Advanced Drug Metabolism
MEDCH 527

"Safety Considerations in Drug Development"

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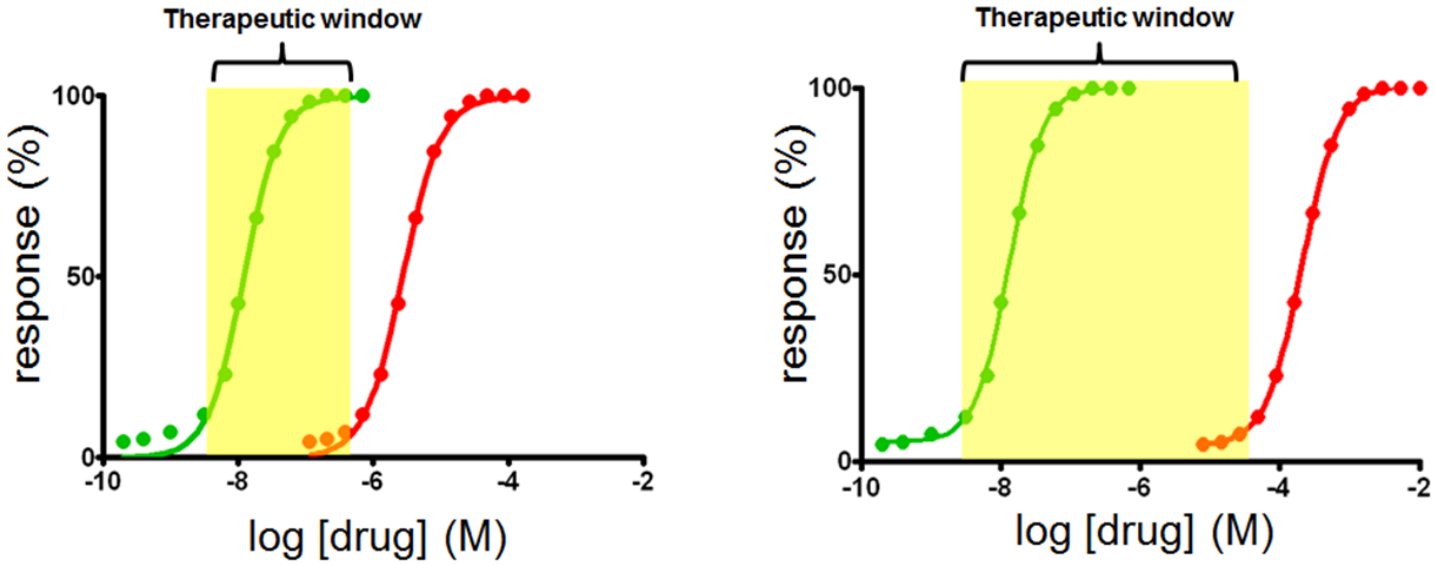
For regulatory agencies, the decision whether to approve a new drug for marketing can be distilled down to two questions:

- **Do the results of well-controlled studies provide substantial evidence of drug effectiveness?**
- **Do the results show the product is safe under the conditions of use in the proposed labeling?**
  - **Safe, in this context extends beyond assessing a drug's Therapeutic Index and embraces the notion that the benefits of the drug appear to outweigh its risks.**



Toxicologically-relevant questions for the Drug Metabolism Scientist

Pharmacology

Are there adverse effects as a result of the desired drug–target interactions?

What are common co-therapies, are pharmacokinetic drug–drug interactions likely to occur?

Chemistry

Are there known adverse drug effects associated with the major chemical structure (scaffolding)?

Are there chemical side chains with known toxicity?

Can the toxicophore be separated from the pharmacophore?

Drug metabolism and pharmacokinetics

Is the chemical entity biotransformed? If so, is it rendered more (toxification) or less (detoxification) toxic?

Are the human metabolites similar or different from the metabolites formed in laboratory animals?

Which animal species is most like human?

How rapidly is the chemical entity cleared?

Is the chemical entity or its metabolites accumulated in specific organs?

Toxicology

Is there toxicity observed with the chemical entity *in vitro* and *in vivo* and is it associated with the chemical structure?

Does drug metabolism or organ distribution contribute to the toxicity observed?

Would there be species differences in toxicity? If so, why?

Risk factors

Physiological: would specific age, gender, race and disease state enhance toxicity?

Is the patient population known to be more susceptible to certain types of adverse drug effects (e.g. hepatotoxicity in the diabetes)?

Environmental: are there environmental conditions that can enhance toxicity?

Are there co-administered drugs or foods that would lead to toxicity due to either pharmacokinetic or pharmacological interactions?

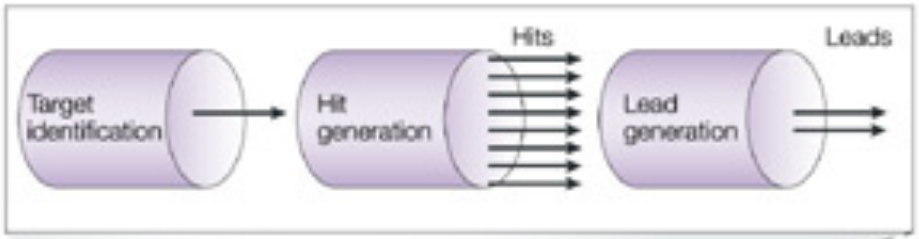
Genetic: are there genetic determinants of susceptibility to drug toxicity (e.g., is there known genetic polymorphism of the toxifying or detoxifying pathways) in the human population?

Outline for Today

- ***Background***
  - **Toxicity as a major source of attrition in drug development**
  - **Toxicity-related drug withdrawals**
  - **Drug Metabolism & Toxicity studies in drug development**
- ***Metabolism-dependent drug toxicities***
  - **Exaggerated Pharmacology and Inhibitory Metabolites**
  - **Cardiovascular toxicity (QTc effects) caused by DDIs**
  - **Reaction Phenotyping as its relates to predicting Drug Toxicity**
  - **Liver injury (chemically reactive drug metabolites)**
- ***Stable drug metabolites***
  - **Active Metabolites**
  - **FDA and ICH* "MIST" Guidance**
  - **Species differences in drug metabolism & toxicity**

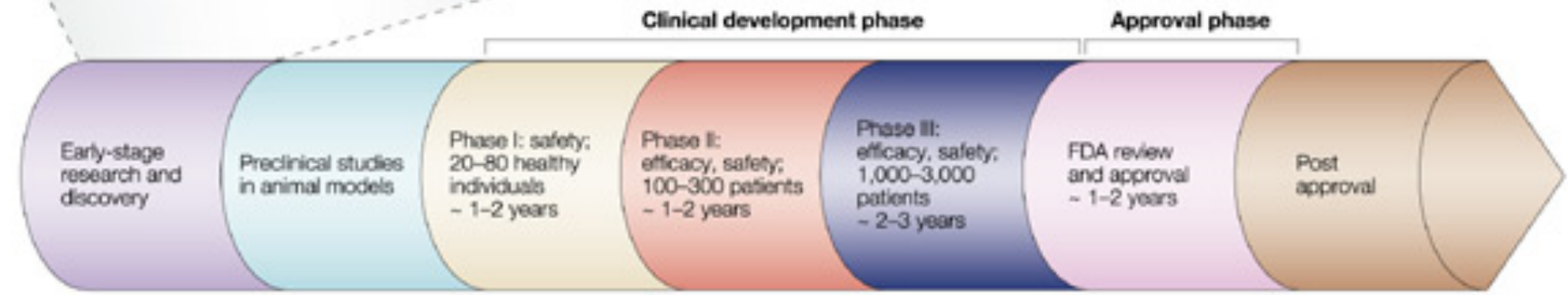
*ICH = International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use

Generic Schematic of the Drug Discovery/ Development Continuum



Phase II and III (patients)

• Long-term safety and efficacy studies that form the basis of regulatory filing (NDA)



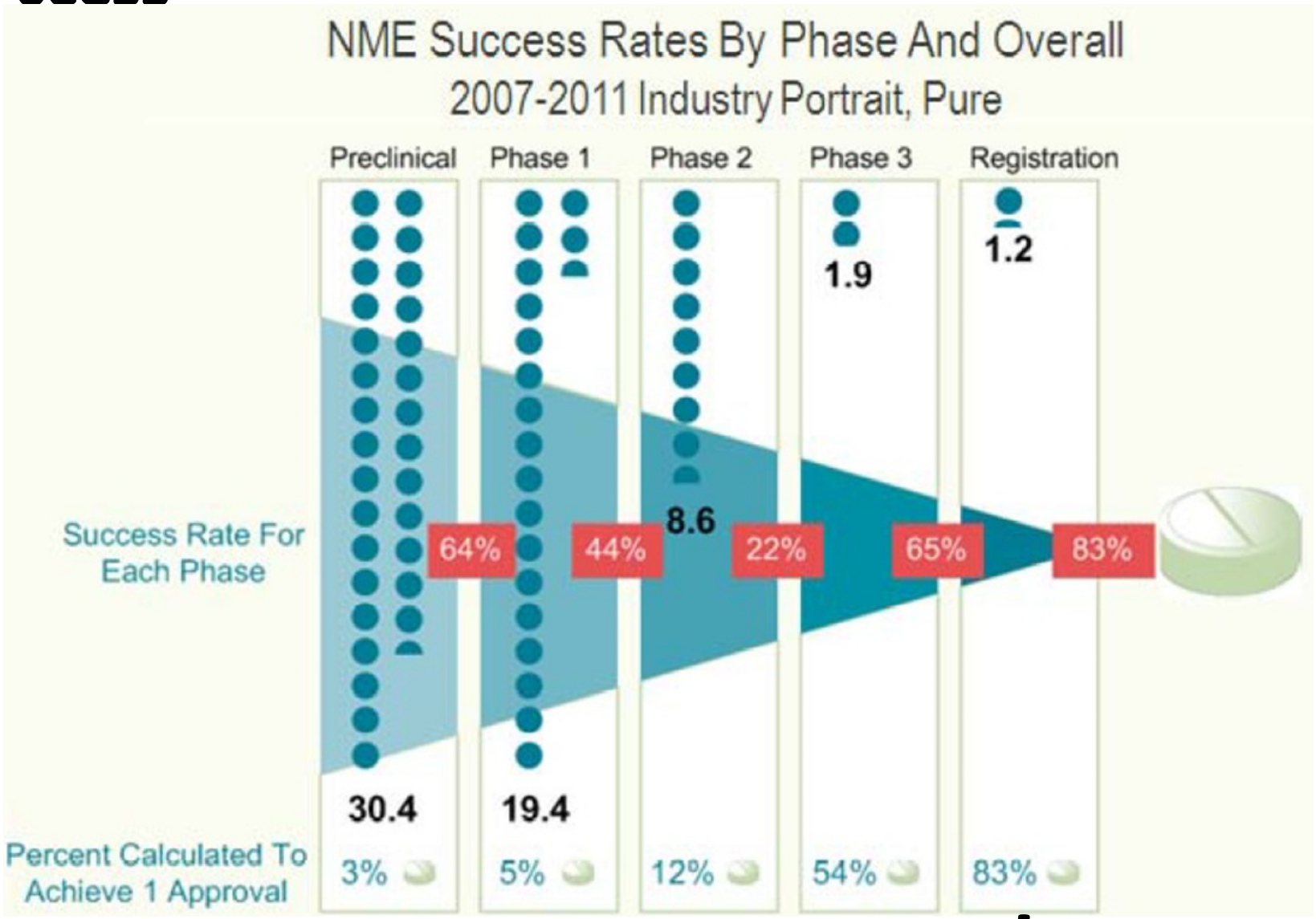
Phase I (healthy volunteers)

- Single ascending dose (SAD) for safety, tolerability, and PK
  - Starting dose selected to give ~100-fold lower AUC than NOAEL in most sensitive animal species
- Multiple ascending dose (MAD) – duration not to exceed that of longest animal studies
  - Detailed analysis of side-effect profile; circulating metabolites, drug interaction studies, etc

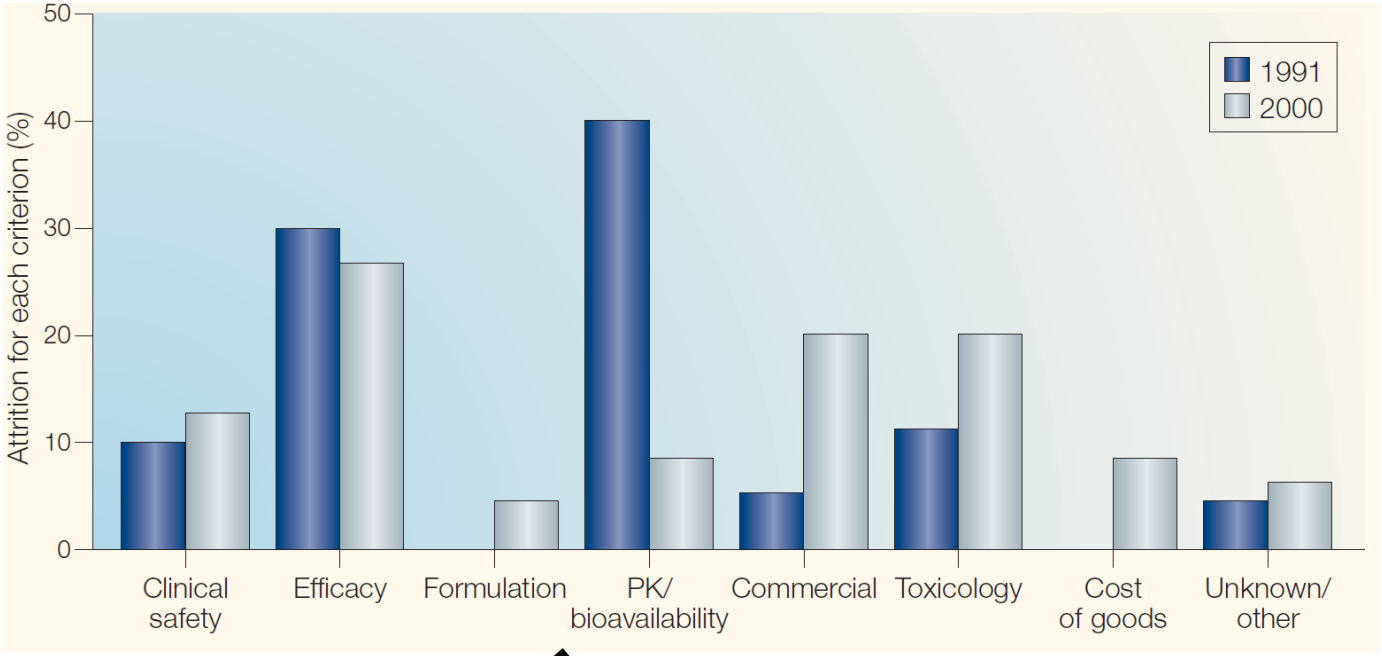
Postmarketing

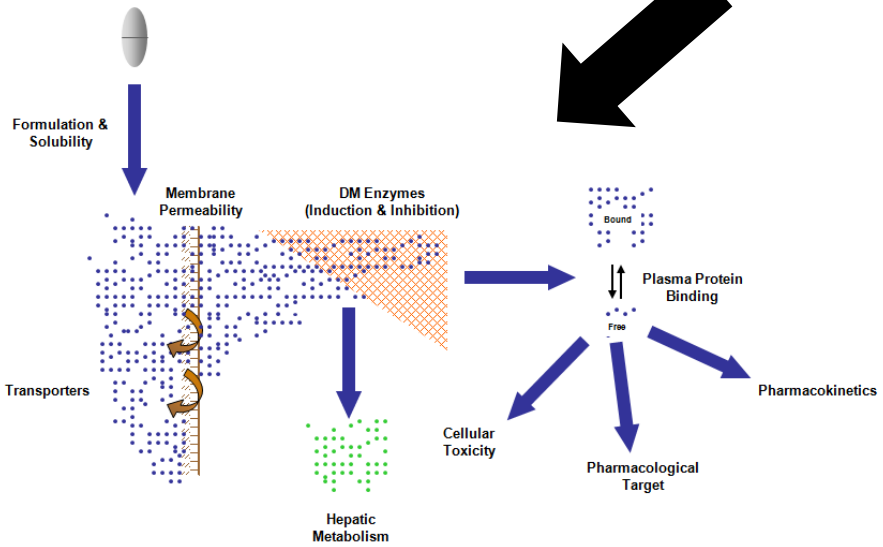
• Pharmacovigilence (adverse event reporting)

Simple Breakdown of the Drug Development Process



Reasons for NCE attrition across two decades





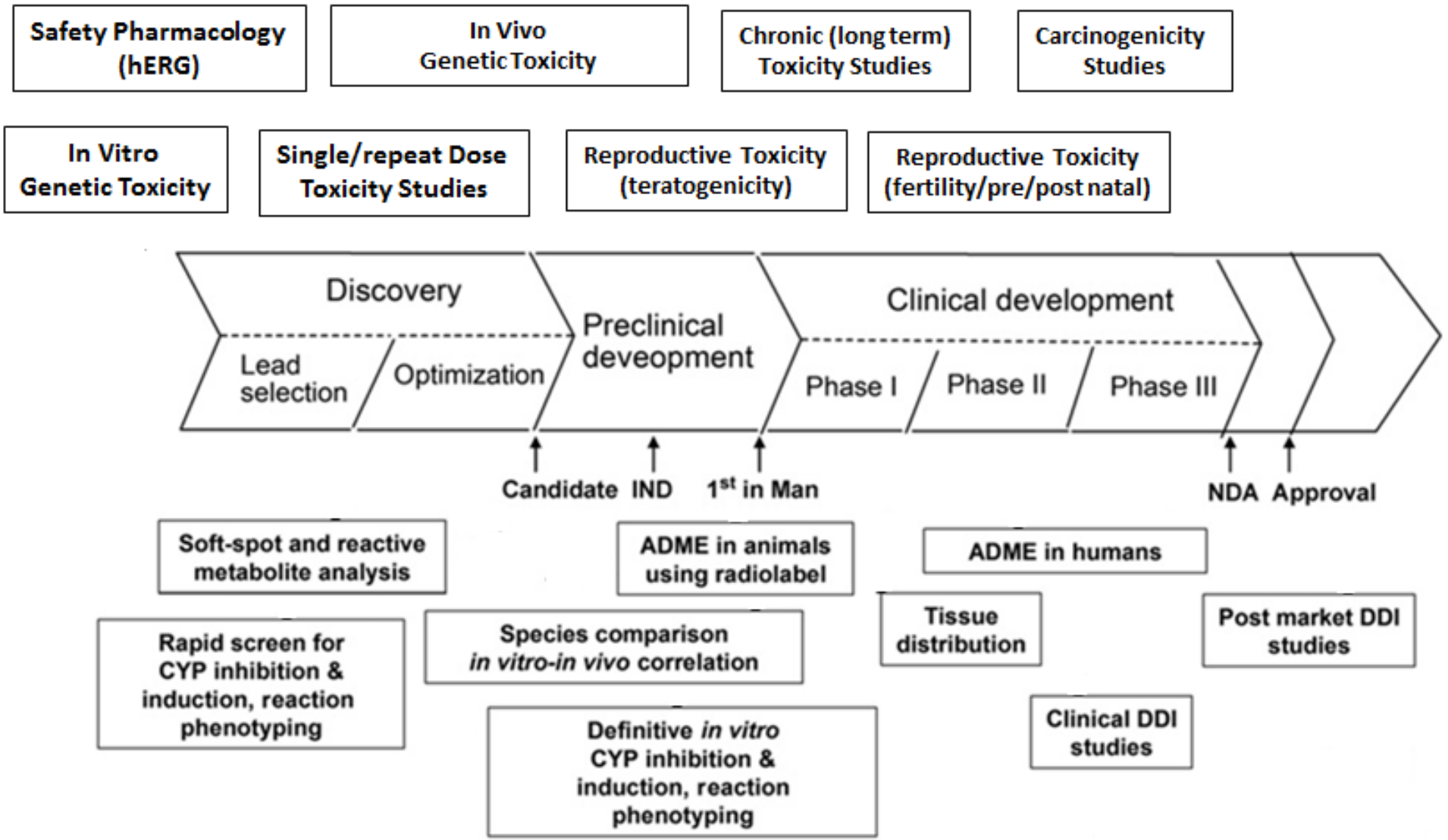
Through the implementation of early ADME screening and rule based drug design, failure shifted from poor PK properties to Toxicity

Considering the Investment, its Clear that Identifying Compound Liabilities Early is Best

Given that attrition rates remain high, it is critical that only the very best candidates from Discovery / Lead Optimization efforts are taken forward into development.

- **The role of scientists engaged in drug discovery has expanded in recent years such that it is now important to consider many issues beyond organic synthesis, pharmacology, etc, notably;**
  - **Drug Metabolism and Pharmacokinetics (DMPK)**
  - **Preclinical Toxicology**
- **Challenges in Drug Discovery**
  - **Minimizing potential for toxicity (esp. cardiovascular and liver toxicity)**
  - **Minimizing potential drug-drug interaction potential**
  - **Dealing prospectively with reactive drug metabolite issues**
  - **Developing strategies to respond to stable drug metabolites ("MIST")**

Drug Metabolism / Toxicity "Touch points" at Various Times Across the Development Process



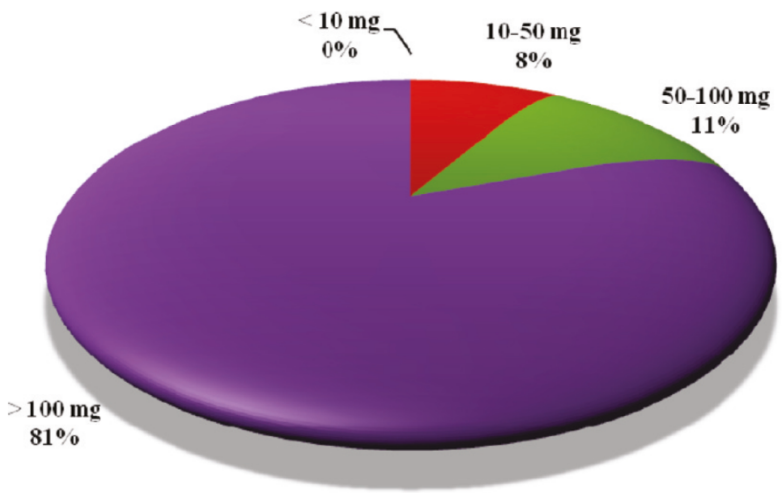
Some Important Determinants of Drug Toxicities

- **Dose of the Drug**
- **Patient variability of a drug and its metabolites**
- **Immune recognition of macromolecular adducts of the drug and/or metabolites**
- **Efficiency of macromolecular repair mechanisms**
- **Chemical structure of a drug and its metabolites**
- **Relative rates of toxic metabolite formation vs detoxifcation**

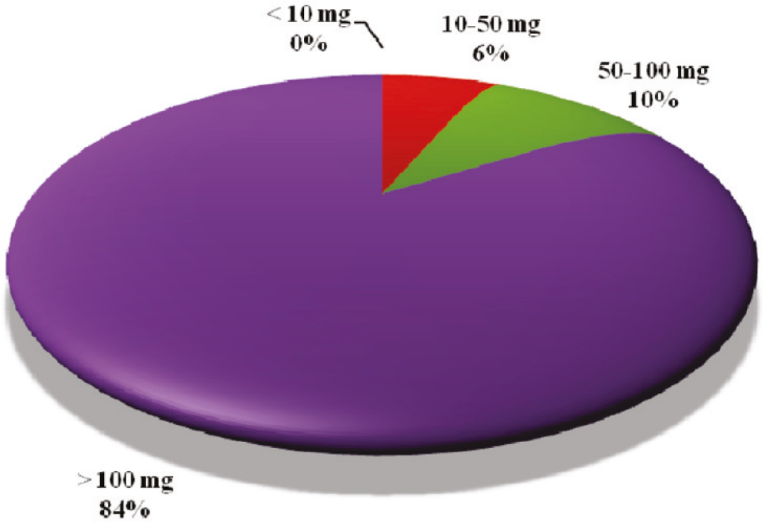
The Dose makes the Poison

"All things are poison and nothing is without poison, only dose permits something not to be poisonous" Paracelsus (1493-1541)

Maximum recommended daily dose assessments for drugs associated with idiosyncratic toxicity.



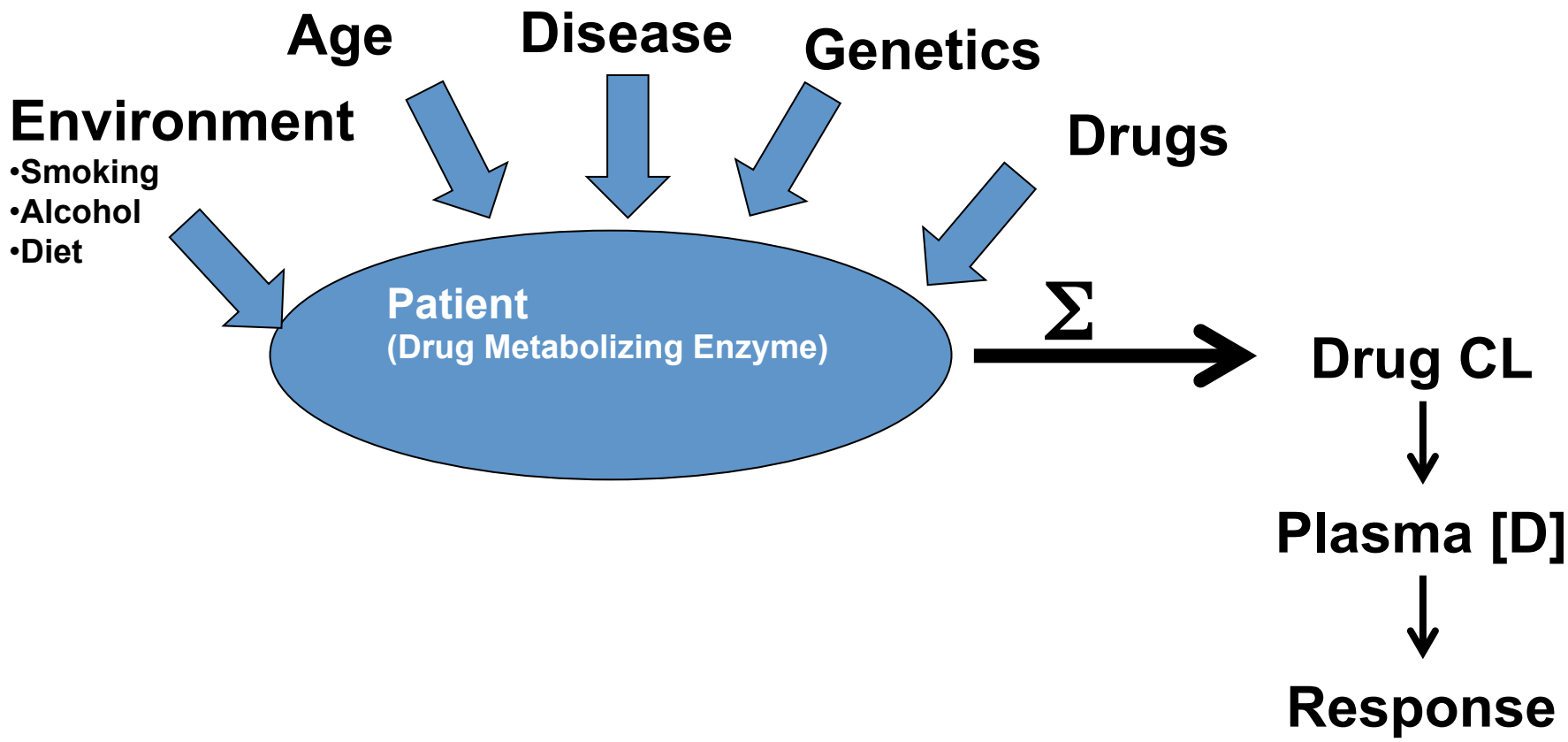
Black Box Warning Due to IADRs



Withdrawn from the Market Due to IADRs

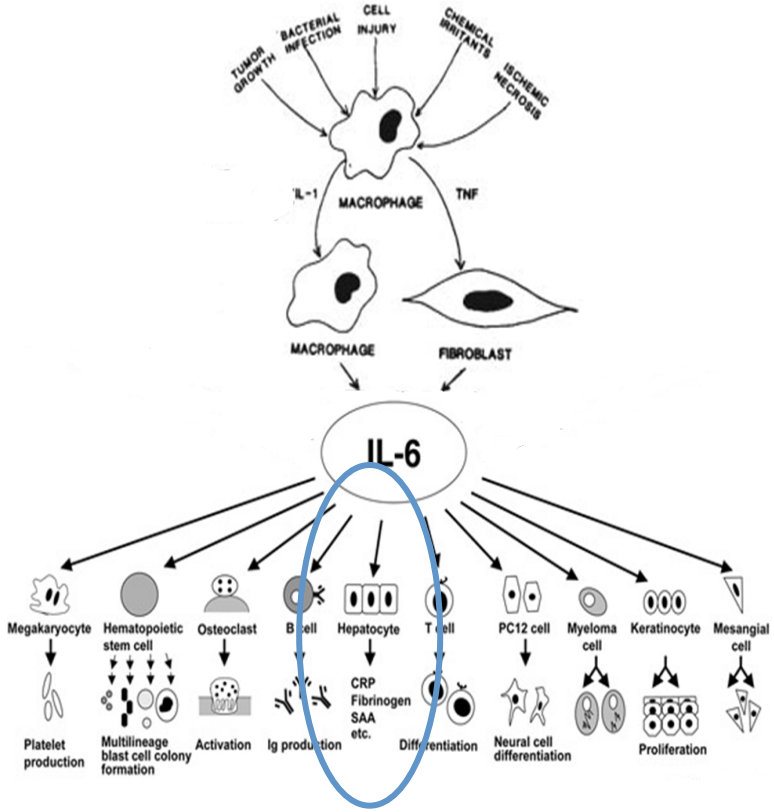
Integration of Factors Associated with Patient Variability in Drug Metabolism.

Clinical trials provide evidence of efficacy and safety at usual doses in controlled populations

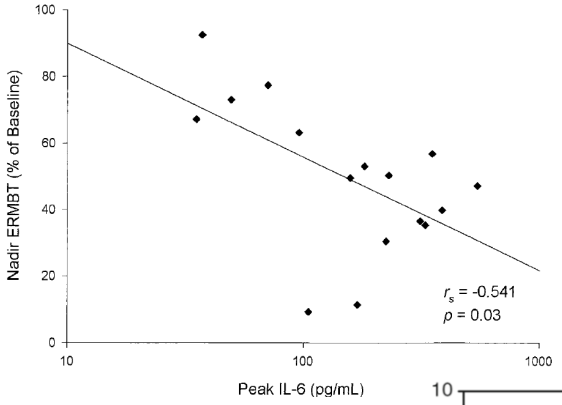


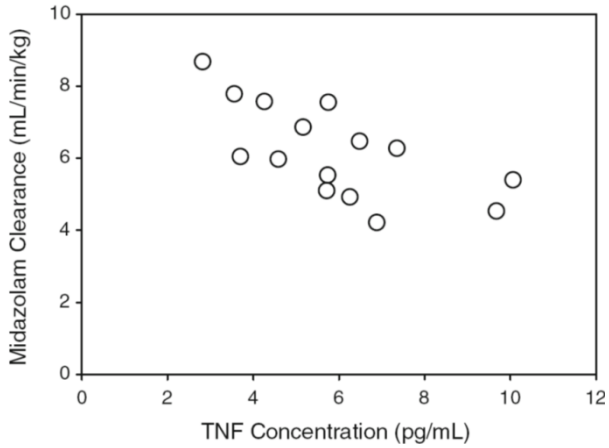
"Variability is the law of life, and as no two faces are the same, so no two bodies are alike, and no two individuals react alike, and behave alike under the abnormal conditions which we know as disease." *Sir William Osler (1849-1919)*

Simplified Schematic Representation of Mechanisms of Disease for Inflammation.



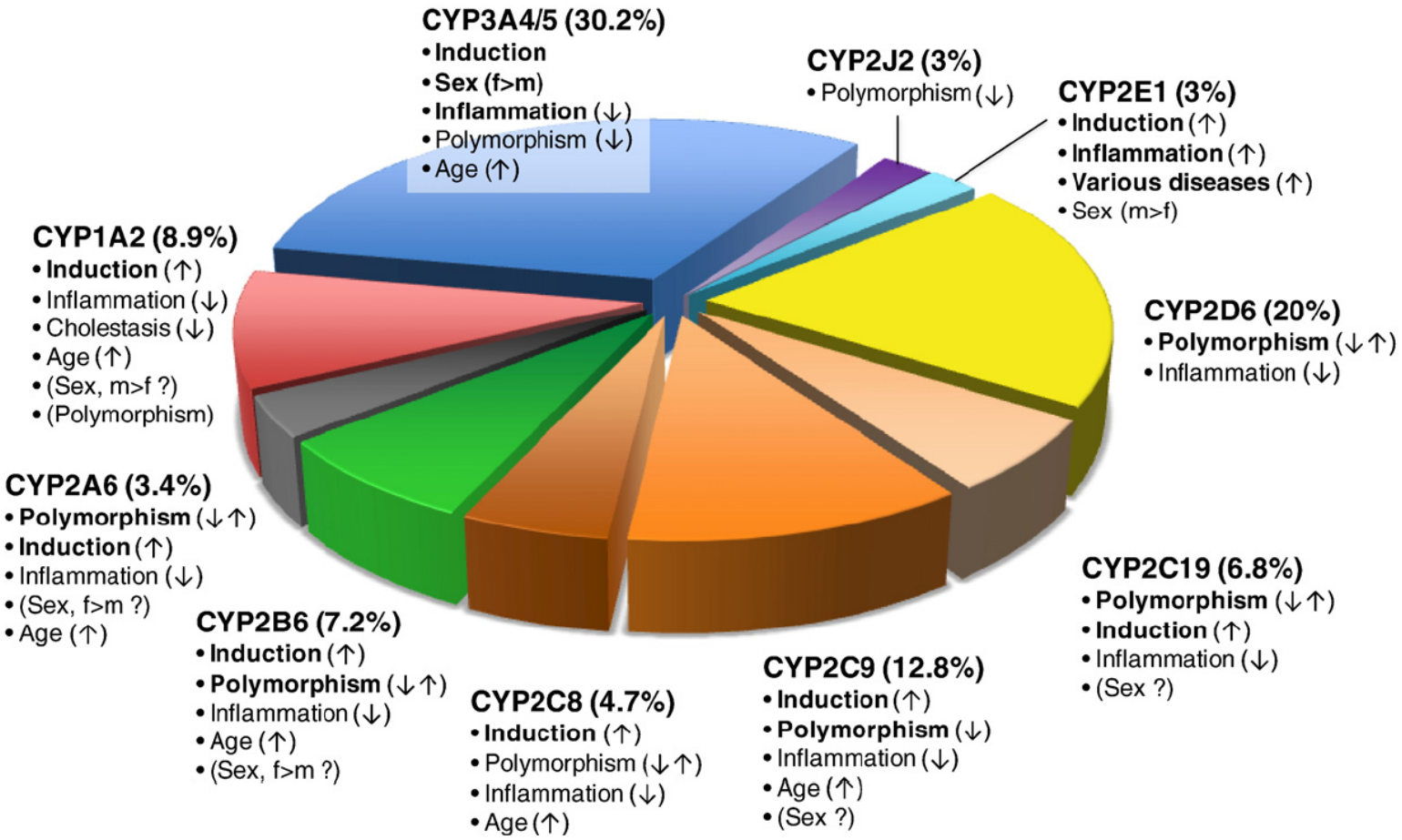
CYP3A4 activity correlates with inflammation biomarkers IL-6 & TNF





- **Plasma concentrations of simvastatin were higher in RA patients than those reported for healthy volunteers.**
- **Actemra (mAb IL-6) reduced significantly the AUC_{last} and C_{max} of simvastatin on Day 15 by 57% (1 week after Actemra infusion).**

Fraction of clinically used drugs metabolized by P450 isoforms and factors influencing variability



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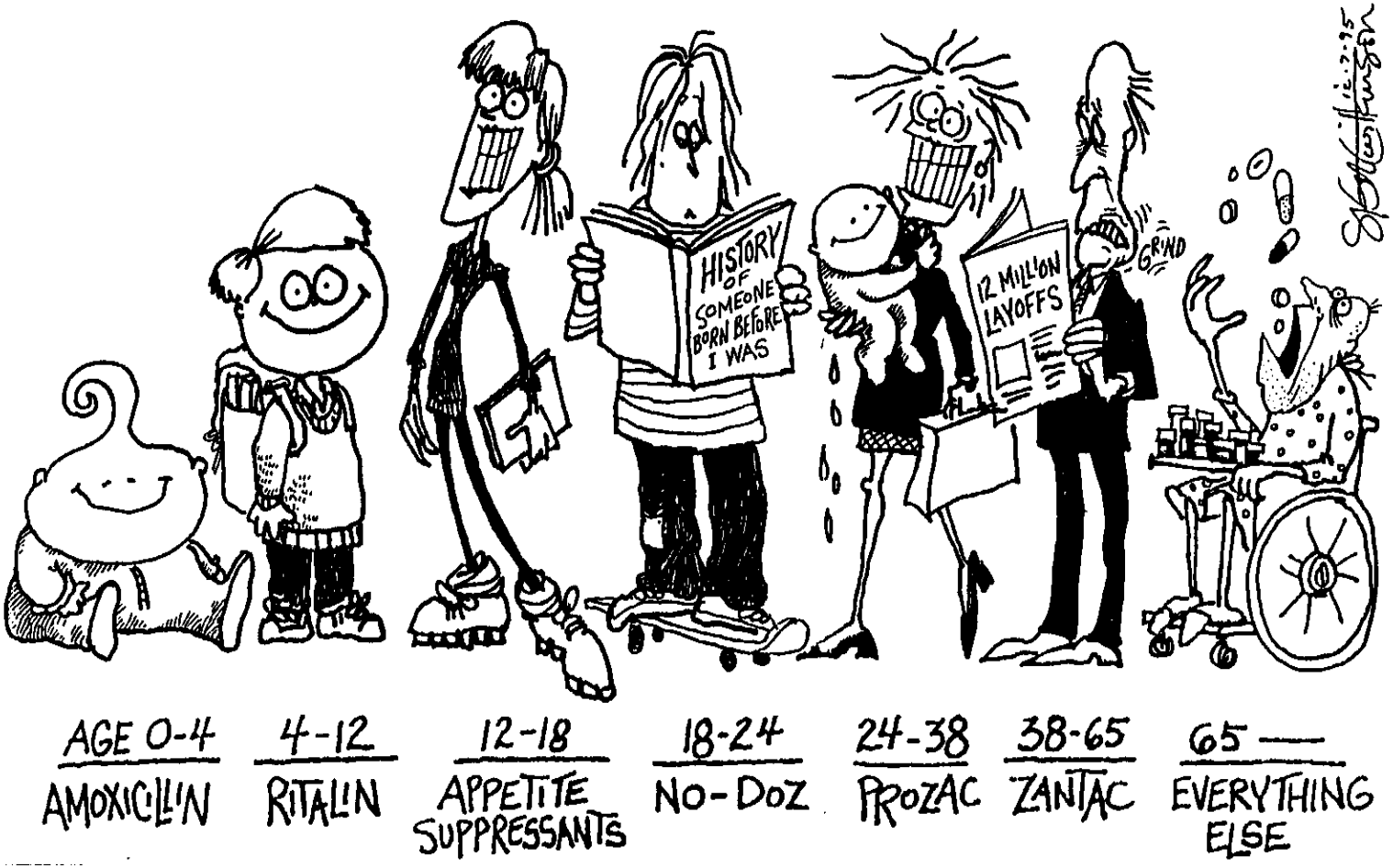
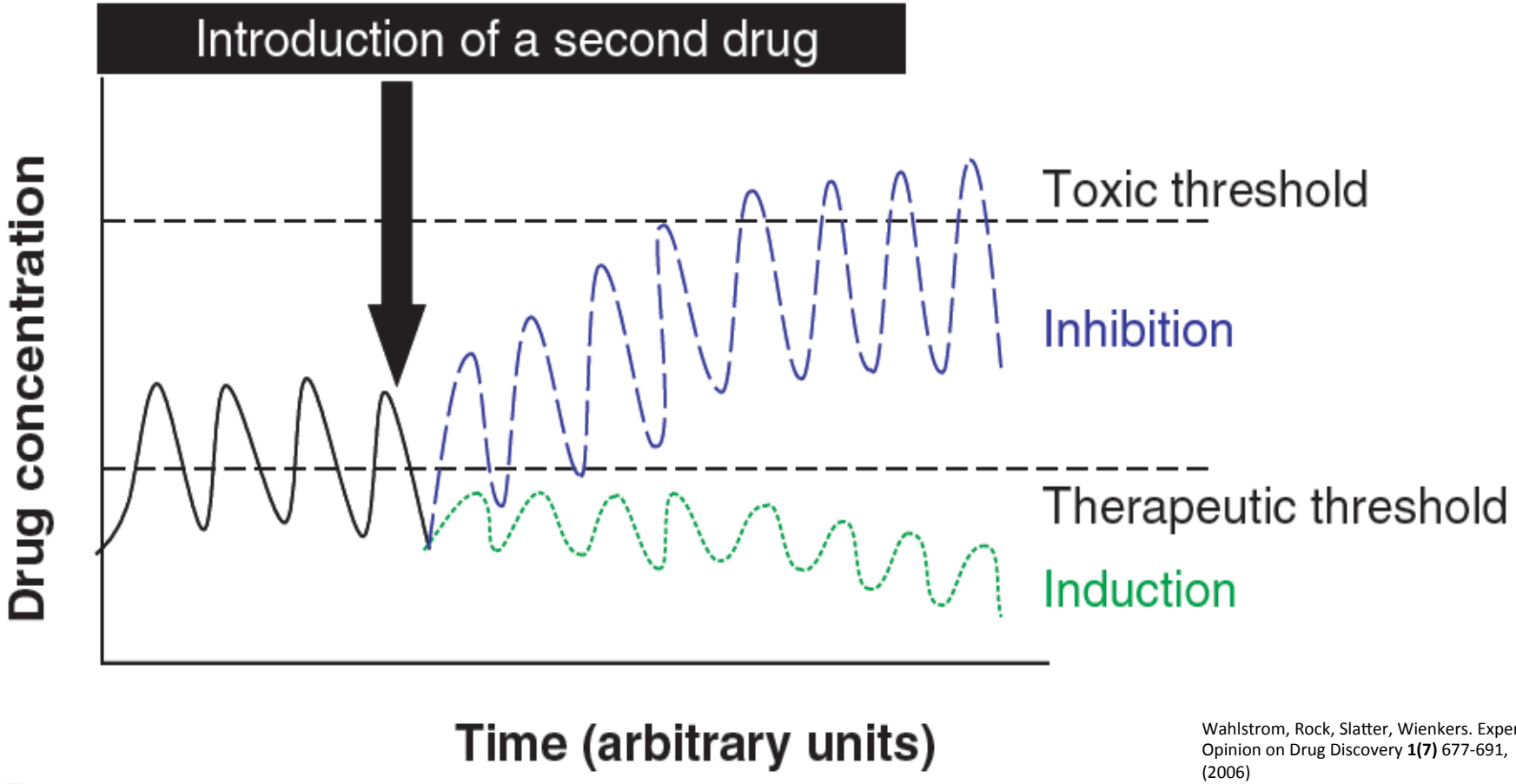
Drug-Drug Interactions



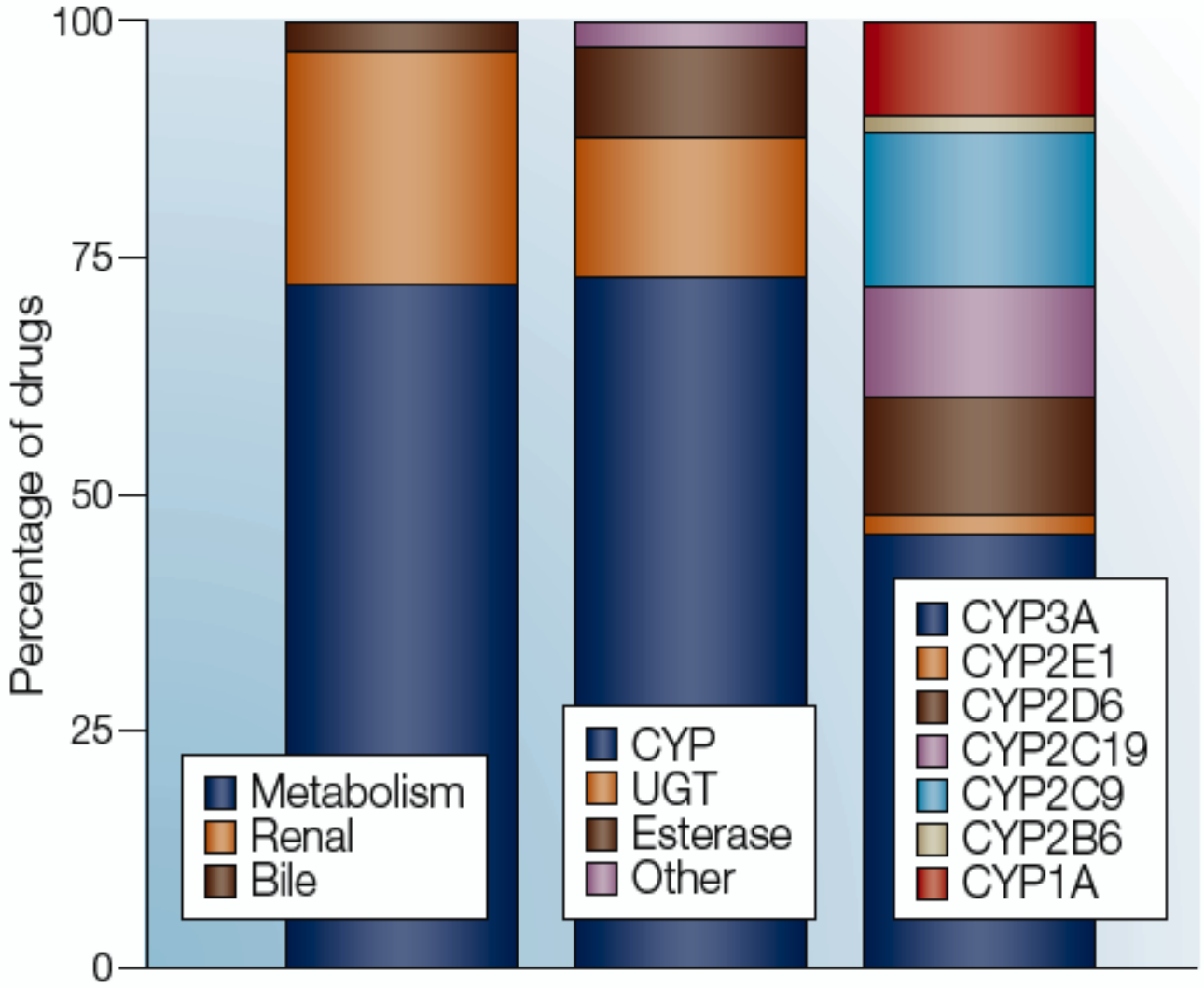
Metabolism-Dependent Drug Toxicities: Drug-drug interactions (DDIs)

It's hard enough to look at the pharmacokinetics, toxicology etc of one drug candidate in isolation, but many patients take several medicines, which can interact with one another. One substance can affect the metabolism of another.



Wahlstrom, Rock, Slatter, Wienkers. Expert Opinion on Drug Discovery **1(7)** 677-691, (2006)

Metabolism by Cytochrome P450 enzymes are a key determinant in governing drug clearance



Wienkers and Heath. Nature reviews Drug Discovery, 4, 825-834. 2005

PRESCRIBING INFORMATION

TAGAMET®

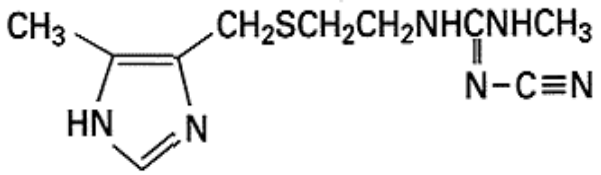
brand of

cimetidine tablets
cimetidine hydrochloride liquid
and
cimetidine hydrochloride injection

DESCRIPTION

Tagamet (cimetidine) is a histamine H_2-receptor antagonist. Chemically it is *N*″-cyano-*N*-methyl-*N*′-[2-[[(5-methyl-1*H*-imidazol-4-yl)methyl]thio]-ethyl]-guanidine.

The empirical formula for cimetidine is $C_{10}H_{16}N_6S$ and for cimetidine hydrochloride, $C_{10}H_{16}N_6SHCl$; these represent molecular weights of 252.34 and 288.80, respectively.



Cimetidine

Cimetidine contains an imidazole ring, and is chemically related to histamine.

(The liquid and injection dosage forms contain cimetidine as the hydrochloride.)

Cimetidine has a bitter taste and characteristic odor.

Solubility Characteristics: Cimetidine is soluble in alcohol, slightly soluble in water, very slightly soluble in chloroform and insoluble in ether. Cimetidine hydrochloride is freely soluble in water, soluble in alcohol, very slightly soluble in chloroform and practically insoluble in ether.

(6) The treatment of pathological hypersecretory conditions (i.e., Zollinger-Ellison Syndrome, systemic mastocytosis, multiple endocrine adenomas).

CONTRAINDICATIONS

Tagamet is contraindicated for patients known to have hypersensitivity to the product.

PRECAUTIONS

General: Rare instances of cardiac arrhythmias and hypotension have been reported following the rapid administration of Tagamet (cimetidine hydrochloride) Injection by intravenous bolus.

Symptomatic response to *Tagamet* therapy does not preclude the presence of a gastric malignancy. There have been rare reports of transient healing of gastric ulcers despite subsequently documented malignancy.

Reversible confusional states (see Adverse Reactions) have been observed on occasion, predominantly, but not exclusively, in severely ill patients. Advancing age (50 or more years) and preexisting liver and/or renal disease appear to be contributing factors. In some patients these confusional states have been mild and have not required discontinuation of *Tagamet* therapy. In cases where discontinuation was judged neces-

Drug Interactions: *Tagamet*, apparently through an effect on certain microsomal enzyme systems, has been reported to reduce the hepatic metabolism of warfarin-type anticoagulants, phenytoin, propranolol, nifedipine, chlordiazepoxide, diazepam, certain tricyclic antidepressants, lidocaine, theophylline and metronidazole, thereby delaying elimination and increasing blood levels of these drugs.

Clinically significant effects have been reported with the warfarin anticoagulants; therefore, close monitoring of prothrombin time is recommended, and adjustment of the anticoagulant dose may be necessary when *Tagamet* is administered concomitantly. Interaction with phenytoin, lidocaine and theophylline has also been reported to produce adverse clinical effects.

300 mg q.i.d. or 800 mg h.s. concomitantly with a 300 mg b.i.d. dosage of theophylline (Theo-Dur®, Key Pharmaceuticals, Inc.) demonstrated less alteration in steady-state theophylline peak serum levels with the 800 mg h.s. regimen, particularly in subjects aged 54 years and older. Data beyond 10 days are not available. (Note: All patients receiving theophylline should be monitored appropriately, regardless of concomitant drug therapy.)

Understanding Metabolism when Attempting to Predict Potential DDI for a New Candidate

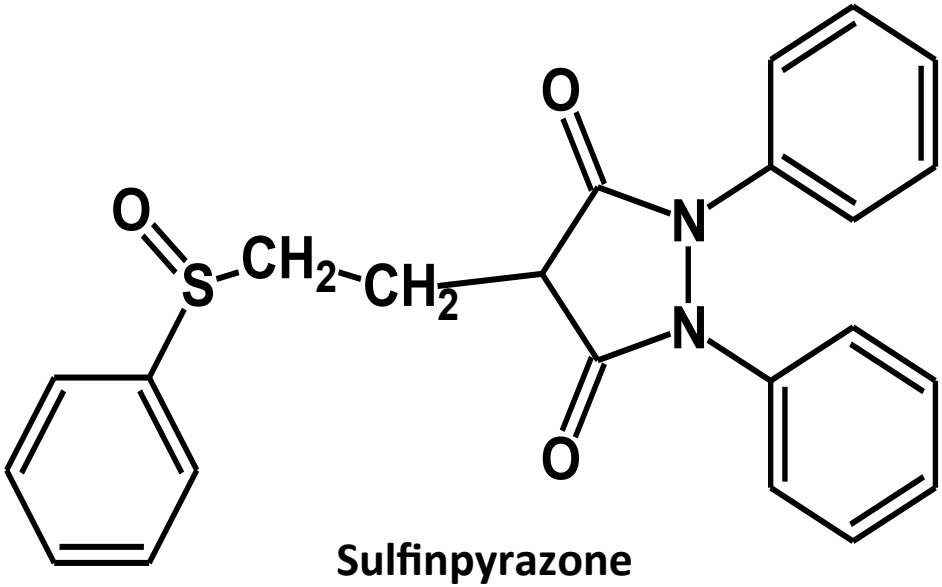
$$\frac{AUC_I}{AUC} = \frac{1}{\frac{f_m}{1+(I/Ki)} + (1-f_m)}$$

fm: fraction of dose metabolized by the affected enzyme

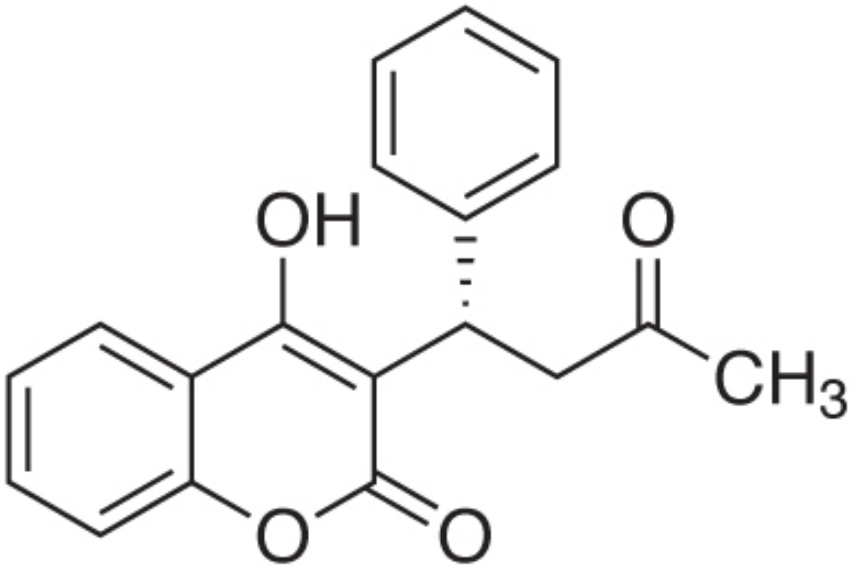
I/Ki: ratio of in vivo inhibitor concentration to the inhibition constant for the affected enzyme

[I]/Ki Ratio	Prediction
[I]/Ki > 1	Likely
0.1 < [I]/Ki < 1	Possible
[I]/Ki < 0.1	Not Likely

Quick Example 1: Sulfinpyrazone and (S)-Warfarin Clinical Drug Interaction



Sulfinpyrazone

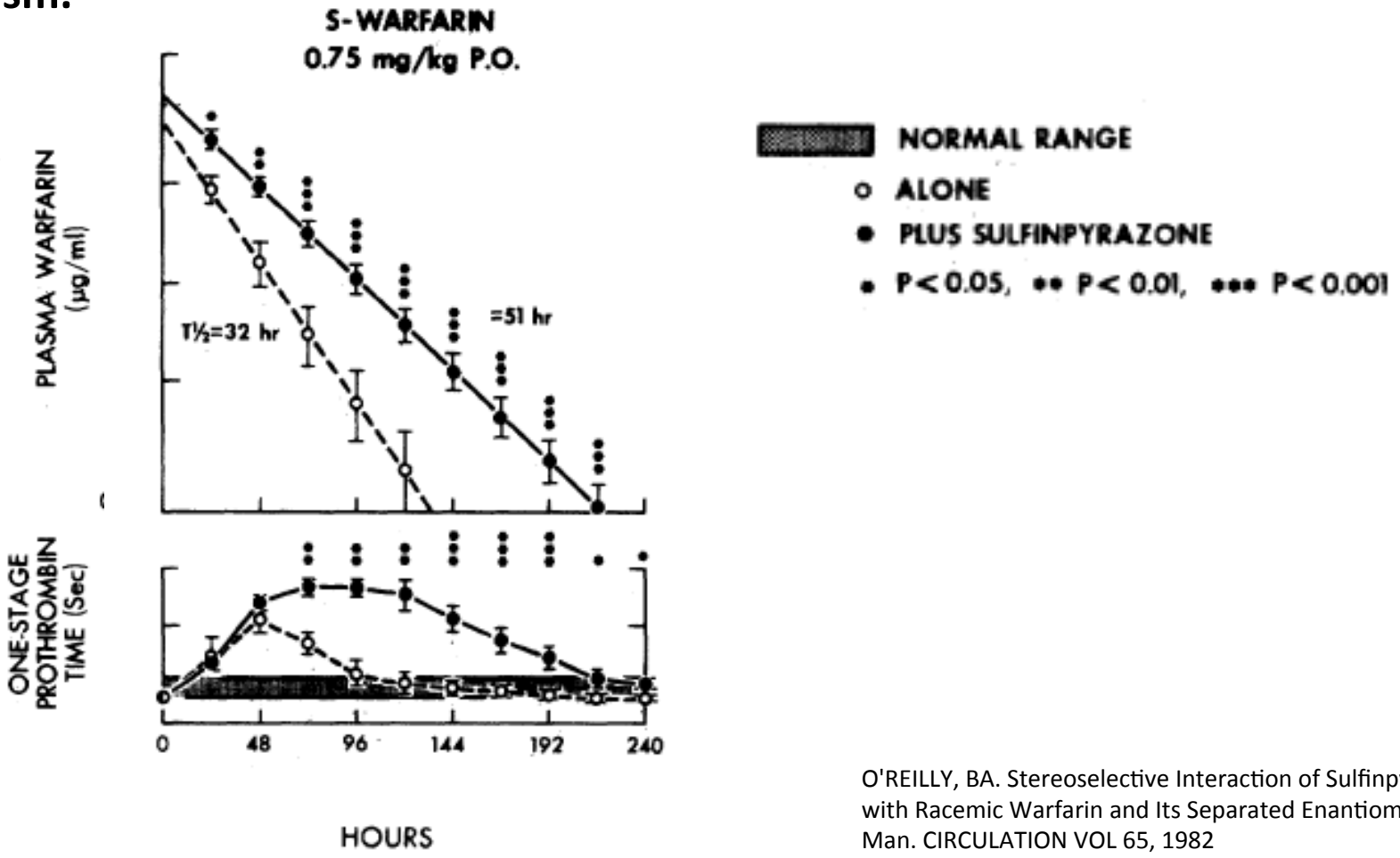


(S)-Warfarin

- **(S)-warfarin is the pharmacologically active enantiomer of warfarin and is almost exclusively cleared *via* CYP2C9 oxidation**
- **Sulfinpyrazone Ki for CYP2C9 = 230μM which resulted in an I/Ki ratio of 0.04**
- **Based on our prediction model these drugs are safe to be coadministered.**

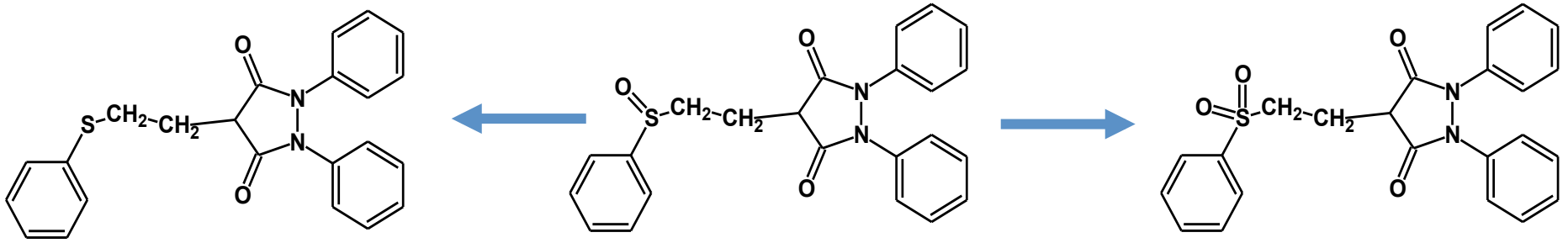
Sulfinpyrazone and (S)-Warfarin Clinical Drug Interaction

Problem: although the initial in vitro Ki values were 25 times greater than therapeutic concentrations of Sulfinpyrazone achieved in vivo, coadministration with (S)-Warfarin resulted in a 3-fold decrease in the active enantiomers metabolism.



O'REILLY, BA. Stereoselective Interaction of Sulfinpyrazone with Racemic Warfarin and Its Separated Enantiomorphs in Man. CIRCULATION VOL 65, 1982

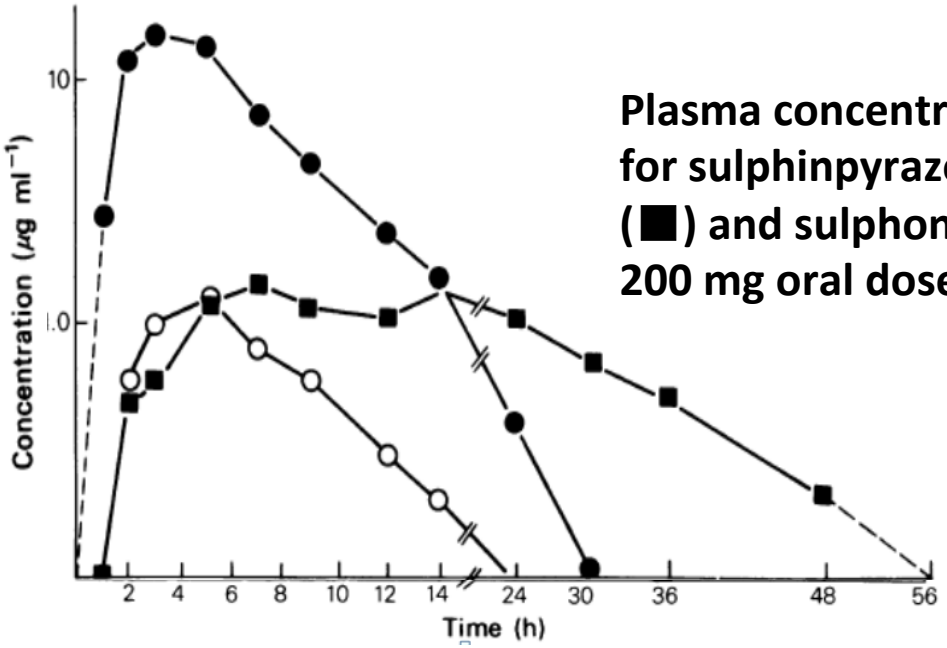
Pharmacokinetic profiles of sulphinpyrazone and it's inhibitory metabolites



Sulfinpyrazone sulfone
CYP2C9 Ki = 73 μM

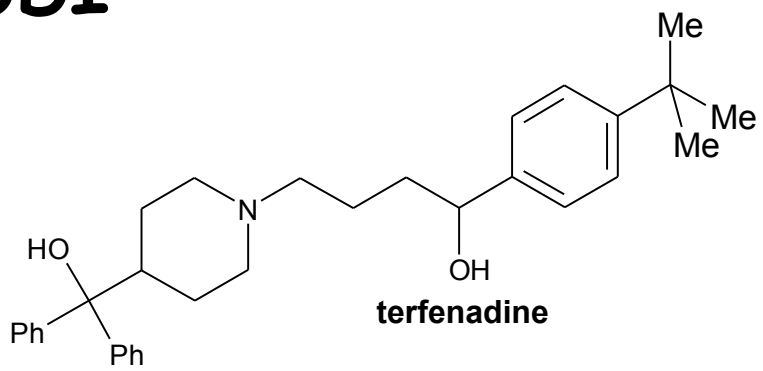
Sulfinpyrazone
CYP2C9 Ki = 230 μM

Sulfinpyrazone sulfide
CYP2C9 Ki = 17 μM

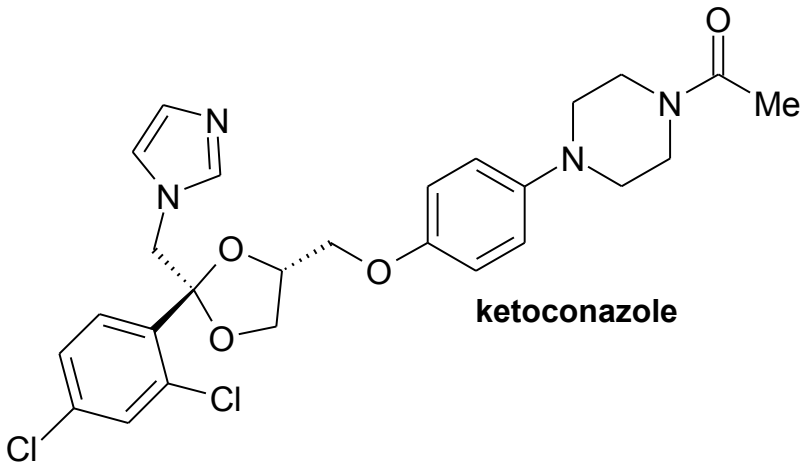


Plasma concentration-time profiles for sulphinpyrazone (●), sulphide (■) and sulphone (○) after receiving 200 mg oral dose.

Quick Example 2: Terfenadine / Ketoconazole DDI

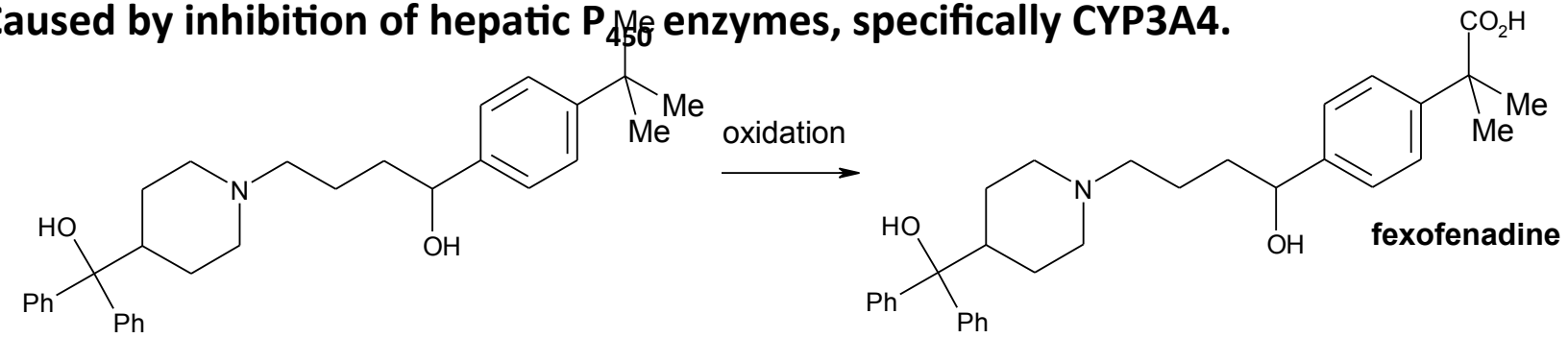


terfenadine



ketoconazole

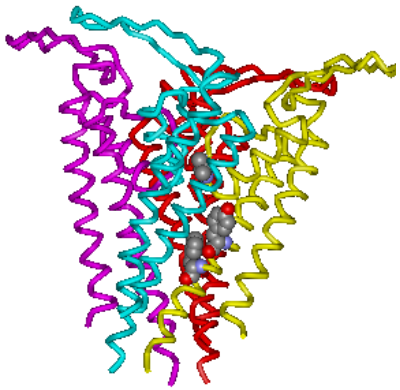
- **Terfenadine – antihistamine drug on market as an 'over the counter' remedy for hayfever.**
- **Found to cause life threatening cardiac arrhythmias when co-administered with medicines such as erythromycin (antibiotic) or ketoconazole (antifungal).**
- **Caused by inhibition of hepatic P450 enzymes, specifically CYP3A4.**



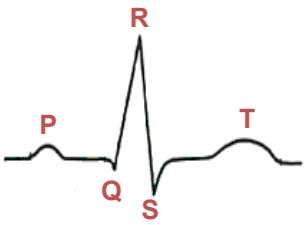
fexofenadine

- **Found that the major metabolite of terfenadine, caused by oxidation of the *tert*-butyl group, is the pharmacologically active species.**
- **Moreover, he major metabolite, fexofenadine, has little hERG activity as it is a zwitterion, and was developed as a medicine.**

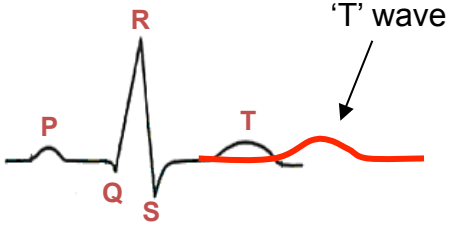
A Brief Overview of hERG



- **hERG = 'human ether-a-go-go related gene'**
- **Potassium channel**
- **Activation causes prolongation of electrical impulses regulating heart beat**
  - A delay of the T wave by 5-10 milliseconds can cause lack of control of the heartbeat, which may lead to a fatal arrhythmia.



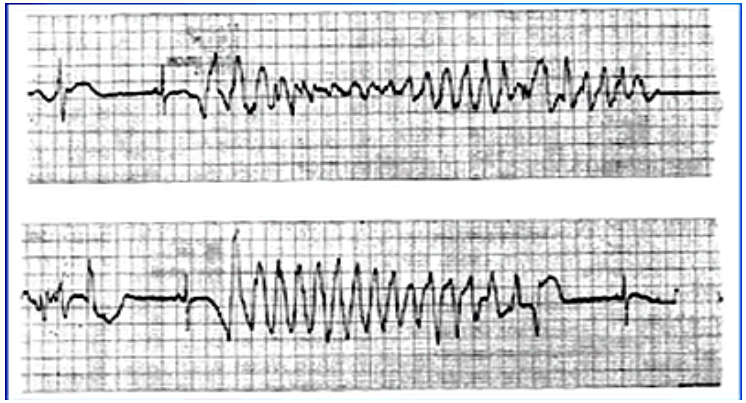
Normal heart beat



Activation of hERG

Torsades de Pointes Occurring in Association With Terfenadine Use

Brian P. Monahan, MD; Clifford L. Ferguson, MD; Eugene S. Killeavy, MD; Bruce K. Lloyd, MD; James Troy; Louis R. Cantilena, Jr, MD, PhD



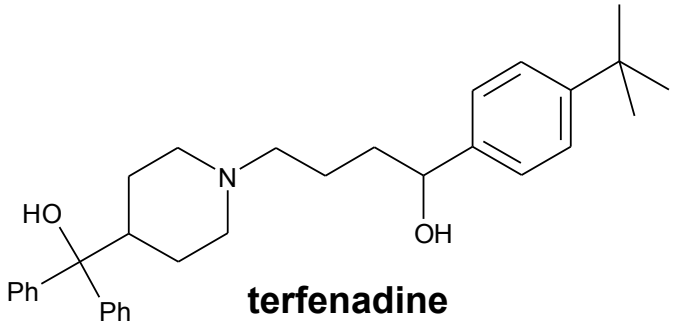
Regulatory guidance for nonclinical (ICH S7B) and clinical (ICH E14) testing strategies published in 2005.

Safety margin based on ratio of:
(hERG IC50 / Cmax) or (NOAEL / Cmax)

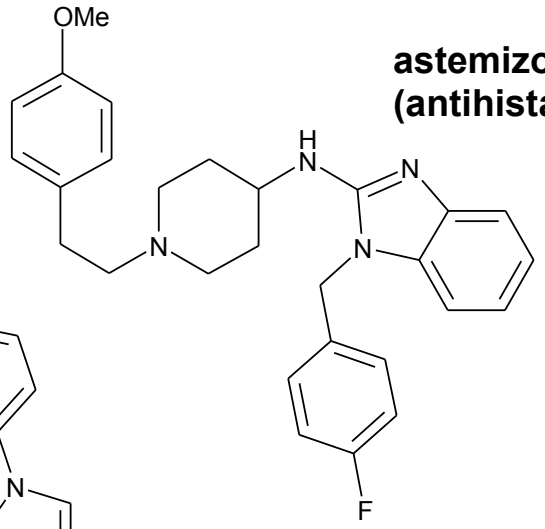
***at expected top dose should be >30, and preferably >100**

Why does hERG receive so much attention?

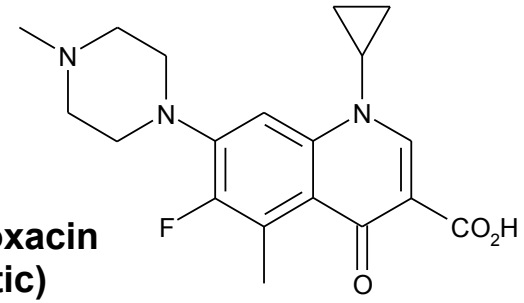
Many drugs with apparently diverse structures are able to bind to it.



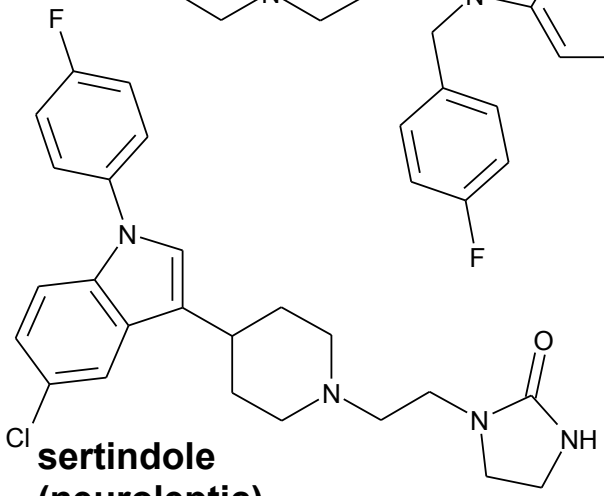
terfenadine (antihistamine)



astemizole (antihistamine)



grepafloxacin (antibiotic)

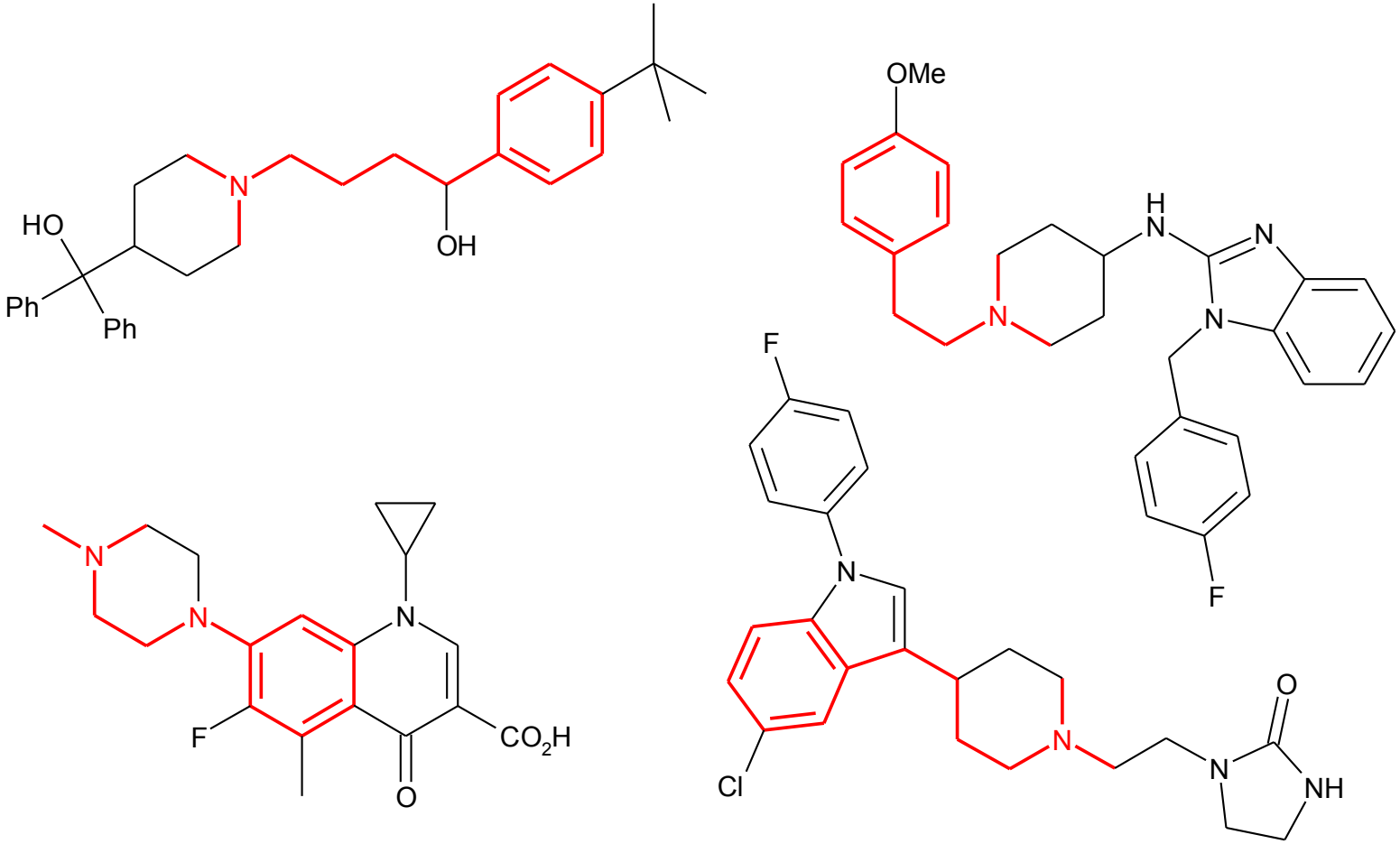


sertindole (neuroleptic)

Drug withdrawals due to QTc effects:

Terodiline (1991), sertindole (1998), terfenadine (1998), astemizole (1999), grepafloxacin (1999), cisapride (2001), droperidol (2001), levacetylmethadol (2001), thioridazine (2005), dofetilide (2004)

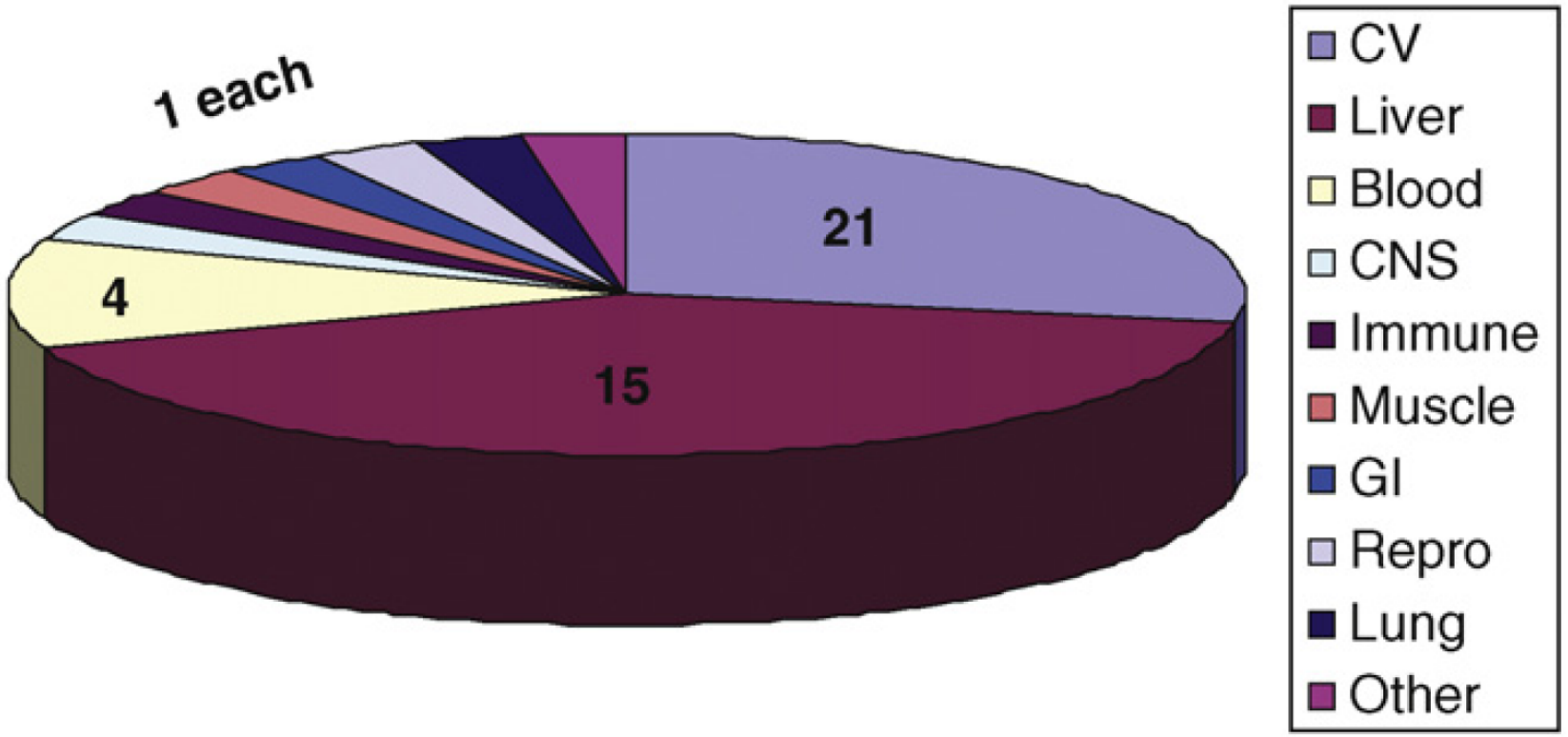
Strategies to Minimize QTc Risks include:



Formation of zwitterions (e.g. terfenedine to fexofenadine)
Modulation of LogP
Attenuation of pKa
Pharmacophore models

Drug-Induced Liver Toxicity

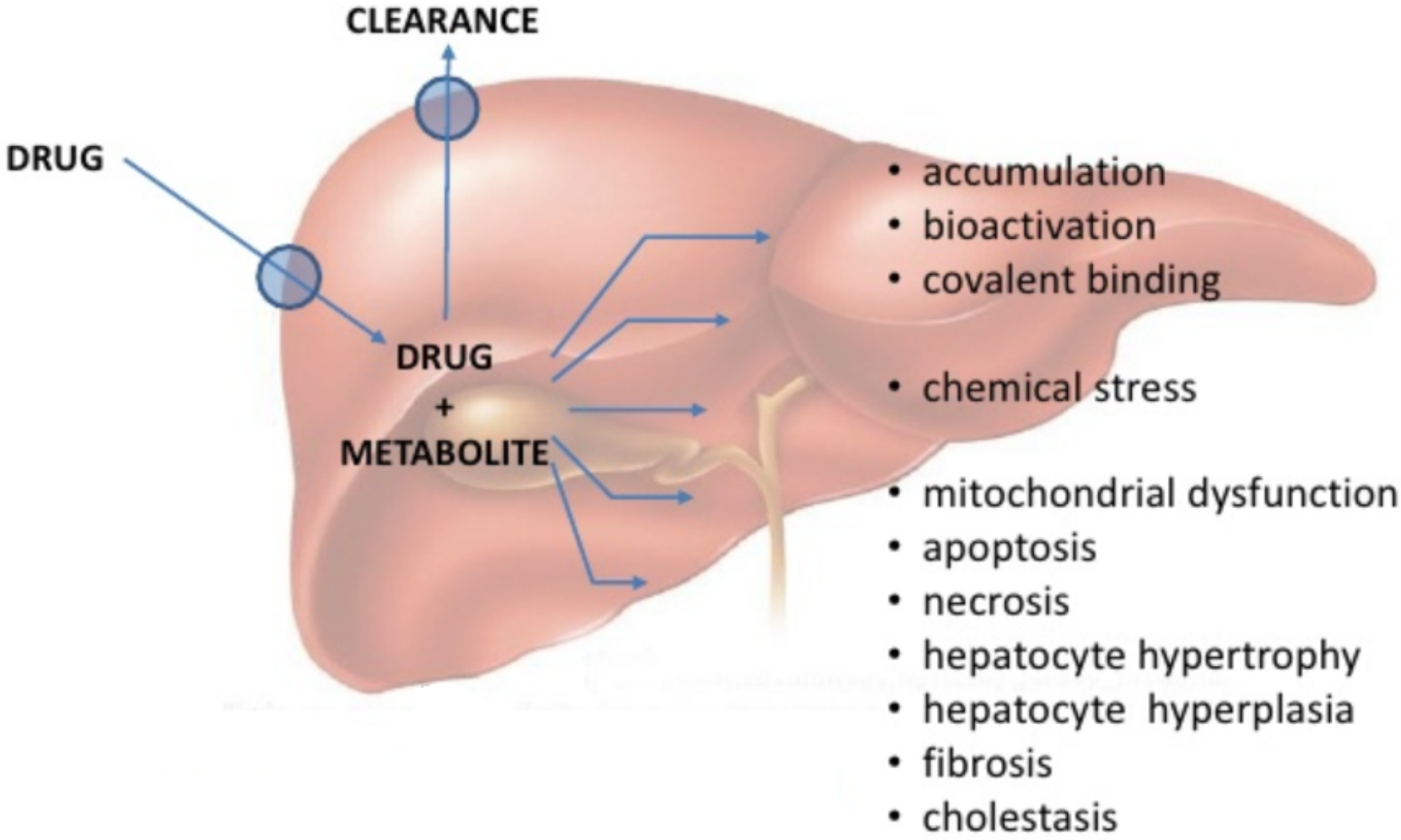
Summary of target organ contributions to drug withdrawals 1975–2007



Toxicity can arise through a variety of mechanisms

- **Predictable, dose-dependent toxicities (animal model, clear dose response relationship, etc)**
- **Metabolism related toxicities account for the majority of observed liver toxicities**

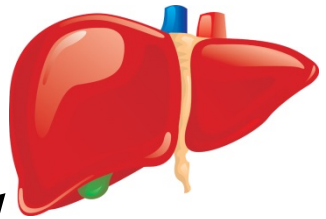
Mechanisms of Drug Induced Liver Injury



Evidence suggests that, in some cases, reactive metabolites may play a causative role in liver toxicity. Idiosyncratic toxicities of greatest concern in drug development

The Role of Drug Metabolism in Liver Toxicity

Drug → **Reactive Metabolite**

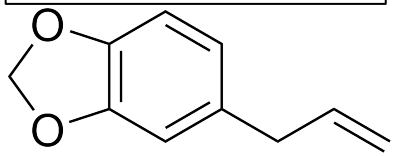


The Liver is Typically the first Organ Impacted

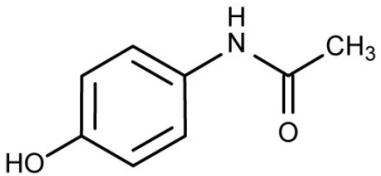
Reaction with DNA

Reaction with Proteins

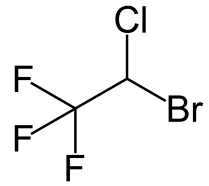
Mutagenicity
Carcinogenicity
Teratogenicity
- **Safrole**



Target Organ Toxicity (reproducible or idiosyncratic)
- **Acetaminophen**
- **Diclofenac**

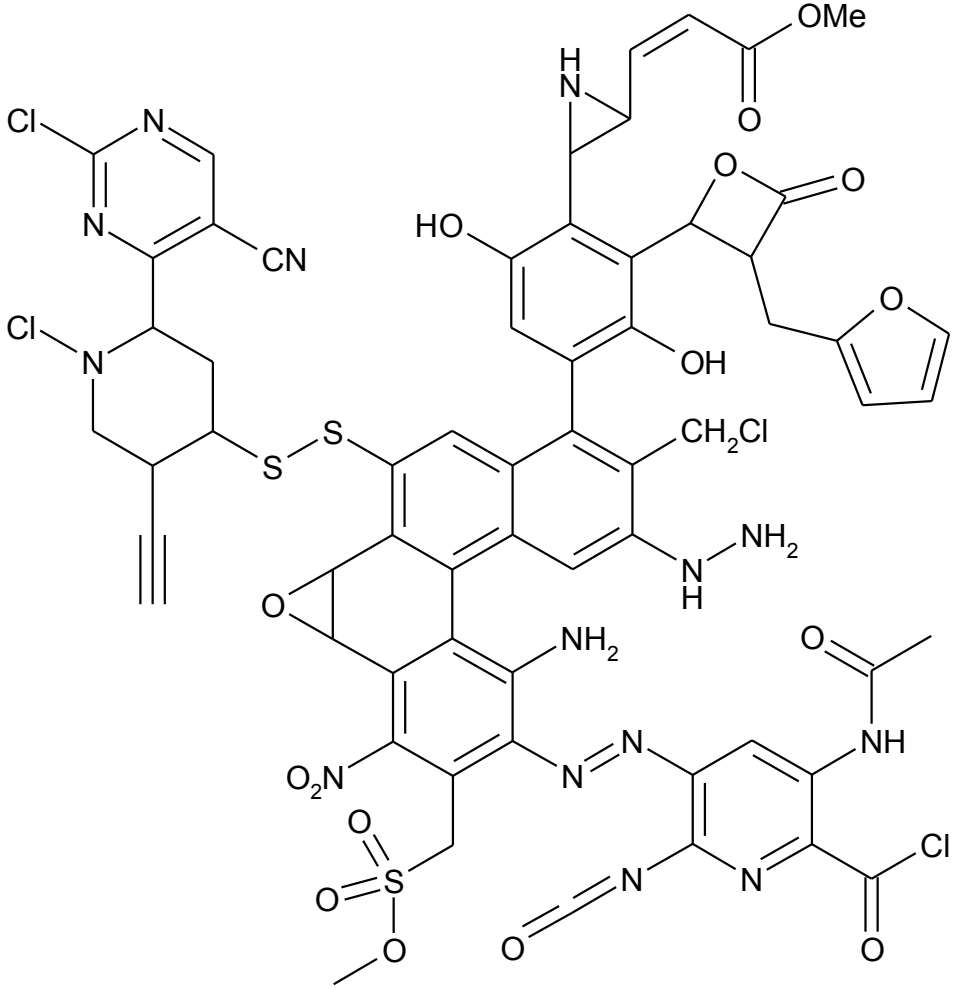


Immune Hypersensitivity reactions (idiosyncratic)
- **Halothane**

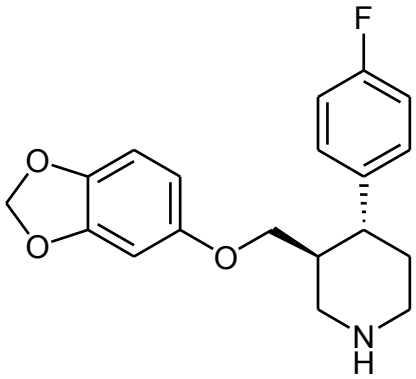


Anticipating Drug Activation & Toxicity

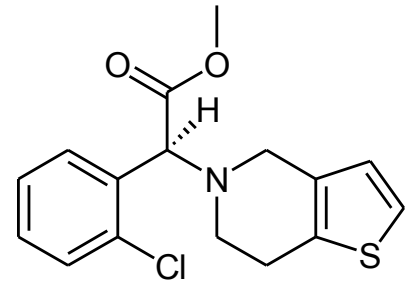
A pharmacophore is an abstract description of molecular features which are necessary for molecular recognition of a ligand by a biological macromolecule. This concept can be extended to some molecular substructures which are susceptible to bioactivation potential toxicity (aka., chemical alerts)



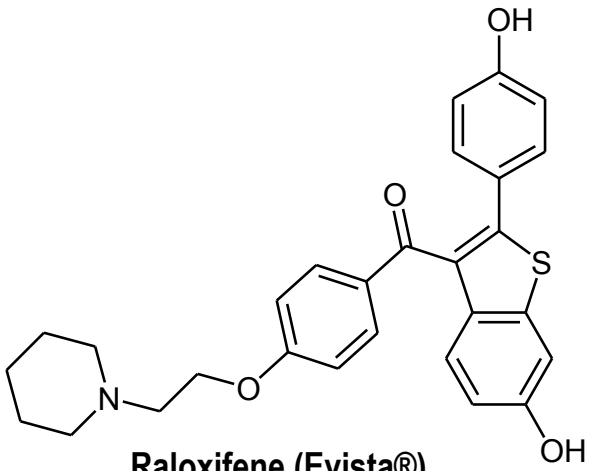
Applying Reason with Respect to Chemical Alerts & Anticipating Metabolism Based Drug Toxicity



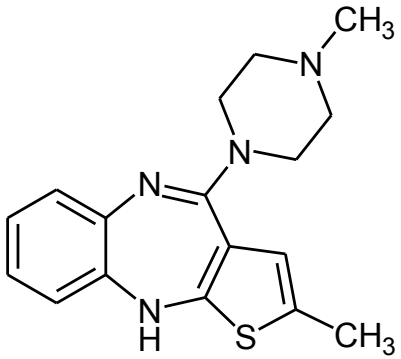
Paroxetine (Paxil®)



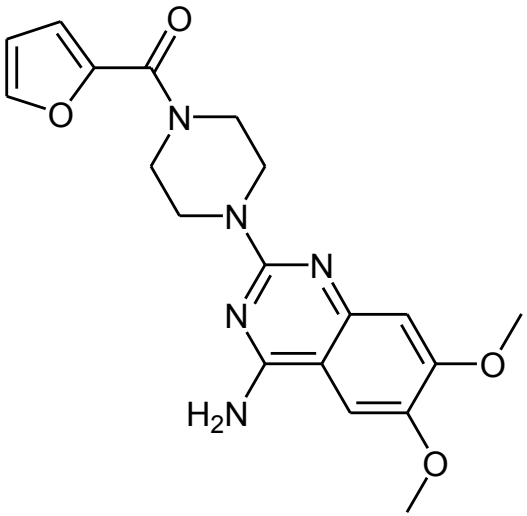
Clopidogrel (Plavix®)



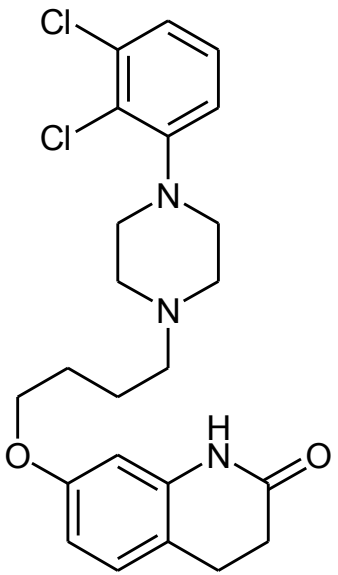
Raloxifene (Evista®)



Olanzapine (Zyprexa®)

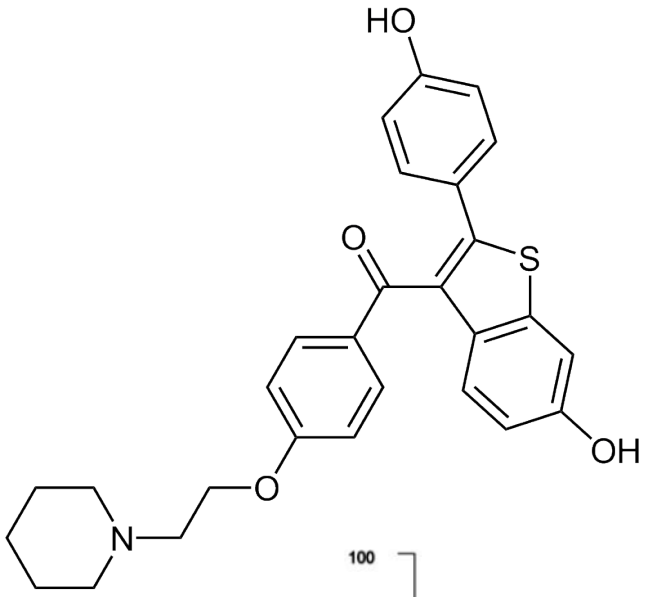


Prazosin (Minipress®)

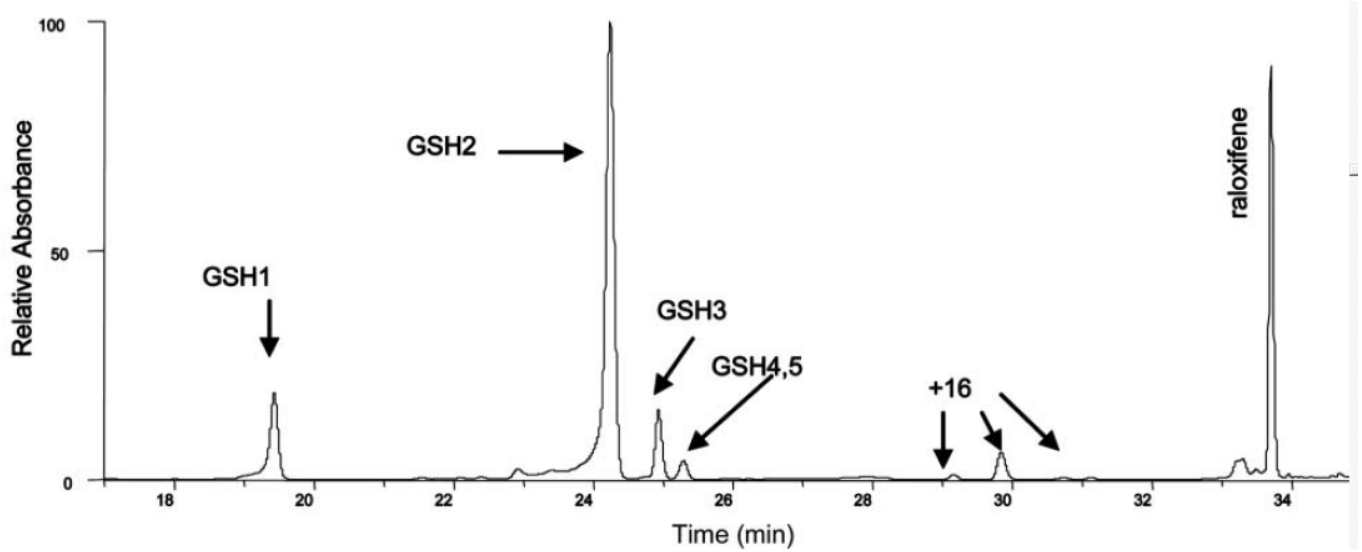


Aripiprazole (Abilify®)

Quick Example 3: Raloxifene Metabolism Glucuronidation versus Oxidation

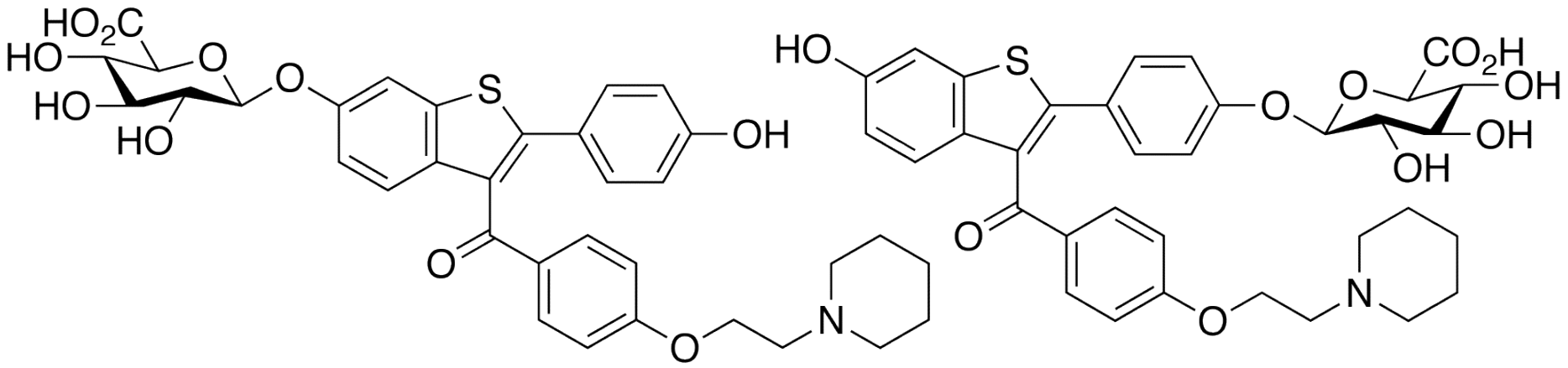


Raloxifene (Evista) is selective estrogen receptor modulator used in the treatment of osteoporosis and for chemoprevention of breast cancer. In vitro raloxifene is bioactivated to reactive intermediates, which covalently bind to proteins and form GSH conjugates upon incubation with NADPH and GSH-supplemented human microsomes.



Despite these in vitro findings, no major raloxifene-related toxic events have been reported upon its oral administration to humans.

Quick Example 3: Raloxifene Metabolism Glucuronidation versus Oxidation



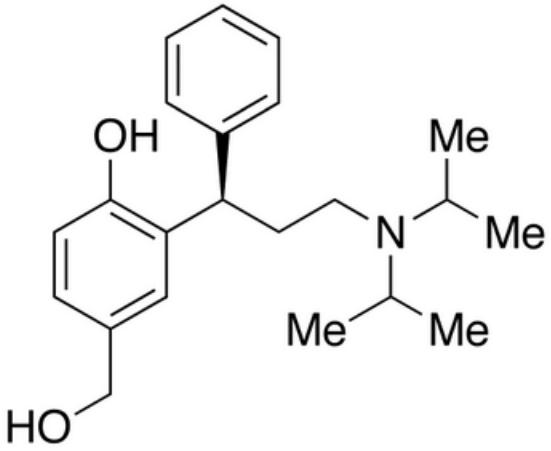
- **UGT1A1 and 1A8 were found to catalyze the formation of both the 6-beta- and 4'-beta-glucuronides.**
- **Raloxifene is rapidly absorbed from the gastrointestinal tract and undergoes extensive first-pass glucuronidation.**
  - **Approximately 60% of an oral dose is absorbed; however, because of extensive presystemic glucuronide conjugation, absolute bioavailability is only 2%.**

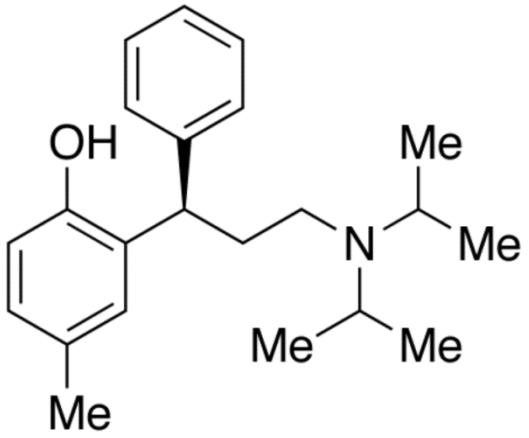
Outline for Today

- ***Background***
  - Toxicity as a major source of attrition in drug development
  - Toxicity-related drug withdrawals
  - Drug Metabolism & Toxicity studies in drug development
- ***Metabolism-dependent drug toxicities***
  - Exaggerated Pharmacology and Inhibitory Metabolites
  - Cardiovascular toxicity (QTc effects) caused by DDIs
  - Reaction Phenotyping as its relates to predicting Drug Toxicity
  - Liver injury (chemically reactive drug metabolites)
- ***Stable drug metabolites***
  - **Active Metabolites**
  - **FDA and ICH* "MIST" Guidance**
  - **Species differences in drug metabolism & toxicity**

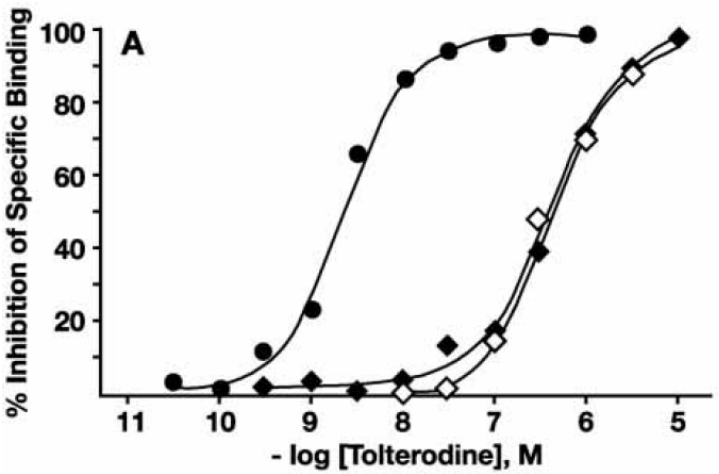
*ICH = International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use

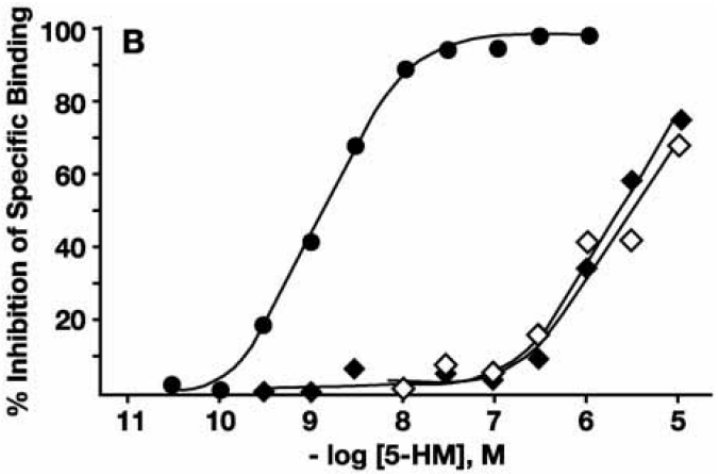
Quick Example 4: Additive Effects of an Active Metabolite (Tolterodine & CYP2D6)





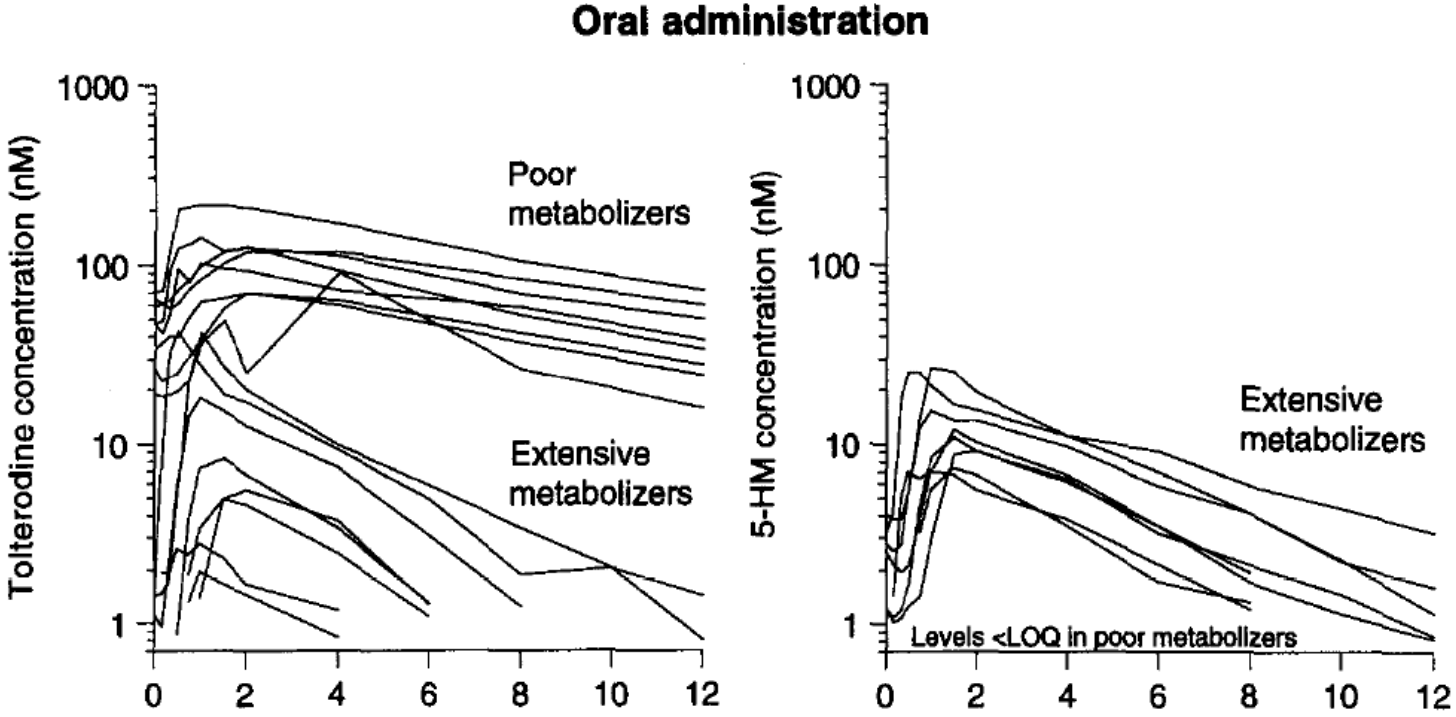
- **The primary metabolic route for tolterodine is via oxidation of the 5-methyl group and is mediated by the CYP2D6 and leads to the formation of a pharmacologically active 5-hydroxymethyl metabolite.**





Quick Example 4: Additive Effects of an Active Metabolite (Tolterodine & CYP2D6)

- **Tolterodine is cleared at a slower rate in poor metabolizers than in extensive metabolizers; this results in significantly higher serum concentrations of tolterodine and in negligible concentrations of the 5-hydroxymethyl metabolite.**



- **Despite the effect on pharmacokinetics, the CYP2D6 polymorphism does not appear to be of great importance in the antimuscarinic effect, probably because of the additive action of parent drug and active metabolite.**

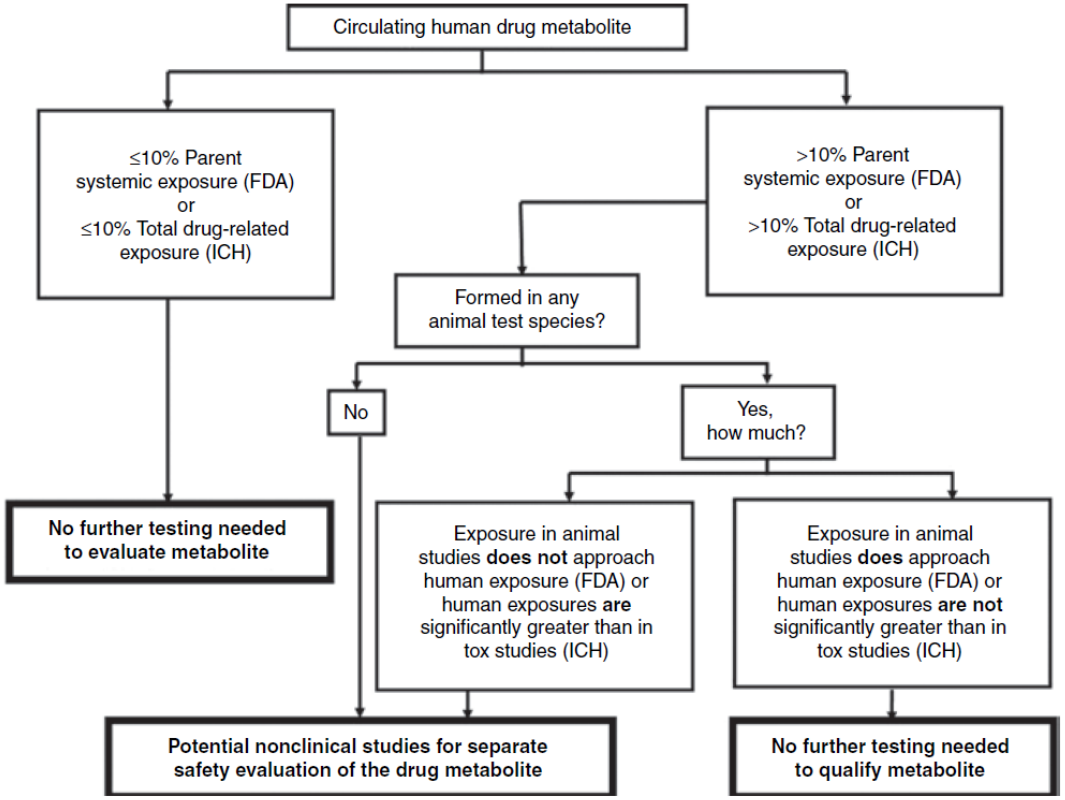
"Are human metabolites of a drug candidate, as well as the parent compound, adequately evaluated for safety during preclinical toxicology studies?"

Guidance for Industry
Safety Testing of Drug Metabolites

- **The Guidance Focuses on:**
  - *stable* metabolites circulating in human plasma
  - unique or "disproportionate" metabolites in humans
- **The Key FDA recommendations:**
  - a stable metabolite whose AUCp at steady-state is <10% that of parent needs no further study
  - if AUCp is >10% of parent, "coverage" (i.e. exposure margin >1) needs to be demonstrated in at least one tox species otherwise, human metabolite is "disproportionate" and may require testing
- **ICH Topic M3 (R2) difference:**
  - Only those human metabolites observed at levels >10% of total drug-related exposure require nonclinical characterization, if they circulate at "significantly greater" levels in humans than the maximum exposure in animal toxicology studies
- **Potential resource and time implications for drug development:**
  - Types of toxicology studies that may be required include general tox (3 months), genotoxicity, embryo-fetal development tox, carcinogenicity

Possible Decision Tree for Drug Metabolites

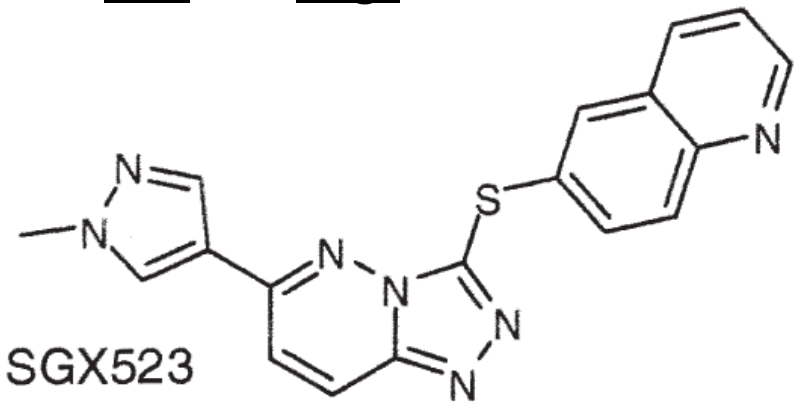
- **Preliminary studies on circulating human metabolites need to be conducted during early clinical development (Phase I / II) such that "disproportionate" metabolites can be identified and addressed preclinically.**



- **The implementation of regulatory guidances from the FDA and ICH requires that a detailed understanding of the metabolic fate of a new drug candidate be established, both in humans and in the animal species used for toxicology studies, prior to the start of large-scale (Phase III) clinical trials**

Quick Example 5: Selecting the most relevant nonclinical species for toxicological evaluation

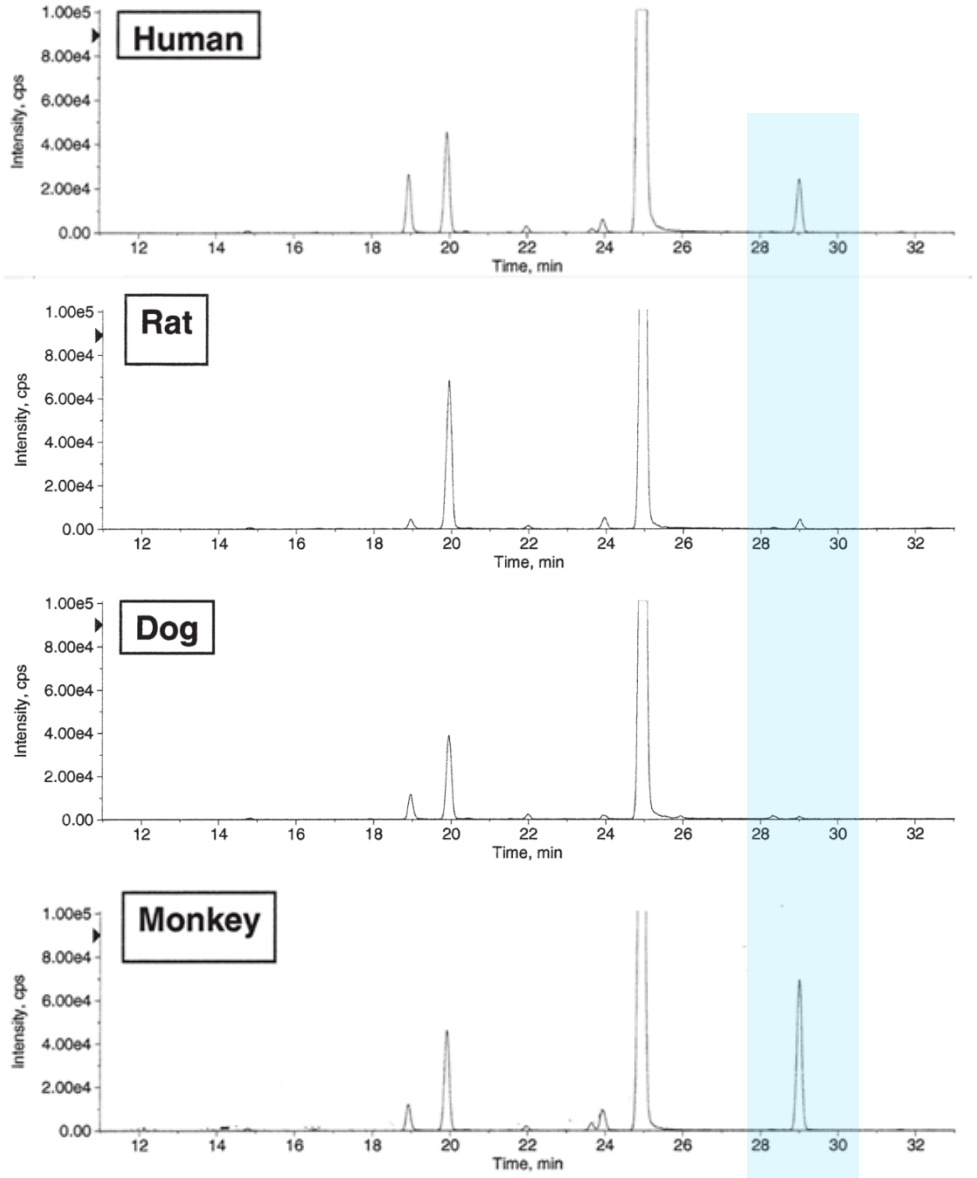
Background: (SGX523) was an orally bioavailable, potent, and selective small molecule inhibitor of c-MET, and was one of the first selective c-MET inhibitors to be evaluated in patients. Because the microsomal metabolism profile of SGX523 was similar among preclinical species and human, investigational new drug-enabling studies were conducted in rats and dogs.

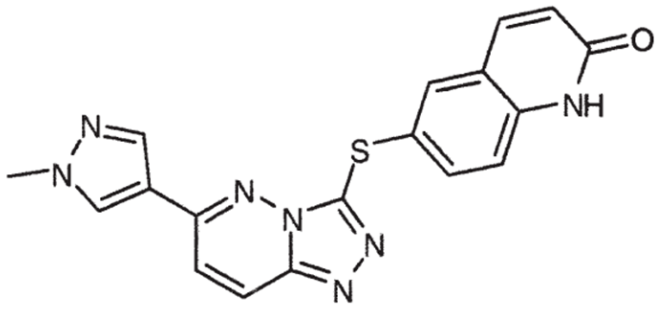


SGX523

Problem: The SGX523 Phase I study was started at a dose of 40 mg in patients. After escalating to doses 80 mg of SGX523 in patients, acute renal failure was observed as evidenced by increased serum creatinine. The analysis of samples from the discontinued clinical trial revealed a metabolism profile different from that of the preclinical species studied.

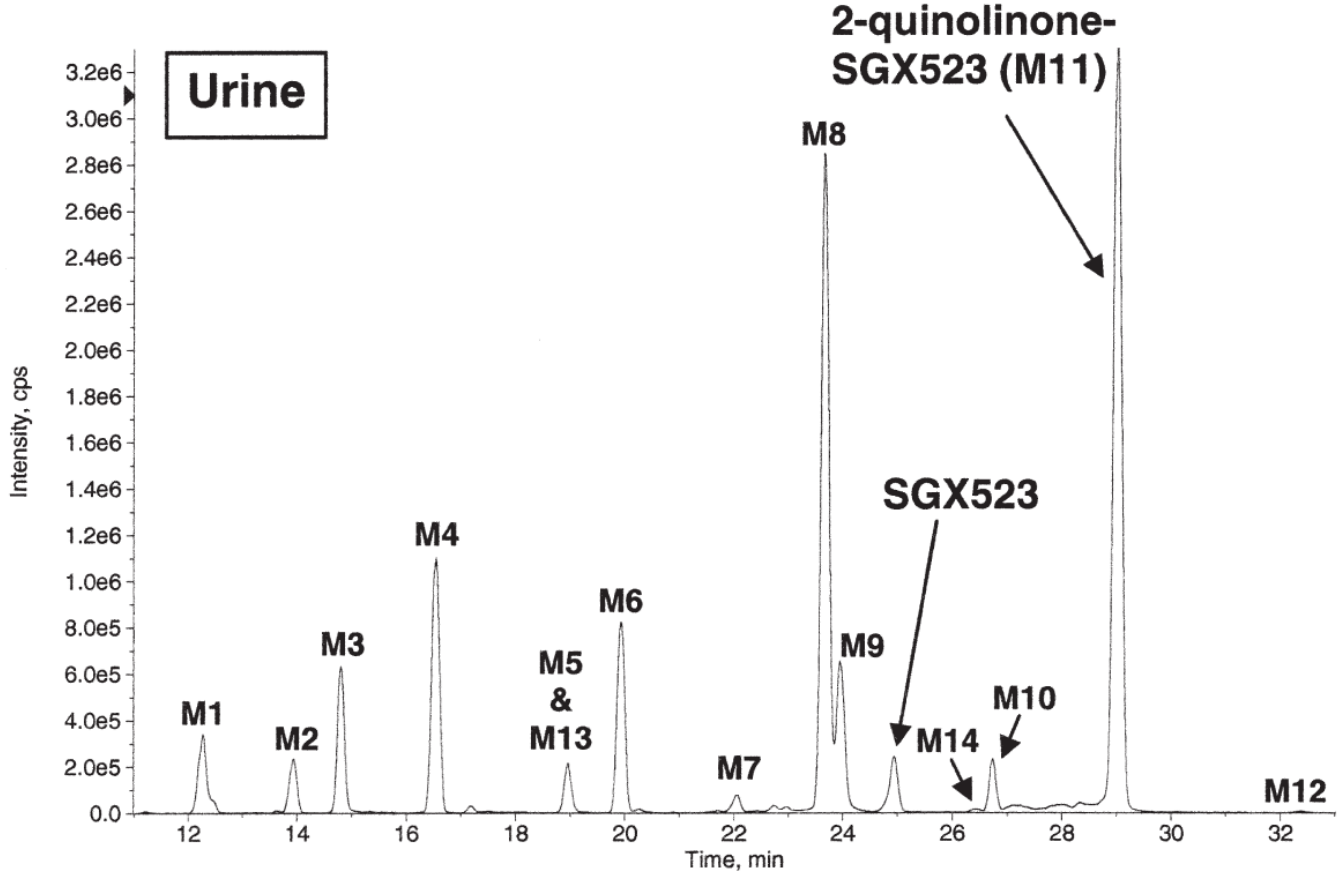
Quick Example 5: Selecting the most relevant nonclinical species for toxicological evaluation





M11 (2-quinolinone-SGX523)

Quick Example 5: Selecting the most relevant nonclinical species for toxicological evaluation



- **Answer: Solubility in monkey urine (pH 8.4): SGX 523 – 13μg/ml M11 – 0.37μg/ml**
- **Summary, SGX523 is metabolized by AO in a species-specific manner**

to a markedly less-soluble metabolite, M11which was likely involved in the observed obstructive nephropathy reported in clinical studies.

Key Messages Regarding Active Metabolites

- Circulating metabolites can…
  - Enhance therapeutic response
  - Lead to new off target affects (QT elongation and CYP inhibition)
- Provides improved patent protection for novel molecular structures
- Increase the resource required for safety testing if exposure is extremely high (pro-drug approach)
- Understanding metabolism of lead can improved diagnosis of at risk populations

It doesn't matter what company you work for, we, for all intent and purposes, are all after the same goal: New advances in pharmacotherapy that are safe and effective; provide acceptable benefit-to-risk ratios for the disease; and are brought to patients who need them with a sense of urgency and diligence.

The goal of Drug Metabolism scientist in this particular undertaking is to answer the right question with the correct experiment at the appropriate time.