ISOA/ARF Drug Development Tutorial

By Jens Eckstein

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This Drug Development Tutorial was produced via the collaborative efforts of the Institute for the Study on Aging (ISOA) and the Alzheimer Research Forum. Drug discovery and development has its own vocabulary, which we attempt to define in the glossary of terms. The list of references encompasses ample reading material for the interested and motivated reader; however, we gladly accept recommendations of additional citations.

General Introduction

- This tutorial is an introduction aimed at academics and other researchers who are new to the field of drug discovery. We outline here the fundamental concepts and processes of drug discovery. Our goal is to guide researchers toward the steps necessary to translate benchside findings into bedside applications, and to locate resources that can help provide reagents and services needed in this process. The views presented here are based on pharmaceutical industry experiences, but are by no means the only perspective on the highly complex and diverse field of drug discovery and development. For more comprehensive textbooks and reviews on this topic, please refer to our list of references below.

Target Discovery—Overview

- Drug discovery and development can broadly follow two different paradigms—physiology-based drug discovery and target-based discovery. The main difference between these two paradigms lies in the time point at which the drug target is actually identified.
- Physiology-based drug discovery follows physiological readouts, for example, the amelioration of a disease phenotype in an animal model or cell-based assay. Compounds are screened and profiled based on this readout. A purely physiology-based approach would initially forgo target identification/validation and instead jump right into screening. Identification of the drug target and the mechanism of action would follow in later stages of the process by deduction based on the specific pharmacological properties of lead compounds.
- By contrast, the road of target-based drug discovery begins with identifying the function of a possible therapeutic target and its role in disease. Given the thousands of human or pathogen genes and the variety of their respective gene products, this can be a difficult task. Furthermore, insight into the "normal" or "native" function of a gene or gene product does not necessarily connect the gene or gene product to disease.
- The two paradigms are not mutually exclusive, and drug discovery projects can employ a two-pronged approach. The genomics revolution has been the main driver of the target-based paradigm over the last decade.
- Currently, all existing therapies together hit only about 400 different drug targets, according to a recent review article [Science, 2000, Vol 287, 1960-1964]. The same review estimates that there are at least 10 times as many potential drug targets that could be exploited for future drug therapy.

Target Discovery—Disease Mechanism

- The disease mechanism defines the possible cause or causes of a particular disorder, as well as the path or phenotype of the disease. Understanding the disease mechanism directs research and formulates a possible treatment to slow or reverse the disease process. It also predicts a change of the disease pattern and its implications.
- Disease mechanisms can be broadly classified into the following groups
    - Defects in distinct genes—genetic disorders
    - Infection by bacteria, fungi, or viruses
    - Immune/autoimmune disease
    - Trauma and acute disease based on injury or organ failure
    - Multicausal disease

Target Discovery—Disease Genes

- Disease genes have been identified based on hereditary patterns even before the knowledge of the DNA sequences of the human genome. Following an original founder mutation, these genetically inherited diseases run in families; examples include phenylketonuria, cystic fibrosis, Huntington disease, Fanconi's anemia, and autosomal-dominant familial Alzheimer's (FAD).
- The specific gene defects or mutations that bring about a hereditary disorder have been identified for a number of diseases. Progress in DNA sequencing technology has enabled rapid identification of disease genes through genetic screening. Early intervention is possible for a limited number of hereditary diseases.
- A large fraction of disease, however, is not based on the mutation of a single gene, but rather on a number of genes that together determine a person's risk of developing a particular disease. For example, certain mutations in the BRCA gene family raise the risk for cancer. However, this risk does not always equal 100 percent certainty, and individuals bearing certain BRCA mutations may never develop cancer. Certain allelic variants can increase susceptibility for diseases, such as the ApoE4 allele does for Alzheimer's.
- Environmental factors such as diet, toxic exposures, trauma, stress, and other life experiences are assumed to interact with genetic susceptibility factors to result in disease. Thus, drug targets may include molecular pathways related to environmental factors.

Target Discovery—Target Type and "Drugability"

- Targets for therapeutic intervention can be broadly classified into these categories:
    - Receptors
    - Proteins and enzymes
    - DNA
    - RNA and ribosomal targets
- The "drugability" of a given target is defined either by how well a therapeutic, such as small molecule drugs or antibodies, can access the target, or by the efficacy a therapeutic can actually achieve. A long list a parameters influences drugability of a given target; these include cellular location, development of resistance, transport mechanisms such as export pumps, side effects, toxicity, and others.
- Some target classes, for example, the G-protein coupled receptors (GPCRs), have been successfully targeted, and a sizable number of drugs prescribed today hit this particular class. Therefore, the GPCR target type is considered drugable.

Target Discovery—Functional Genomics
- Functional genomics can be broadly defined as the systematic analysis of gene activity in healthy versus diseased organisms/organs/ tissues/cells.
- Specifically, functional genomics employs the large-scale exploration of gene function that includes the analysis of regulatory networks, biochemical pathways, protein-protein interactions, the effects of gene knockouts or gene upregulation or gain-of-function, and the results of functional complementation of knockouts.
- Functional genomics aims to determine disease mechanisms and to identify disease genes and disease markers. It also aims to guide the understanding of signal transduction pathways that either lead to disease or indicate therapeutic strategies for the development of novel therapeutics.
- Functional genomics relies heavily on disease models that are based on the high homology of genes and their function in a variety of organisms ranging from nematodes to mammals.
- Functional genomics employs high-throughput sequencing and high-density arraying of gene expression and activity of gene products. The information content of functional genomics experiments is exceedingly large; it requires sophisticated statistical analysis, which has accelerated the discipline of bioinformatics.

Target Validation—Overview
- Target validation requires a demonstration that a molecular target is critically involved in a disease process, and that modulation of the target is likely to have a therapeutic effect.
- The validation of a molecular target in vitro usually precedes the validation of the therapeutic concept in vivo; together this defines its clinical potential. Validation involves studies in intact animals or disease-related cell-based models that can provide information about the integrative response of an organism to a pharmacological intervention and thereby help to predict the possible profile of new drugs in patients.

Target Validation—Knockout/in, Gain-of-Function, Transgenic Models
- Transgenic animals where the target gene is knocked out have become an important experimental approach for the determination of the function of targets (genes) in a whole organism.
- Knockouts of genes that are essential in development are usually lethal. Inducible knockouts, i.e., transgenic models where the transgene can be switched on or off at will in the adult animal, can be used to study the function of such essential genes.
- Disease models are transgenic animal models which present a phenotype that bears the hallmarks of a certain disease. These can be combined with knockouts to study the effect of modulating or inhibiting the function of the drug target.
- Knockins or gain-of-function models reactivate gene expression of the target gene, and often ameliorate or even reverse the disease phenotype. Knockins also are used to create disease models.
- Knockins or gain-of-function can also be lethal. For example, switching on or restoring the function of cell cycle genes in postmitotic cells often leads to cell death. Selective switching-on of genes might present a therapeutic strategy if such restoration of gene function can be engineered in a tissue- or organ-specific fashion.
- Most neurodegenerative disease models have been generated by introducing mutant genes that cause autosomal-dominant forms of the disease in humans. In these models, the mutant gene (such as APP, presenilin, tau, superoxide dismutase-1, expanded huntingtin) is assumed to result in a toxic gain of function, but the actual mechanisms by which the mutations cause disease phenotypes remain debated.

Target Validation—Pathways

- The action and interaction of genes and their gene products is complex. Research aimed to define pathways that control and regulate processes in living organisms provides valuable information for drug discovery.
- The knowledge of a pathway allows definition and separate targeting of upstream or downstream targets. Inhibition or modulation of selected targets could lead to the same therapeutic with fewer side effects or better drugability.
- Knowledge of pathways and their relation to each other helps researchers understand side effect profiles.
- Identification of one disease target can lead to a number of alternative drug targets in the same pathway and increase the possibilities for a novel therapeutic. Examples include the drugs acting on the cholesterol synthesis pathway.

Target Validation—Clinical Data

- The best validation of a target is clinical efficacy and safety data.
- Second- and third-generation therapeutics often have better efficacy and side effect profiles based on the clinical trials and track record of first-generation drugs.
- Efficacy in clinical trials, i.e., amelioration or reversal of disease in human patients, is the ultimate validation of a target.
- Efficacy in animal disease models does not always predict the outcome in patients. The reliability of disease models for the prediction of human clinical trials varies widely among diseases and needs to be assessed on a case-by-case basis.

Target Validation—Antisense DNA/RNA and RNAi

- Antisense DNA/RNA are oligonucleotides or analogs thereof that are complementary to a specific sequence of RNA or DNA. The underlying concept of antisense therapeutics is that the antisense compound binds to the native target to form a double-stranded sequence and thus inhibits its normal function. An antisense drug for viral retinitis has been approved.
- Interfering RNA or RNAi is a gene silencing phenomenon, whereby specific double-stranded RNAs (dsRNAs) trigger the degradation of homologous messenger RNA (mRNA). The specific dsRNAs are processed into small interfering RNA (siRNA), which initiates the cleavage of the homologous mRNA in a complex named the RNA-induced silencing complex (RISC).
- Introduction of either dsRNA or siRNA into cells leads to inhibition of the biological function encoded in the targeted mRNA—the underlying concept of RNAi therapeutics. For example, this approach is being investigated to silence mutant alleles of tau, APP, ataxin, and SOD1.

Target Validation—Chemical knockouts and Chemical Biology

- The prevailing approach for target validation involves the study of the biology of a disease. Knowledge of the disease mechanism and the underlying biological pathways leads to the identification and characterization of drug targets.
- A fundamentally different approach involves using compound collections to screen for phenotypes generated by exposure to molecules; this connects chemical structures to biological effects from the start irrespective of the molecular targets/pathways that are hit.
- This "chemical biology" takes a holistic and random approach to drug discovery. It may complement traditional, deductive approaches.
- In chemical biology, chemical knockouts are a new method whereby the effect of a chemical compound, not a genetic manipulation, knocks out the function of a gene and thus leads to a readable phenotype. Chemical biology and chemical knockouts rely on the creation of diverse

chemical libraries of many thousands of compounds. Cornerstone technologies for this new way of drug discovery are combinatorial chemistry and genetic manipulation of biosynthetic pathways in microbes for the production of new compounds.

Assay Development—Overview

- The key to drug discovery is an assay that fulfills several important criteria:
    - Relevance: Does the readout unequivocally relate to the target?
    - Reliability/Robustness: Are results reproducible and statistically significant?
    - Practicality: Do time, reagents, and effort correlate with quality and quantity of results?
    - Feasibility: Can assay be run with resources at hand?
    - Automation: In order to screen large numbers of compounds, can assay be automated and run in highly parallel format?
    - Cost: Does cost of the assay permit scale-up for high-throughput screening?
- The quality of an assay determines the quality of data, i.e., compromising on assay development can have substantial downstream consequences.

Assay Development—in Vitro/Cell-based

- In-vitro assays monitor a surrogate readout. Examples for such a readout are the catalytic action of an isolated enzyme, the binding of an antibody to a defined antigen, or the growth of an engineered cell line.
- An in-vitro assay system can be designed using only recombinant reagents, reagents that were isolated from lysates, whole crude lysates, or intact cells.
- Cell-based assays range in their complexity from simple cytotoxicity assays or cell growth to reporter gene assays that monitor activation or upregulation of certain genes or their gene products.
- In-vitro functional assays are usually more complex. They combine several molecular components to mimic the function of a biological process, such as activation of a signal transduction pathway. Biological processes that can be monitored in cell-based functional assays include changes in cell morphology, cell migration, or apoptosis.
- In general, in-vitro assays are more robust and cost-effective, and have fewer ethical implications than whole-animal experiments. For these reasons they are usually chosen for high-throughput screening, where tens of thousands of data points are generated in the hunt for novel drug molecules.

Assay Development—in Vivo/Animal Models

- In-vivo testing involves whole organisms. It assesses both pharmacology and biological efficacy in parallel.
- Animal models have specific characteristics that mimic human diseases. The technologies for the creation of transgenic animals, where certain genes are either deleted, modulated, or added, have progressed tremendously in the last decade. As a consequence, the predictive power of animal models for human disease and pharmacology is improving. Even so, human biology and disease is so complex that for many diseases or pharmacological parameters, the human remains the definitive model. For some disease, e.g., hepatitis C, adequate models still do not exist.
- It is important to note that some experts in the pharmaceutical industry and the U.S. Food and Drug Administration (FDA) believe that inadequate animal models, or the lack of animal models altogether, are a major hurdle in drug discovery and development.
- Pharmaceutical companies have long used model organisms in preclinical efficacy and safety studies. With the emerging knowledge of whole genomes, researchers are now increasingly

seeking animal models not only of specific diseases, but also of their underlying particular pathways to broaden assays from pharmacology to include mechanism of action.
- Regarding current animal models of Alzheimer disease, scientists debate whether they adequately model the disease. Amyloid-depositing models, for example, have scant, if any, cell loss. Disease models are usually incomplete models of pathology or mechanism, and their utility in drug screening is limited by the validity of the pathway in human disease pathogenesis.

Assay Development—HTS
- High-throughput screening (HTS) aims to rapidly assess the activity of a large number of compounds or extracts on a given target. The term HTS is used when assays are run in a parallel fashion using multi-well assay plates (96-, 384-, 1536-well).
- Assays run in 1536-well plates with minuscule volumes (single-digit microliter to nanoliter scale) are sometimes referred to as ultra high-throughput screening or UHTS.
- Today, HTS/UHTS commonly involves semi-automation or full automation for liquid handling, sample preparation, running of the actual assays, as well as data analysis. HTS laboratories frequently employ robots and the latest detection technologies for assay readouts.
- Assay development for HTS/UHTS faces formidable challenges in terms of reagent stability and cost, environmental robustness (temperature, oxidation, agitation), and statistics (signal-to-noise ratios, Z and Z' quality measures). Therefore, the ultimate design of a HTS/UHTS assay often differs from its respective lower- throughput format.
- Large HTS/UHTS operations are significant investments. Optimal and cost-effective use, as well as minimization of down-time, are important issues in today's drug discovery environment.
- It is common in today's HTS environment to run a primary screen through a 1,000,000 compound library in a matter of days. However, while the actual screen may only take a few days, assay development usually involves weeks of engineering and fine-tuning to achieve sufficient speed and robustness, as well as cost-effectiveness.

Screening and Hits to Leads—Overview
- After successful development of an assay, screening of compound libraries follows. Primary screens will identify hits. Subsequently, confirmation screens and counter screens will identify leads out of the pool of hits. This winnowing process is commonly referred to as "hits-to-leads."
- The success of screening depends on the availability of compounds, as well as their quality and diversity. Efforts to synthesize, collect, and characterize compounds are an essential and costly part of drug discovery.

Screening and Hits-to-Leads—Compound Libraries
- Compound libraries are the "bread and butter" of screening. There are several sources for compounds:
  - Natural products (NPs) from microbes, plants, or animals. NPs are usually tested as crude extracts first, followed by isolation and identification of active compounds.
  - (Random) collections of discreetly synthesized compounds.
  - Focused libraries around certain pharmacophores.
  - Random libraries exploring "chemical space."
  - Combinatorial libraries.
- The total number of possible small organic molecules with a molecular mass of less than 500 that populate "chemical space" is estimated to exceed 10^{60}—vastly more than were ever made

or indeed will ever be made.
- Given this near- infinite number of theoretical compounds, one can either focus the search around known molecules or pharmacophores with biological activity, or sample the chemical compound universe with a random selection of diverse representatives. Both approaches are used, and complement each other, in today's drug discovery efforts.
- In contrast to the theoretical small-molecule universe, the idea of "privileged" structures has been advanced. Such structures represent a discreet selection of compounds with the highest probability of having biological activity, i.e., of interacting with the universe of biological diversity that has developed on Earth. Likewise, this biological diversity can be viewed as privileged, because all organisms on Earth together do not contain anywhere near the theoretical number for 300 amino acid proteins, 10^{390}.
- An important practical measure for the value of a random library is chemical diversity, which analyzes how similar one compound in the library is to one other.

Screening and Hits to Leads—in Silico/CADD and SBDD
- Advances in computing power and in structure determination by x-ray crystallography and NMR have made computer-aided drug design (CADD) and structure-based drug design (SBDD) essential tools for drug discovery.
- Elucidation of protein/DNA/RNA structures has been industrialized in recent years, such that structural information about a given drug target, or the binding conformation of a drug, are available to the scientist at earlier stages of drug discovery. HIV protease inhibitor drugs are a prominent success story for SBDD.
- Virtual (in-silico) screening sifts through large numbers of compounds based on a user-defined set of selection criteria. Selection criteria can be as simple as a physical molecular property such as molecular weight or charge, a chemical property such as number of heteroatoms, number of hydrogen-bond acceptors or donors. Selection criteria can be as complex as a three-dimensional description of a binding pocket of the target protein, including chemical functionality and solvation parameters.
- In-silico screening can involve simple filtering based on static selection criteria (i.e., molecular weight). Alternatively, it can involve actual docking of ligands to a target site, which requires computer-intensive algorithms for conformational analysis, as well as binding energies.
- Selection criteria are often combined, either in Boolean fashion or otherwise, to generate complex queries which, for example, describe a SAR established from experimental data. Scoring functions are used to rank compounds that meet selection criteria.
- Initially, in-silico screening was intended to filter out the majority of compounds that have little chance of hitting a target. In this way, one can either reduce the actual number of compounds being screened in a benchtop assay, or enrich a yet-to-be-screened library with compounds that have a chance of hitting the target.
- With increasingly sophisticated algorithms describing the interaction of ligands and receptors, in-silico screening is more commonly being implemented in drug discovery. In-silico screening has been particularly helpful in projects where a wide-ranging SAR around a discreet pharmacophore is known (QSAR), or where high-resolution three-dimensional structural information is available (SBDD).

Screening and Hits to Leads—Synthesis and Combinatorial Chemistry
- Screening relies on the availability and chemical synthesis of compounds.
- Today, a chemist typically supplies new compounds to the screener in milligram or even sub-milligram amounts. Compound synthesis often involves the synthesis of precursors, which can serve as the starting point for a compound series. Such precursors tend to involve scale-up procedures, since larger amounts are needed for subsequent analoging.

- By rule of thumb, one chemist synthesizes, purifies, and characterizes about 100 novel compounds per year, fewer if the task is complex. It takes approximately 10,000 different compounds to develop a drug that will make it to market.
- The large capacity and appetite of screening operations has motivated chemists to develop new approaches involving parallel synthesis of many compounds. Such parallel synthesis is called fast analoging when chemical space is explored around a defined pharmacophore, or combinatorial chemistry when compounds are created by combining arrays of building blocks employing the same underlying chemistry. Both technologies have led to large libraries of synthetic compounds that are used for screening.

Screening and Hits to Leads—Primary Screen
- A primary screen is designed to rapidly identify hits from compound libraries.
- The goals are to minimize the number of false positives and to maximize the number of confirmed hits. One philosophy often quoted by people in screening operations, especially HTS environments, is not to fret about compounds that were missed but to really care about the quality of data for the compounds that repeat.
- Depending on the assay, hit rates typically range between 0.1 percent and 5 percent. This number also depends on the cutoff parameters set by the researchers, as well as the dynamic range of a given assay.
- Typically, primary screens are initially run in multiplets (i.e., two, three, or more assay data points) of single compound concentrations. Readouts are expressed as percent activity in comparison to a positive (100 percent) and a negative (0 percent) control.
- Hits are then retested a second time (or more often, depending on the assays' robustness). The retest is usually run independently of the first assay, on a different day. If a compound exhibits the same activity within a statistically significant range, it is termed a confirmed hit, which can proceed to dose-response screening.

Screening and Hits to Leads—Potency and Dose-Response
- Initial potencies of hits are either reported in milligrams per milliliter (mgs/mL), where the molecular weight of compounds is not weighed in, or in micromolar (uM), which takes into account the different molecular weights of compounds.
- Most hits have potencies between 1 and 100 uM, somewhat dependent on the dynamic range and cutoff of assays.
- Hits with potencies in the nanomolar (nM) range are rare.
- Establishing a dose-response relationship is an important step in hit verification. It typically involves a so-called secondary screen. In the secondary screen, a range of compound concentrations usually prepared by serial dilution is tested in an assay to assess the concentration or dose dependence of the assay's readout.
- Typically, this dose-response is expressed as an IC50 in enzyme-, protein-, antibody-, or cell-based assays, or as an EC50 in in-vivo experiments.
- The shape of a dose-response curve, where drug concentration is recorded on the x-axis and drug effect on the y-axis, often provides information about the mechanism of action (MOA).

Screening and Hits to Leads—Counterscreens and Selectivity
- Confirmed hits proceed to a series of counterscreens. These assays usually include drug targets of the same protein or receptor family, for example, panels of GPCRs or kinases. In cases where selectivity between subtypes is important, counterscreens might include a panel of homologous enzymes, different protein complexes, or heterooligomers. Counterscreens profile the action of a confirmed hit on a defined spectrum of biological target classes.
- Selectivity toward a drug target decreases the risk of so-called off-target side effects.

Selectivity and potency are often coupled, i.e., selectivity increases with better potency.
- Counterscreens are also used to confirm the mechanism of action. For example, if a drug molecule is believed to interfere with a particular amino acid side-chain in a protein, it will not affect a mutant protein where that residue is changed to a different amino acid. If a drug molecule is interacting with target class-specific residues involved in catalysis, it will not affect a different target class.
- The number and stringency of counterscreens can vary widely and depend on the drug target.

Screening and Hits to Leads—Mechanism of Action (MOA)
- One of the goals throughout the discovery of novel drugs is to establish and confirm the mechanism of action. In an ideal scenario, the MOA remains consistent from the level of molecular interaction of a drug molecule at the target site through the physiological response in a disease model, and ultimately in the patient.
- As an example, let's assume the drug target is a protein kinase. A confirmed hit inhibits the in-vitro catalytic activity of the kinase in the primary screen, where a surrogate or known physiological substrate is phosphorylated. In the next step, whole cells are exposed to the same inhibitor, the cells are lysed, and the physiological or native substrate is isolated and its phosphorylation state determined. Next, in a disease model dependent on a pathway regulated by the target kinase, one assesses the effect of the inhibitor on the pathway and the phenotype. If the drug action in all three steps is consistent, an MOA is established.

Lead Optimization—Overview
- Lead optimization is the complex, non-linear process of refining the chemical structure of a confirmed hit to improve its drug characteristics with the goal of producing a preclinical drug candidate. This stage frequently represents the bottleneck of a drug discovery program.
- Lead optimization employs a combination of empirical, combinatorial, and rational approaches that optimize leads through a continuous, multi-step process based on knowledge gained at each stage. Typically, one or more confirmed hits are evaluated in secondary assays, and a set of related compounds, called analogs, are synthesized and screened.
- The testing of analog series results in quantitative information that correlates changes in chemical structure to biological and pharmacological data generated to establish structure-activity relationships (SAR).
- The lead optimization process is highly iterative. Leads are assessed in pharmacological assays for their "druglikeness." Medicinal chemists change the lead molecules based on these results in order to optimize pharmacological properties such as bioavailability or stability. At that point the new analogs feed back into the screening hierarchy for the determination of potency, selectivity, and MOA. These data then feed into the next optimization cycle. The lead optimization process continues for as long as it takes to achieve a defined drug profile that warrants testing of the new drug in humans.

Lead Optimization—Medicinal Chemistry
- Medicinal chemistry blends synthetic chemistry, molecular modeling, computational biology, structural genomics, and pharmacology to discover and design new drugs, and investigate their interaction at the molecular, cellular, and whole-animal level.
- Medicinal chemistry combines empirical knowledge from the structure-function relationships of known drugs with rational designs optimizing the physicochemical properties of drug molecules.
- For example, medicinal chemists improve drug efficacy, particularly with respect to stability and bioavailability, by developing mechanism-based pro-drugs. Pro-drugs are engineered in such a way that they undergo chemical transformation either in the bloodstream or specific

tissues such as the liver. Upon transformation, biologically active metabolites are released, which are the actual drugs.

Lead Optimization—Animal PK/PD/ADME

- Animal pharmacokinetics (PK), pharmacodynamics (PD), and absorption, distribution, metabolism, and excretion (ADME) assess the general pharmacology and mechanisms of action of drugs.
- Lead molecules are administered via different routes: intravenous (iv), intraperitoneal (ip), subcutaneous (sc), intramuscular (im), rectal, intranasal (IN), inhalational, oral (po), transdermal, topical, etc. The main models used are rodents including mouse and rat, but larger animals such as dogs, pigs, and, more rarely, monkeys, are also used under certain circumstances. The main objective is to understand the effects on the whole organism of exposure to a novel chemical entity, and to predict the new drug's behavior in humans.
- PK/PD/ADME studies are an integral part of lead optimization. They feed back into the medicinal chemistry effort aiming to optimize the physicochemical properties of new leads in terms of minimal toxicity and side effects, as well as of maximum efficacy toward disease.
- PK/PD/ADME studies are expensive and usually have limited throughput. Some PK/PD studies require specific formulations, pro-drugs, or radioisotope labeling of lead molecules, all of which tend to draw heavily on medicinal chemistry resources.
- PK/PD/ADME studies rely heavily on analytical methods and instrumentation. The recent innovation and progress in mass spectroscopy, (whole-body) imaging, and chromatography technology (HPLC, LC-MS, LC-MS-MS) have tremendously increased the quantity and quality of data generated in PK/PD experiments.
- A large number of parameters is assessed. Here is a partial list: (ADME); bioavailability (F) and protein binding; stability and half-life (t1/2); maximum serum concentration (Cmax); total exposure or area under the curve (AUC); clearance (Cl); volume of distribution (Vd); drug-drug interactions; onset of drug action; multicompartmental analysis of blood, liver, and other tissues.

Lead Optimization—Toxicity

- The definition of toxicity is the degree to which a substance or mixture of substances can harm humans or animals. Acute toxicity involves harmful effects in an organism through a single or short-term exposure. Chronic toxicity is the ability of a substance or mixture of substances to cause harmful effects over an extended period, usually upon repeated or continuous exposure that can last for the entire life of the exposed organism. This may well apply to many Alzheimer's drugs.
- These days, the screening process includes a series of standard assays early on: P450 inhibition (using either recombinant cytochrome P450 enzymes or liver microsome), MTT-like cytotoxicity assays, effects on cardiac HERG channels. Toxicity in these relatively simple in- vitro assays flags hits or leads and goes into the risk-benefit evaluation of which lead series can advance into preclinical studies.
- Animal models are used for escalating dose studies aimed at determining a maximum tolerated dose (MTD). This step involves monitoring a series of parameters, such as body weight, food intake, blood chemistry (BUN), and liver activity. Biopsies are usually stored in freezers for subsequent pathological analysis.
- Animal toxicity studies require relatively large amounts of compound. The purity of the compound needs to be very high in order to exclude toxicities stemming from impurities. The norm for short-term animal toxicity is one- or two-week studies. Long-term testing in animals ranges in duration from several weeks to several years. Some animal testing continues after human tests have begun in order to learn whether long-term use of a drug may cause cancer or

birth defects.
- Empirically, medicinal chemists find it difficult to "engineer away" existing toxicity. Hence, time and money is spent instead on lead series that come without early liabilities.

Lead Optimization—Formulation and Delivery
- The formulation and delivery of drugs is an integral part of the drug discovery and development process. Indeed, formulation problems and solutions influence the design of the lead molecules; they feed back into the iterative lead optimization cycle, as well as the preclinical and clinical evaluations.
- In turn, formulation and delivery are closely linked. For example, intravenous delivery of a novel drug might call for a different formulation than oral delivery, because parameters such as metabolic stability or solubility can differ significantly.
- If formulation substances are not generally recognized as safe (GRAS), they become part of the safety assessment and their PK/PD/ADME behavior, as well as toxicity profile, needs to be documented in the IND (investigational new drug) application. In fact, side effects such as local irritation or allergic reactions are often attributable to drug formulation, not the active pharmaceutical ingredient (API).
- Formulation substances might exhibit different biological activity than the actual drug. For example, certain formulations enhance absorption through their interaction with the cell membrane of the gastrointestinal tract.
- Formulation and delivery are highly specialized fields of research, and formulation scientists are now part of serious drug discovery and development programs from the early stages.
- Indeed, a sizable number of drug discovery and development programs in the pharmaceutical and biotech industry are centered around new ways of formulating already known and even marketed drugs to increase their efficacy or safety profiles.

Development—Overview
- The decision to take a new drug candidate into the development phase entails a significant commitment in terms of money, resources, and time.
- The attrition rate for making it to market is a disheartening nine in 10 compounds, and development costs per approved drug amount to $800 million, according to a study by the Tufts CSDD. The average time to develop a new drug was 12 years and 10 months in 2002.
- The number of new chemical entities (NCEs) gaining market approval has decreased over the last decade down to 20 per year. At the same time, the estimated average of new NCEs needed for the pharmaceutical and biotech industry to sustain a 5 percent growth rate is 50.
- In terms of standards, drug development requires attention to the following:
  - GLP—Good Laboratory Practice refers to nonclinical laboratory studies that support or are intended to support applications for research or marketing permits;
  - GMP—Good Manufacturing Practice, also known as cGMP ("current" GMP), is a set of regulations requiring that quality, safety, and effectiveness be built into foods, drugs, medical devices, and biological products.
  - 21 CFR—describing the code of regulations for food and drugs. Part 11 has become particularly relevant describing the standards and regulations on electronic data and electronic signatures.

Development—Preclinical Data Package
- Under FDA requirements, a sponsor must first submit data showing that the drug is reasonably safe for use in initial, small-scale clinical studies. Depending on whether the compound has been studied or marketed previously, the sponsor may have several options for fulfilling this requirement: (1) compiling existing nonclinical data from past in-vitro laboratory or animal

studies of the compound; (2) compiling data from previous clinical testing or marketing of the drug in the United States or another country whose population is comparable to the U.S. population; or (3) undertaking new preclinical studies designed to provide the evidence necessary to support the safety of administering the compound to humans.

- During preclinical drug development, a sponsor evaluates the drug's toxic and pharmacologic effects through in-vitro and in-vivo laboratory animal testing. Genotoxicity screening is performed, as well as investigations on drug absorption and metabolism, the toxicity of the drug's metabolites, and the speed with which the drug and its metabolites are excreted from the body. At the preclinical stage, the FDA will generally ask, at a minimum, that sponsors: (1) develop a pharmacological profile of the drug; (2) determine the acute toxicity of the drug in at least two species of animals, and (3) conduct short-term toxicity studies ranging from two weeks to three months, depending on the proposed duration of use of the substance in the proposed clinical studies.

Development—Process Development/CMC/API

- Upon nomination of a development candidate, it becomes imperative that the highest-quality compound can be provided for preclinical and clinical development repeatedly and consistently at reasonable cost and in a timely manner.
- The initial synthetic route will be revised and optimized to achieve:
    - Accessibility of readily available and cost-effective starting material
    - Minimization of synthetic and purification steps
    - Feasibility of scale-up from microgram to gram, and possibly to kilogram scale.
    - Reduced cost of goods (COGS)
- The FDA will require a Chemistry, Manufacturing and Controls (CMC) documentation package for any drug entering clinical trials.
- CMC:
    - Active Pharmaceutical Ingredients (API)
    - Description and characterization
    - Manufacturer
    - Synthesis/method of manufacture
    - Process controls
    - Specifications (list of tests, methods and acceptance criteria)
    - Purity profiles
    - Container/closure system for drug substance (DS) storage
    - Container/closure system for drug product (DP) shelf life
    - Stability
- Examples abound where quality or cost of compounds led to delays or failure of clinical trials, underscoring the importance of process development for the overall success of a new drug.

Development—IND Application

- In many ways, the investigational new drug (IND) application is the result of a successful preclinical development program. The IND is also the vehicle through which a sponsor advances to the next stage of drug development known as clinical trials (human trials).
- Generally, this includes data and information in three broad areas:
    - Animal Pharmacology and Toxicology Studies: Preclinical data to permit an assessment of whether the product is reasonably safe for initial testing in humans.
    - Manufacturing Information: Information pertaining to the composition, manufacture, stability, and controls used for manufacturing the drug substance and the drug product. This information is assessed to ensure the company can adequately produce and supply consistent batches of the drug.

- Clinical Protocols and Investigator Information: Detailed protocols for proposed clinical studies to assess whether the initial-phase trials will expose subjects to unnecessary risks. Also, information on the qualifications of clinical investigators to assess whether they are qualified to fulfill their clinical trial duties. Clinical investigators are professionals, generally physicians, who oversee administration of the experimental compound.
- Types of INDs: "Commercial INDs" are applications that are submitted primarily by companies whose ultimate goal is to obtain marketing approval for a new product. However, there is another class of filings broadly known as "non-commercial" INDs, which, in fact, account for the vast majority of INDs filed. Submitted by NIH and other sponsors, these INDs include "Investigator INDs," "Emergency Use INDs," and "Treatment INDs."

Clinical Trials—Overview

- Clinical trials are a peculiar hybrid between a formalized and strictly regulated process on the one hand and a sophisticated stratagem on the other, particularly when it comes to patient selection, statistical methodology, disease markers, and endpoints employing cutting-edge research. They are also expensive, accounting for 50 to 70 percent of the drug discovery and development cost. They can be very long, lasting many years depending on therapeutic area.
- Ninety percent of NCEs entering clinical trials fail. Forty percent of compounds fail in Phase 1, 62 percent of successful Phase 1 compounds fail in phase 2, 40 percent of successful Phase 2 compounds fail in Phase 3, and a surprising 23 percent of successful Phase 3 compounds fail at the registration stage, when the FDA denies approval for a completed New Drug Application (NDA.)
- In 1991, the main reason for failure was problems in PK/bioavailability (40 percent) followed by lack of efficacy (30 percent) and toxicology (12 percent). In 2000, the main reason for failure was lack of efficacy (27 percent), followed by commercial and market reasons (21 percent) and toxicology (20 percent).
- Success rates vary with therapeutic area: Cardiovascular (20 percent), arthritis/pain (17 percent) and infectious disease drugs (16 percent) fare better than drugs for CNS diseases (8 percent), oncology (5 percent,) or women's health (4 percent).
- All current clinical trials registered with the FDA are listed at this website: http://clinicaltrials.gov/

Phase 1—Overview

- Phase 1 includes the initial introduction of an investigational new drug into humans. These studies are closely monitored and may be conducted in patients, but are usually conducted in healthy volunteer subjects. These studies are designed to determine the metabolic and pharmacologic actions of the drug in humans, the side effects associated with increasing doses, and, if possible, to gain early evidence on efficacy. During Phase 1, sufficient information about the drug's pharmacokinetics and pharmacological effects should be obtained to permit the design of well-controlled, scientifically valid Phase 2 studies.
- Phase 1 studies also evaluate drug metabolism, structure-activity relationships (SAR), and the mechanism of action (MOA) in humans. These studies also determine which investigational drugs are used as research tools to explore biological phenomena or disease processes. The total number of subjects included in Phase 1 studies varies with the drug, but is generally in the range of 20 to 80.
- In Phase 1 studies, CDER (Center for Drug Evaluation and Research) can impose a clinical hold (i.e., prohibit the study from proceeding or stop a trial that has started) for reasons of safety, or because of a sponsor's failure to accurately disclose the risk of study to investigators. Although CDER routinely provides advice in such cases, investigators may choose to ignore any advice regarding the design of Phase 1 studies in areas other than patient safety.

Phase 1—Safety and Dosage

- The first Phase 1 study is usually a single-dose study where healthy volunteers receive a range of single doses of the investigational drug. The design and determination of the dose range relies on data such as the maximum tolerated dose (MTD) determined in preclinical animals studies. Vital signs and physiological parameters, such as blood chemistry, are closely monitored in the volunteers and the PK parameters in humans are determined for a single dose.
- The safety and PK data from the single-dose study serve as guide posts for a subsequent multiple-dose study in healthy volunteers, where indicated.
- Occasionally, Phase 1 testing is divided into two steps known as Phase 1a and Phase 1b. Phase 1a studies normally are conducted as a short-term study to ensure safety before embarking on a longer and more comprehensive Phase 1b study. Phase 1b studies can include actual patients and might provide first indications about drug efficacy against disease.
- Establishing the safety of a new drug molecule is paramount in Phase 1. Also essential is the determination of the best dosage or dosage regimen for subsequent, larger phase 2 trial(s), where the assessment of drug effectiveness in patients moves to the fore.

Phase 2—Overview

- Phase 2 includes early controlled clinical studies conducted to obtain some preliminary data on the efficacy of the drug for a particular indication (or indications) in patients with the disease. This testing phase also helps determine common short-term side effects and risks associated with the drug.
- Decisive or pivotal trials are usually run as randomized controlled trials (RCT). Randomization introduces a deliberate element of chance into the assignment of treatments to trial patients.
- Phase 2a: Pilot trials to evaluate efficacy and safety in selected populations of about 100 to 300 patients who have the condition to be treated, diagnosed, or prevented. They often involve hospitalized patients who can be closely monitored. Objectives may focus on dose-response, type of patient, frequency of dosing, or any of a number of other issues involved in safety and efficacy.
- Phase 2b: Well-controlled trials to evaluate safety and efficacy in patients who have the condition to be treated, diagnosed, or prevented. These trials usually represent the most rigorous demonstration of a medicine's efficacy.

Phase 3—Overview

- Phase 3 studies are expanded, controlled, and uncontrolled trials. They are performed after preliminary evidence of effectiveness has been obtained in Phase 2, and are intended to gather the additional information about safety and effectiveness needed to evaluate the overall benefit-risk relationship of the drug. Phase 3 trials should provide an adequate basis for extrapolating the results to the general population and conveying that information in the physician labeling. These studies usually include several hundred to several thousand people.
- In both Phase 2 and 3, the Center for Drug Evaluation and Research (CDER), a branch of the FDA, can impose a clinical hold if a study is unsafe or if the protocol design is deficient in meeting its stated objectives. The FDA aims to ensure that this determination reflects current scientific knowledge, agency experience with clinical trial design, and experience with the class of drugs under investigation.
- FDA approval/disapproval decisions are based on the results of pivotal studies. To be considered pivotal, a study must meet at least these 4 criteria:
  - Be controlled using placebo or a standard therapy.
  - Have a double-blinded design when such a design is practical and ethical.

- Be randomized.
- Be of adequate size.

NDA—Overview

- Although the amount of information and data submitted in NDAs varies, the components of NDAs are uniform. The components of any NDA are, in part, a function of the nature of the subject drug and the information available to the applicant at the time of submission. As outlined in Form FDA-356h (Application to Market a New Drug for Human Use Or As An Antibiotic Drug For Human Use) NDAs can consist of as many as 15 different sections:
  - Index;
  - Summary;
  - Chemistry, Manufacturing, and Control (CMC);
  - Samples, Methods Validation Package, and Labeling;
  - Nonclinical Pharmacology and Toxicology;
  - Human Pharmacokinetics and Bioavailability;
  - Microbiology (for anti-microbial drugs only);
  - Clinical Data;
  - Safety Update Report (typically submitted 120 days after the NDA's submission);
  - Statistical;
  - Case Report Tabulations;
  - Case Report Forms;
  - Patent Information;
  - Patent Certification; and
  - Other Information.

Review—Overview

- In the primary review process, reviewers attempt to confirm and validate the sponsor's conclusion that a drug is safe and effective for its proposed use. The review is likely to involve a reanalysis or an extension of the analyses presented in the NDA. For example, the medical reviewer may seek to reanalyze a drug's effectiveness in a particular patient subpopulation not analyzed in the original submission. Similarly, the reviewer may disagree with the sponsor's assessment of evaluable patients and seek to retest effectiveness claims based on the patient populations defined by the reviewer.
- Review team members communicate extensively with each other. If a medical reviewer's reanalysis of clinical data produces results different from those of the sponsor, the reviewer will forward this information to the statistical reviewer with a request for a statistical reanalysis of the data. Likewise, the pharmacology reviewer may work with the statistical reviewer in evaluating the statistical significance of potential side effects in long-term animal studies.
- When the technical reviews are complete, each reviewer develops a written evaluation of the NDA that presents their conclusions and their recommendations on the application. The division director or office director then evaluates the reviews and recommendations and decides the action that the division will take on the application. The result is an action letter that provides an approval, approvable, or non-approvable decision and a justification for that recommendation.

Phase 4—Overview

- Phase 4 trials are done after a drug has received a market approval. These trials are monitoring drugs that are available for doctors to prescribe, rather than experimental drugs that are still being developed.

Pharmaceutical companies run Phase 4 trials to find out:
- More about safety and side effects of the drug.
- What the long-term risks and benefits are.
- How well the drug works when it is used more widely than in clinical trials.

- Below are some current examples of approved drugs that were retracted:

Drug (Indication)	Approved	Withdrawn	Years Delay	Reason Drug Is Pulled	Company
Fenfluramine (weight loss)	1973	1997	24	Pulmonary hypertension, heart valve disease	Wyeth-Ayerst
Posicor (hypertension, angina)	1985	1998	13	Reduced liver enzymes	Roche
Seldane (allergies)	1985	1997	12	Heart problem when taken with other drugs	Hoescht Marion Roussel
Hismanal (allergies)	1988	1999	11	Heart arrhythmia	Janssen Pharmaceutica
Propulsid (nocturnal heartbeat)	1993	2000	7	Cardiac arrhythmia	Janssen Pharmaceutica
Vioxx (pain)	1999	2004	5	Heart attack, stroke	Merck
Baycol (anti-cholesterol)	1997	2001	4	Muscle deterioration	Bayer
Rezulin (anti-diabetes)	1997	2000	3	Liver toxicity	Pfizer
Razar (antibiotic)	1997	1999	2	Severe cardiovascular problems	Glaxo
Raplon (airway muscle relaxant)	1999	2001	2	Bronchospasm	Organon
Duract (pain)	1997	1998	1	Hepatitis, liver failure	Wyeth-Ayerst
Lotronex (IBD)	2000	2000	9 months	Ischemic colitis, constipation	Glaxo

References

Bolten, B.M. and DeGregorio, T. (2002) Trends in development cycles. Nature Reviews Drug Discovery 1, 335-336.

Chanda, S.K. and Caldwell, J.S. (2003) Fulfilling the promise: drug discovery in the post-genomic area. Drug Discovery Today 8, 168-174.

DiMasi, J.A., Hansen, R.W. and Grabowski, H.G. (2003) The price of innovation: new estimates of drug development costs. J. Health Economics 22, 151-185.

Drews, J. (2000) Drug Discovery: a historical perspective. Science 287, 1960-1964.

FDA (2004) Innovation and Stagnation: Challenge and Opportunity on the Critical Path to New Medical Products. FDA White Paper.

Handen, J.S. (2002) The industrialization of drug discovery. Drug Discovery Today 7, 83-85.

Kola, I. And Landis, J. (2004) Can the pharmaceutical industry reduce attrition rates? Nature Reviews Drug Discovery 3, 711-715.

Lindsay, M.A. (2003) Target discovery. Nature Reviews Drug Discovery 2, 831-838.

Nature Insight: Chemical Space (2004) Nature 432, 824-865.

Sams-Dodd, Frank (2005) Target-based drug discovery: is something wrong? Drug Discovery Today 10, 139-147.

Related Web Sites

- FDA Center for Drug Evaluation and Research

- Tufts Center for the Study of Drug Development

- ClinicalTrials.gov