodent tolerability studies, and (2) IND-enabling safety evaluation. Exploratory studies should be conducted early in the discovery process and can be performed at the home research institution. Preliminary tolerability studies in rodents include maximum tolerated dose (MTD) and repeated dose tolerability, and predict whether a compound will be safely tolerated in larger-scale animal toxicity studies required for IND-enabling. IND-enabling safety evaluation requires the evaluation of *in vitro* and *in vivo* genetic toxicity, *in vitro* and *in vivo* safety pharmacology of a compound in the CNS, cardiovascular, and respiratory systems, general toxicology, and exposure assessment in two species, at least one of which is not rodent. All IND-enabling safety studies are required to be conducted in **good laboratory practice** (GLP), FDA-certified labs. GLP regulations ensure that training, monitoring, documentation, and archiving are of the highest quality and produce reliable data.

5.2 Chemistry Manufacture Control (CMC)

Advanced planning for the manufacture and scalability of a lead compound should be considered throughout the drug discovery process and prior to synthesis. The **CMC** is a document that details all of the manufacturing steps and controls to ensure that the **active pharmaceutical ingredient** (API) is pure, free of metal impurities, stable, consistent, scalable, safe, and effective for use in humans, and is manufactured in a facility that complies with **current good manufacturing practices** (cGMP) regulations for the production of the drug when it is approved.

6. Clinical Studies

Clinical studies are required to determine the safety and efficacy, as well as the optimal dose of an investigational treatment in humans. The results of these studies are packaged together and presented to the relevant regulatory agencies in order to be approved and made commercially available.

6.1 Clinical Phase 1

Phase 1 studies are performed to determine the safety, tolerability, and MTD of an investigational drug or biologic in a small cohort of human subjects, typically healthy volunteers. These studies may additionally provide target engagement data and evaluate the PK/PD relationship.

6.2 Clinical Phase 2

Phase 2 trials are designed to evaluate the safety and efficacy of an investigational drug or biologic in patients (50-300) and to show clinical proof-of-concept. Phase 2 studies typically employ biomarkers as either primary or secondary end points and explore the optimal dose range for Phase 3 studies.

6.3 Clinical Phase 3

Phase 3 trials are designed to evaluate efficacy and monitor safety in large patient cohorts (300-3000). Positive Phase 3 studies are required for approval by the relevant regulatory agency.

6.4 New Drug Application (NDA)

Prior to approval and commercialization of a new drug, a **new drug application** (NDA in the US) must be submitted and approved by the relevant regulatory agency. Data gathered in the IND application and the clinical data package is presented in the form of an NDA. The goals of the NDA are to provide enough information to permit reviewers to assess whether the drug is safe and effective for its proposed use(s), the benefits outweigh the risks, the proposed labeling is appropriate, and whether the manufacturing methods are appropriate.

7. Biologics

For some targets, biologics (blood and blood products, vaccines and allergenics, conventional and novel biotechnology-derived products, recombinant proteins, monoclonal antibodies, and antigenic peptides) are the only therapeutic approach that provides the sensitivity and specificity needed to successfully impact disease progression. However, due to their larger size, many biologics are poorly brain penetrant.

7.1 Antibody Development

The development of therapeutic monoclonal antibodies should consider cloning requirements, protein recovery and purification, humanization, analytical assays, scale-up, and production. It is advisable to integrate process development (humanizing options, sterility characterization) and manufacturing (production cell lines, clonal cell sources, and working cell banks) at an early stage to ensure product control and consistency.

7.2 Peptide and Peptide Mimetics

Peptides are frequently identified as hits in early biological studies of drug targets. However, natural peptides are not sufficiently diverse to cover all potential targets, and are generally poor drug candidates due to enzymatic degradation, low bioavailability, and lack of specificity. Improving the druggability of peptides and peptide mimetics often includes an extensive chemical synthesis campaign to improve stability, blood brain barrier and cellular permeability, pharmacokinetic properties, and specificity.

7.3 Toxicology for Biologics

Toxicology for biologics includes a tissue cross-reactivity panel to assess monoclonal antibody cross-binding with non-target tissues expressing the same or related epitope. Immunogenicity assays, while not required at the IND stage, should also be taken into consideration.

References

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