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Pharmacokinetics/ADME In Drug Discovery

An Example

Identified a new compound for memory loss

Animal studies

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PK/ADME in Drug Discovery
Overview

- Pharmacokinetics, absorption, distribution, metabolism, elimination
  - Pharmacodynamics
- Why these are important in early research, target validation and discovery programs
- When should they be determined
  - Early in the process
- Examples

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Drug Development

- Nine of every ten new drugs fail in clinical testing.
  - A drug in phase III testing has 32% chance of failure.
  - Even in Phase I, 37% fail.
  - Most drugs fail in phase II.
- Why
  - Laboratory rat and mice strains are too genetically homogeneous
  - The animal model is wrong, or the theory behind the disease is wrong
  - The pharmacology is done incorrectly
  - Other reasons??
  - **Rats and humans have different metabolic pathways**
  - **There are unforeseen side effects**

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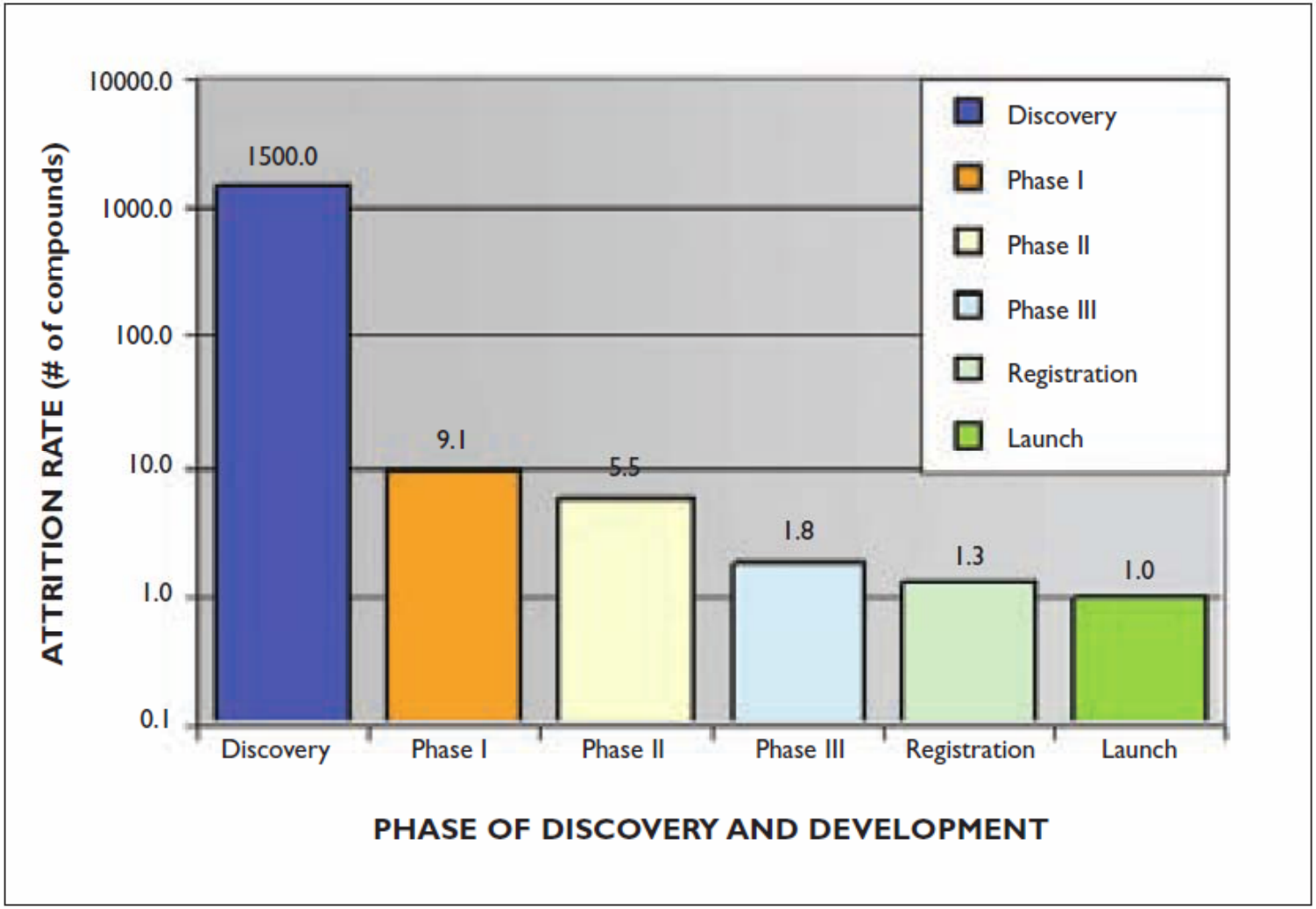
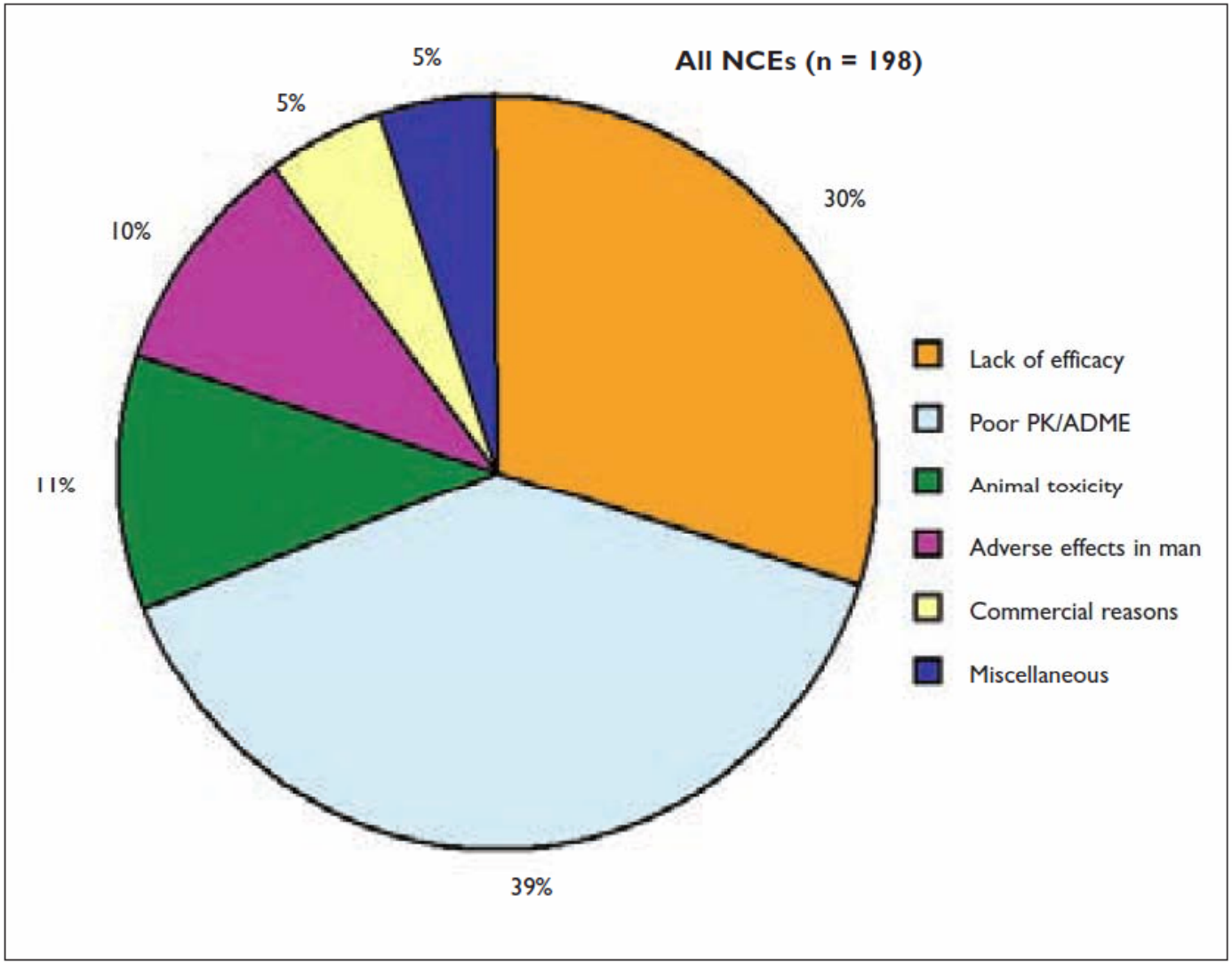
Attrition Rate



Compound Failures



Absorption, Distribution, Metabolism and Excretion

- Absorption - route of drug delivery
  - Where absorbed
- Distribution - where does the drug go, where does it need to go and what are the implications
- Metabolism - this will occur and could impact several variables
  - Could be used to your advantage - Prodrugs
- Excretion – how is the drug eliminated

- **Pharmacokinetics is concerned with the variation in drug concentration with time as a result of absorption, metabolism, distribution and excretion**
  - Drug dose, route of administration, rate and extent of absorption, distribution rate (particularly to site of action) and rate of elimination
  - **Pharmacokinetics** may be simply defined as what the body does to the drug
  - **Pharmacodynamics** defined as what the drug does to the body

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Drug Delivery - Enteral Routes

- Oral- by far the most common route. The passage of drug from the gut into the blood is influenced by biologic and physicochemical properties.

- Sublingual (buccal) - Certain drugs are best given beneath the tongue or retained in the cheek pouch and are absorbed from these regions into the local circulation.

- Rectal -The administration of suppositories is usually reserved for situations in which oral administration is difficult. This route is more frequently used in small children.

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Parental Routes

- Intravenous injection
  - Used when a rapid clinical response is necessary, e.g., an acute asthmatic episode.
  - Achieve relatively precise drug concentrations in the plasma, since bioavailability is not a concern.
- Intra-arterial injection
  - Used in certain special situations, notably with anticancer drugs, in an effort to deliver a high concentration of drug to a particular tissue. Typically, the injected artery leads directly to the target organ.
- Intrathecal injection
  - The blood-brain barrier limits the entry of many drugs into cerebrospinal fluid. life-threatening, antibiotics, antifungals and anticancer drugs are given via lumbar puncture and injection into the subarachnoid space.

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Parental (Cont)

- Intramuscular injection
  - Drugs may be injected into the arm, thigh or buttocks.
- Subcutaneous injection
  - Some drugs, notably insulin, are routinely administered SC. Drug absorption is generally slower SC than IM, due to poorer vascularity.
- Inhalation
  - Volatile anesthetics, as well as many drugs which affect pulmonary function, are administered as aerosols. Drugs administered via this route are not subject to first-pass liver metabolism.
- Topical application
  - Eye, intravaginal, intranasal, skin.
  - Alleviation of local symptoms.

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Drug Absorption
Biological Factors

- Membrane structure and function - The cell membrane is a semi-permeable lipoid sieve containing numerous aqueous channels, as well as a variety of specialized carrier molecules.

- Passive lipid diffusion is probably the most important absorptive mechanism. Lipid-soluble drugs dissolve in the membrane, and are driven through by a concentration gradient across the membrane.

- Carrier-mediated facilitated transport occurs for some drugs, particularly those which are analogs of endogenous compounds for which there already exist specific membrane carrier systems.
  - For example, methotrexate, an anticancer drug which is structurally similar to folic acid, is actively transported by the folate membrane transport system.

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Oral Drug Absorption

- The blood supply draining the gut passes through the liver before reaching the systemic circulation.
  - First-pass effect may reduce the amount of drug reaching the target tissue.
- Drug binding
  - Many drugs will bind strongly to proteins in the blood or to food substances in the gut.
  - Plasma protein binding will increase the rate of passive absorption by maintaining the concentration gradient of free drug.
- Food effects
  - Absorption can be reduced by the presence of food in the gut
  - Absorption can be enhanced by food (bile secretion)
  - Some drugs are irritating and should be administered with meals to reduce adverse effects.

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Plasma Protein Binding

- Drugs can bind to plasma proteins
  - Human serum albumin, lipoprotein, glycoprotein, and α, β, and γ globulins
- Protein binding can influence the drug's biological half-life
  - Fraction bound or free fraction
  - Warfarin is 97% protein bound
- Free fraction is an important consideration when looking at in vivo activity
- Protein binding can have implications in drug-drug interations

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Distribution

- Once in the blood, drugs are simultaneously distributed throughout the body and eliminated.
  - Distribution is much more rapid than elimination, accomplished via the circulation, and influenced by regional blood flow.
- Compartments
  - Central Compartment- The central compartment includes the well-perfused organs and tissues (heart, blood, liver, brain and kidney) with which drug equilibrates rapidly.
  - Peripheral Compartment(s)- The peripheral compartment(s) include(s) those organs (e.g., adipose and skeletal muscle) which are less well-perfused, and with which drug therefore equilibrates more slowly.
  - Special Compartments - The cerebrospinal fluid (CSF) and central nervous system (CNS) is restricted by the structure of the capillaries and pericapillary glial cells.
  - Drugs also have relatively poor access to pericardial fluid, bronchial secretions and fluid in the middle ear.

Metabolism
Drug Transformation

- Phase I and Phase II metabolism
  - Most products of drug metabolism are less active than the parent compound.
- Metabolites may be responsible for toxic, mutagenic, teratogenic or carcinogenic effects
  - For example, acetaminophen hepatotoxicity is due to a minor metabolite which reacts with liver proteins.
- Metabolism of so-called prodrugs, metabolites are actually the active therapeutic compounds
  - Cyclophosphamide, an inert compound which is metabolized by the liver into a highly active anticancer drug.

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Sites of Drug Metabolism

- The liver is the primary organ of drug metabolism.
- The gastrointestinal tract is the most important extrahepatic site
  - Some orally administered drugs (e.g., isoproterenol) are conjugated extensively in the intestinal epithelium, resulting in decreased bioavailability.
- The lung, kidney, intestine, skin and placenta can also carry out drug metabolizing reactions
  - Lung has an enormous perfusion rate and may exert a first-pass effect for drugs administered IV.

Phase I Metabolism

- Most enzymes involved in drug metabolism are located within the lipophilic membranes of the smooth endoplasmic reticulum (SER).
  - Microsomal preps can be isolated and used to evaluate new compounds (rat, dog, human microsomes)
  - Most of the enzymes carry out oxidation reactions (mixed function oxidase, MFO) and require a reducing agent (NADPH), molecular oxygen, and a complex of microsomal enzymes)

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Cytochrome P450 Enzymes

- Cytochrome P450, a heme protein so named because its carbon monoxide derivative absorbs light at 450 nm
- Family of enzymes which differ primarily with regard to their substrate specificities (70 distinct P450 genes)
- Critical for assessing drug-drug interactions
  - Induction or inhibition
- Fluorescent assays looking at the enzymes, microsomal assays, cultured hepatocytes

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Cytochrome P450's

P_{450} Gene Family/Subfamily	Characteristic Substrates	Characteristic Inducers	Characteristic Inhibitor
CYP 1A2	Acetominophen Estradiol Caffeine	Tobacco, Char-Grilled Meats, Insulin	Cimetidine, Amiodarone, Ticlopidine
CYP 2C19	Diazepam, Omeprazole Progesterone	Prednisone , Rifampin	Cimetidine, Ketoconazole, Omeprazole
CYP 2C9	Tamoxifen Ibuprofen Fluoxetine	Rifampin Secobarbital	Fluvastatin, Lovastatin, Isoniazid
CYP 2D6	Debrisoquine Ondansetron Amphetamine	Dexamethasone Rifampin	Cimetidine, Fluoxetine , Methadone
CYP 2E1	Ethanol, Benzene Halothane	Ethanol Isoniazid	Disulfiram, Water Cress
CYP 3A4, 5, 7	Cyclosporin, Clarithromycin Hydrocortisone Simvastatin	Barbiturates Glucocorticoids Carbamazepine St. John's Wort	Cimetidine, larithromycin Ketoconazole, Grapefruit Juice , many others

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Phase II Metabolism

- Phase I reactions convert a drug to a more polar compound by introducing or unmasking polar functional groups such as - OH, -NH2, or -SH.

- Phase I products are still not eliminated rapidly, and hence undergo Phase II reactions involving conjugation of the newly established polar group with endogenous compounds such as glucuronic acid, sulfuric acid, acetic acid, or amino acids (typically glycine).

- Glucuronide formation is the most common phase II reaction.
  - Sometimes, the parent drug may undergo phase II conjugation directly.
  - A drug may undergo a series of consecutive reactions resulting in the formation of dozens of metabolites.

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ADME ASSAYS

Solubility	Oral absorption
Rate of dissolution	Oral absorption
Membrane permeability (PAMPA, cell models (CaCo2, MDCK)	Oral absorption and BBB penetration
Active transport	Oral absorption and drug-drug interaction
Ionisation Constant (pKa)	Oral absorption and binding mechanism
Lipophilicity (LogP, LogD)	Oral absorption, cell membrane penetration, distribution
Chemical stability	Chemical integrity in body fluids, tissues and oral absorption
Metabolic clearance	Bioavailability and clearance
CYP450 inhibition	Metabolism and drug-drug interaction
Protein-binding	Clearance, distribution and bioavailability
Metabolite Identification	Metabolic mechanism

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Drug-like Molecules

- Good ADME properties

- Rule of 5
  - Rule of 4.5?
- MW <500, ClogP 5, H-bond donors, 5 H-bond acceptors (sum of N and O atoms) 10
- Remarks: No more than one violation; not applicable for substrates of transporters and natural products
- Extensions
  - Polar surface area, sum of H-bond donors, and acceptors, rotatable bonds

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Druglikeness

- Optimal solubility to both water and fat
  - Orally administered drug has to go through the intestinal lining, carried in aqueous blood and penetrate the lipid cellular membrane to reach the inside of a cell.
    - cLogP, is used to estimate solubility.
- High potency (IC_{50} or EC_{50})
  - Reduces the risk of non-specific, off-target pharmacology at a given concentration
  - Low clearance, high potency also allows for low total dose, which lowers the risk of idiosyncratic drug reactions
  - The less you give the better

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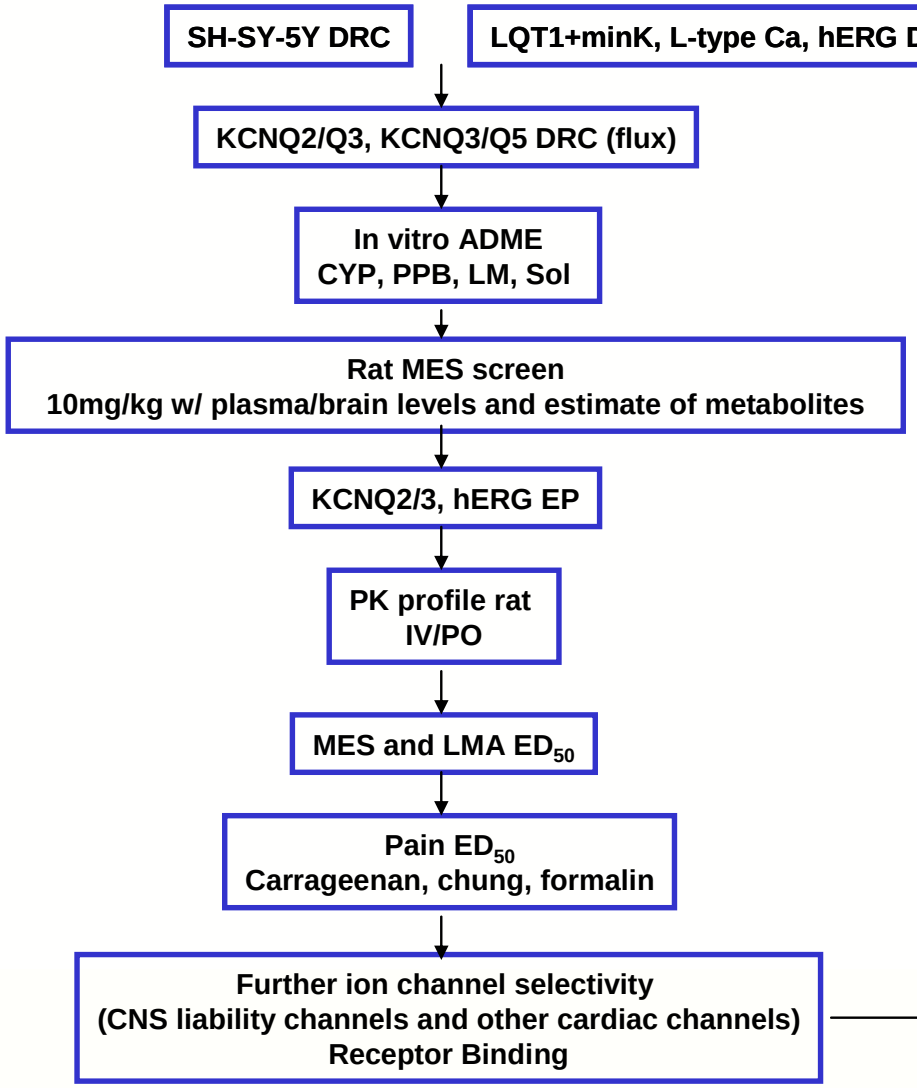
CNS Target Example

Assay Progression Scheme



In vitro selectivity

Early assessment of *in vitro* ADME

In vivo activity and early *in vivo* PK

Electrophysiology

Definitive *in vivo* PK

Drug/drug Interactions

Therapeutic index

Efficacy in pain models

Safety

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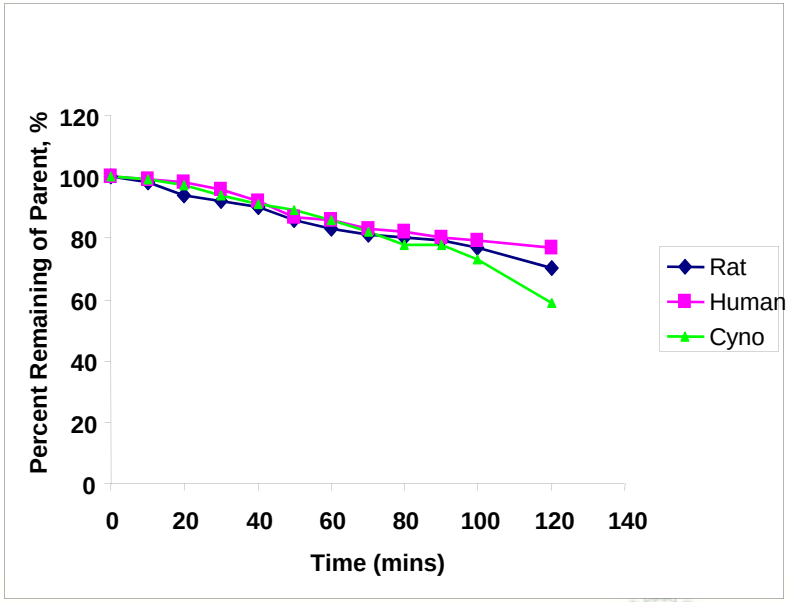
In Vitro ADME

- **Plasma protein binding**
  - 88% rat, 91% human and cynomolgus monkey
- **Brain tissue protein binding (rat)**
  - 92%
- **Good permeability – no efflux**
  - MDR-MDCK assay
- **Microsomal Stability**
  - CL_{int} *in vitro* 3/2/3 (µL/min/mg)



Cytochrome P450 Interactions

- **No inhibition, no time-dependent inhibition**

Enzyme/Compound	ICA-183998
3A4 Midazolam	>100 µM
TDI: 3A4 Midazolam	>100µM
2C9 Diclofenac	>100 µM
TDI: 2C9 Diclofenac	>100 µM
2C9 Tolbutamide	51 µM
2A6 Coumarin	>100 µM
2D6 Bufuralol	2.5 µM
2C19 Omeprazole	69 µM

- **An induction study was done at 3, 10 and 30µM**
  - CYP3A4 – No signal in enzymatic activity assay, increased mRNA expression at 30 and 10µM
  - CYP2B6 - Small signal in enzymatic activity in one donor
  - CYP1A2- No signal in enzymatic activity assay or mRNA signal.

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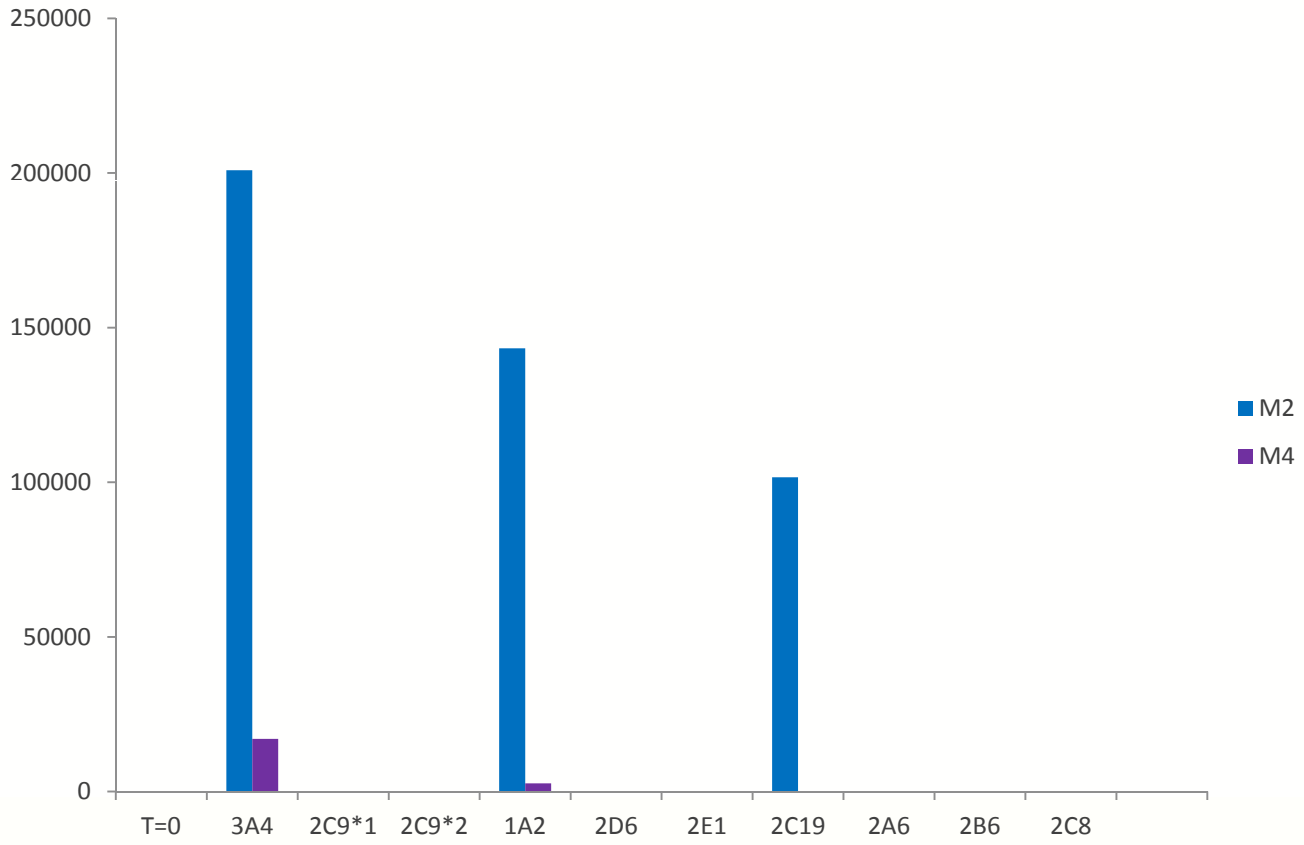
Multiple Metabolic Pathways

- Hydroxyl containing metabolites generated by 3 different cytochrome P450's



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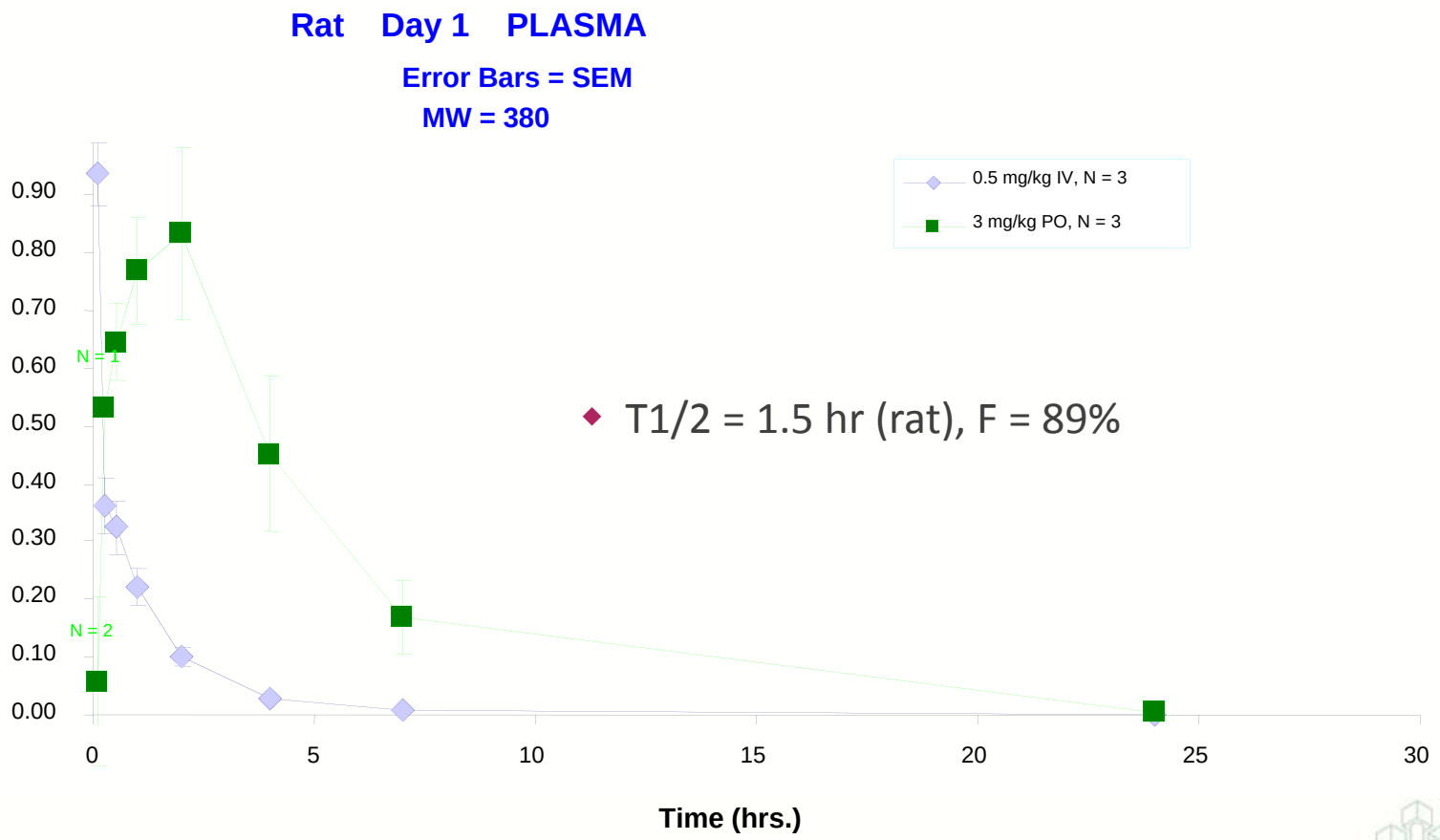
In Vivo PK Study



- T1/2 = 1.5 hr (rat), F = 89%

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New Approaches
Phase 0 Clinical Trial

- Exploratory, first in human trial
  - Micro-dose study- sub-therapeutic doses based on reduced manufacturing and toxicologic requirements
- Establishing very early on whether the drug behaves in human subjects as was expected from preclinical studies
  - Gather preliminary data on the compounds pharmacodynamics (what the drug does to the body) and pharmacokinetics (what the body does to the drug)
- **Provides no data on safety or efficacy, being by definition a dose too low to cause any therapeutic effect**

- Phase 0 studies are used to rank drug candidates in order to decide which has the best pharmacokinetic parameters in humans to take forward into further development

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Questions