Metabolism/Safety Considerations in Drug Development

MEDCH/PCEUT 527 – ADVANCED DRUG METABOLISM
March 6, 2019

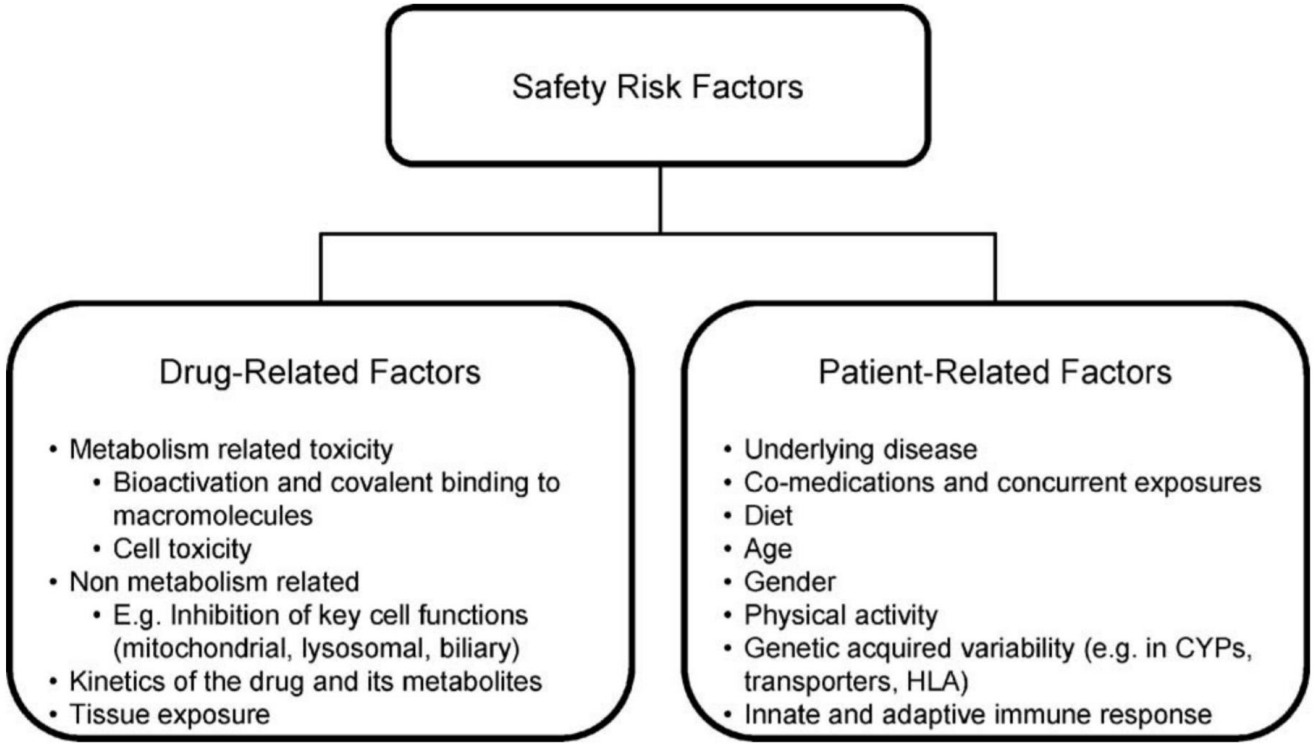
Outline for Today

- ***Background***

Overview of the drug development process

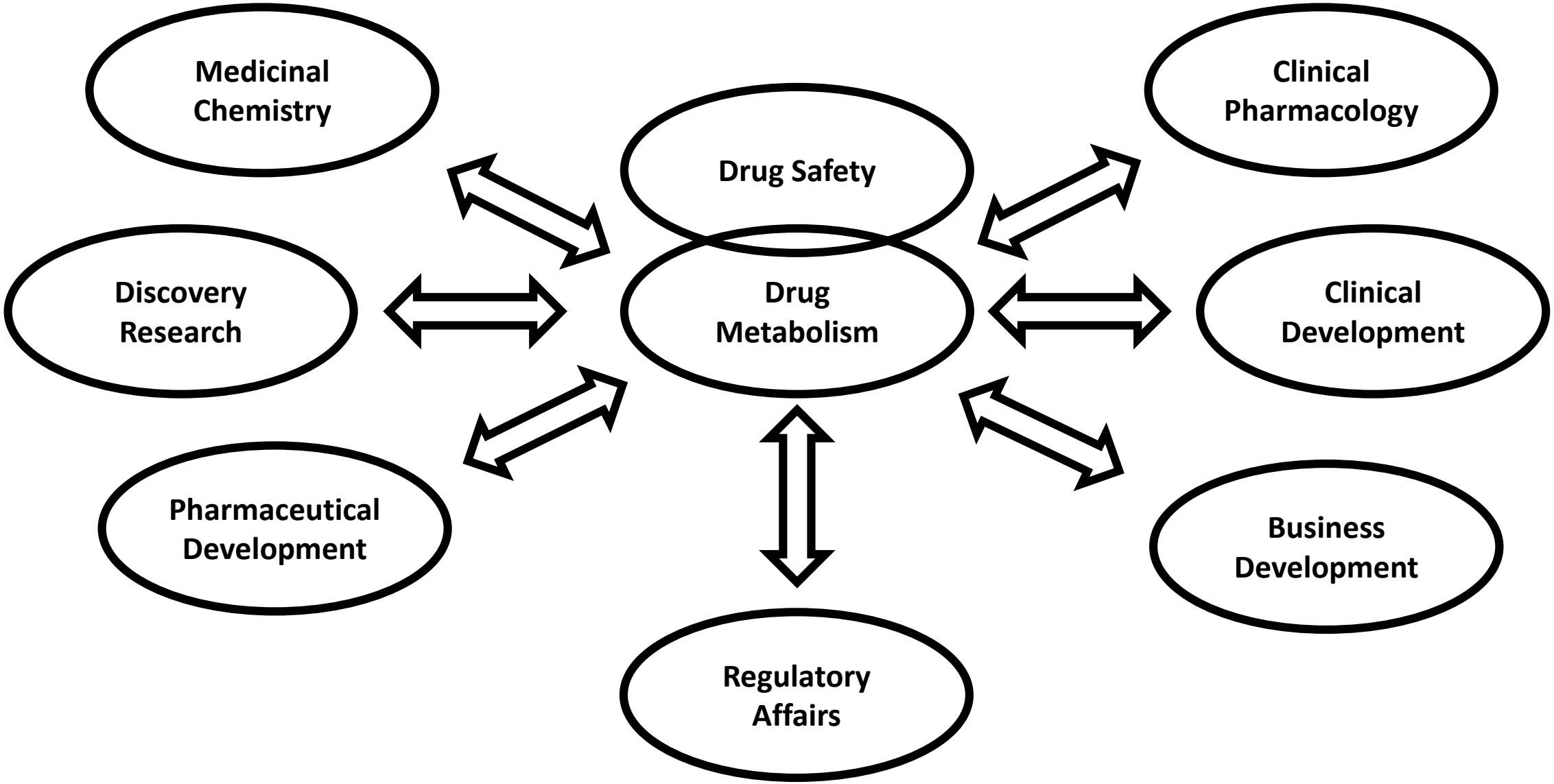
Toxicity as a major source of attrition in drug development

Drug Metabolism/Toxicity studies (dissecting a multifactorial problem)

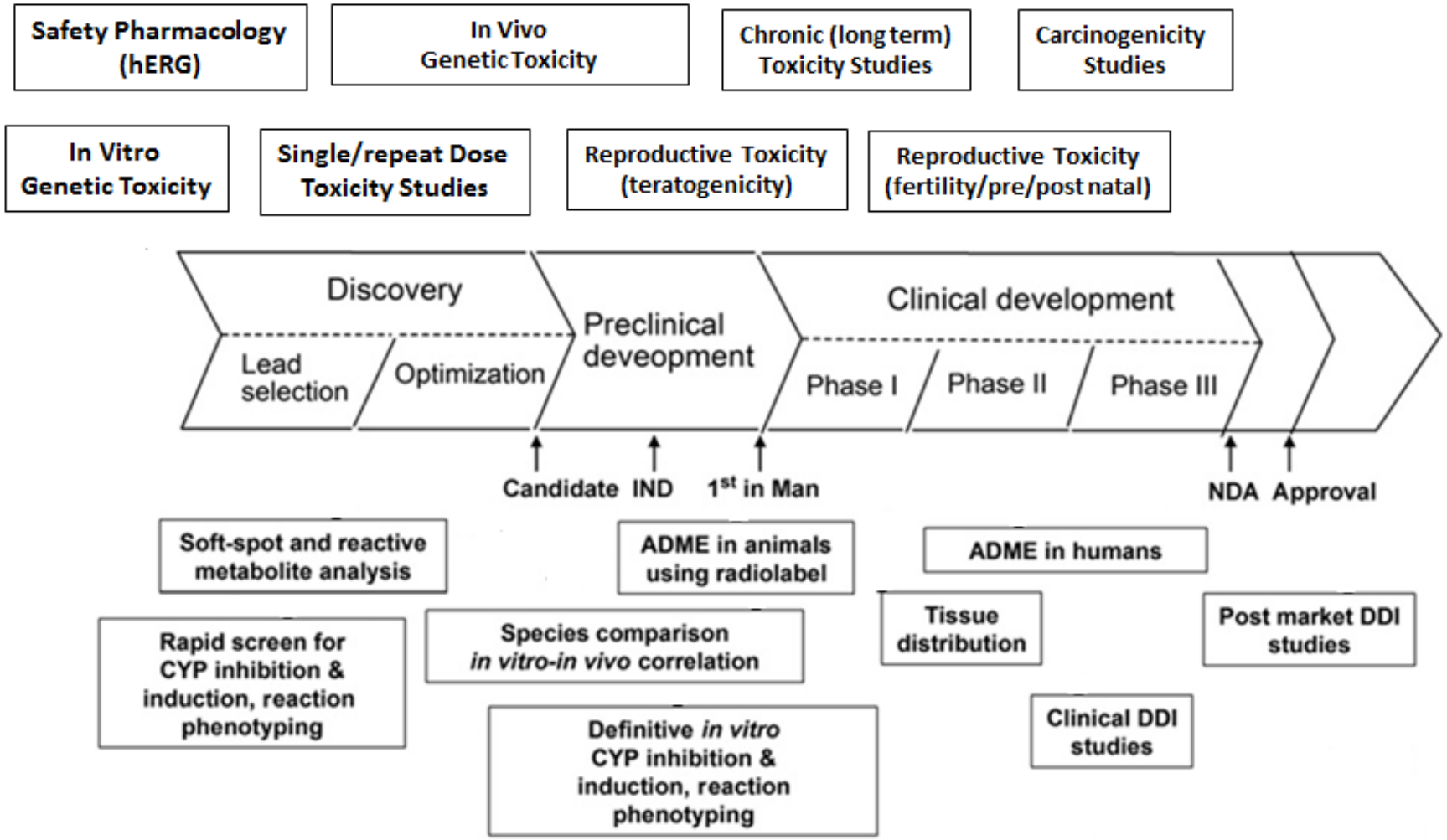


- **Active Drug Metabolites**
- **Metabolism-dependent Drug Safety Concerns**

Simple Relationship Map for DMPK Scientists

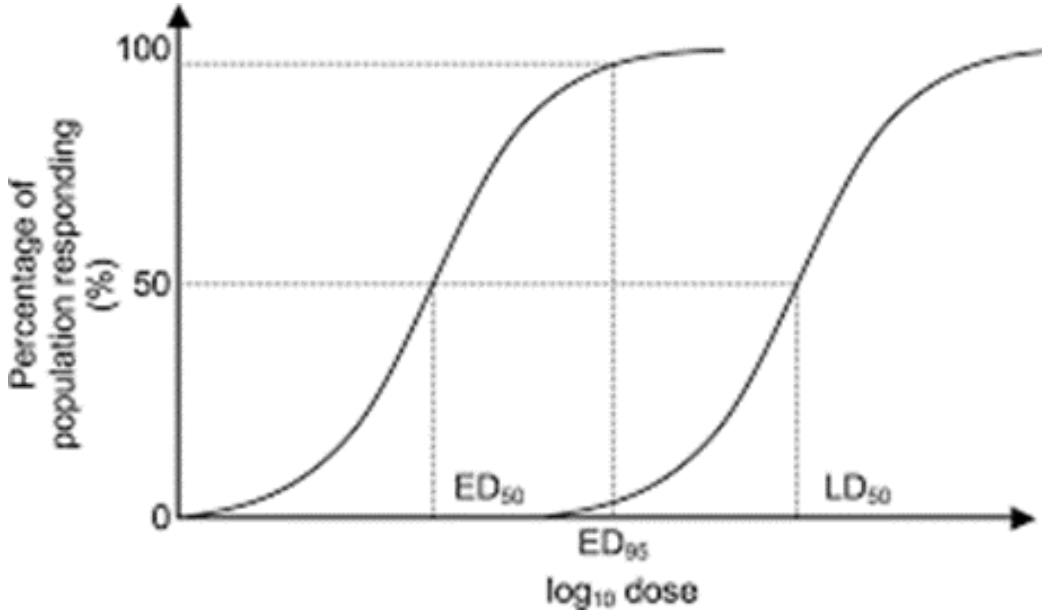


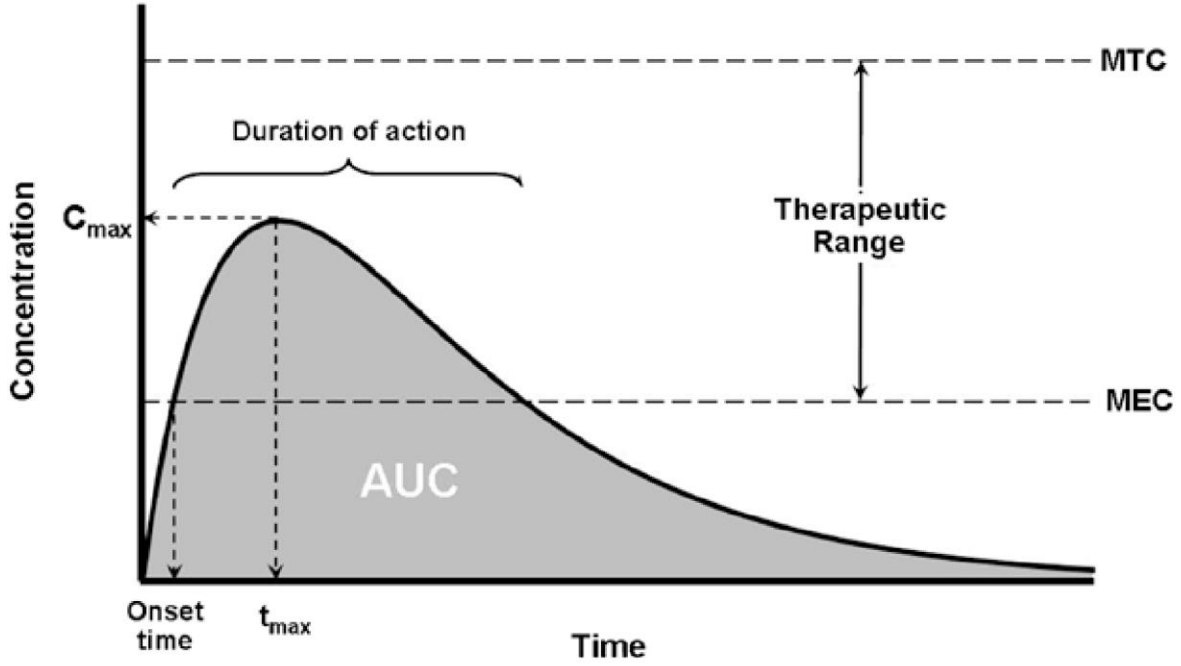
Drug Metabolism / Drug Safety "Touch Points" at Various Times Across the Development Process



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

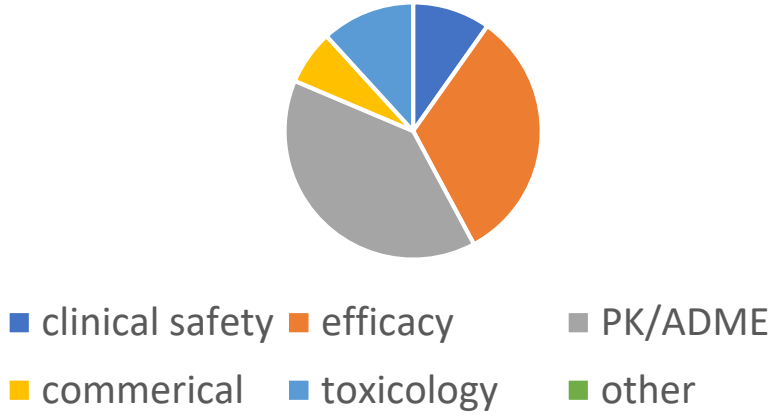
- **Do the results of well-controlled studies provide substantial evidence of drug effectiveness?**
- **Do the results show the product is safe under the conditions of use in the proposed labeling?**
  - **Safe, in this context extends beyond assessing a drug's Therapeutic Index and embraces the notion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



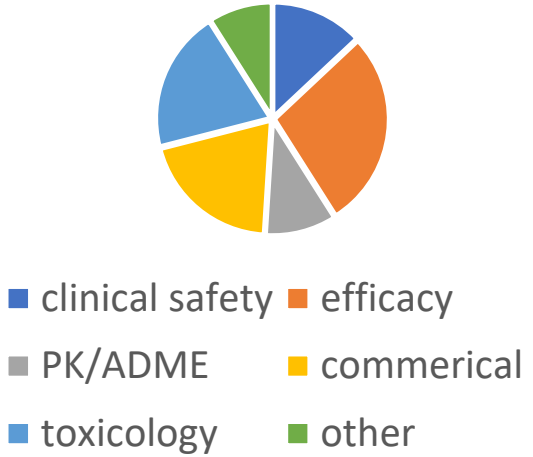


Pharmaceutical industry success rates and the primary reasons for attrition through the years

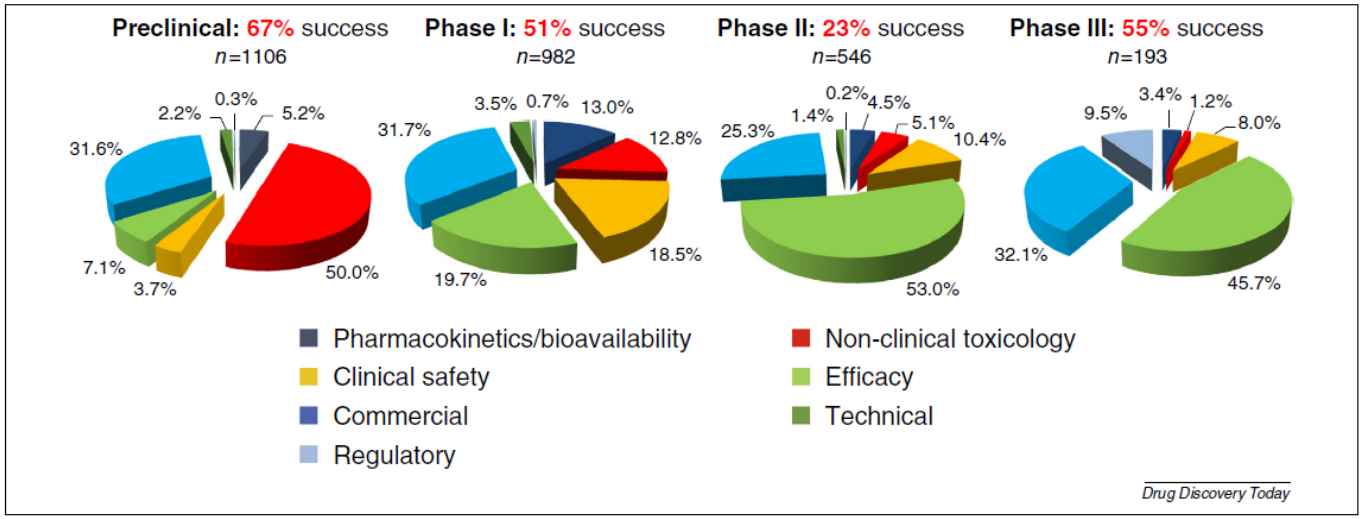
Reason for drug failure: 1990



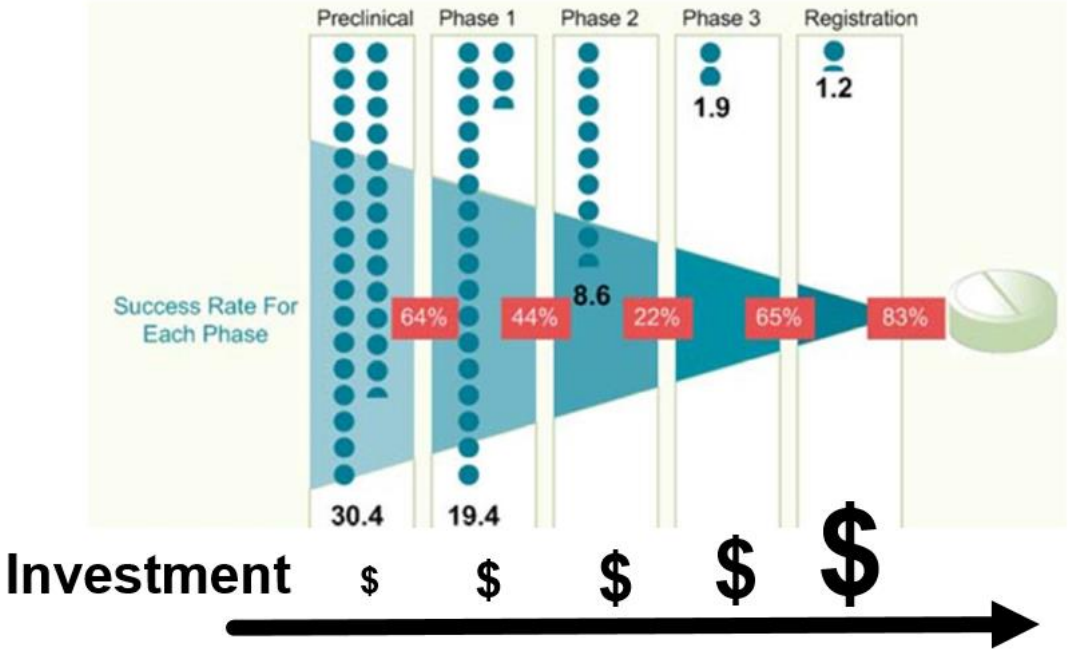
Reason for drug failure: 2000

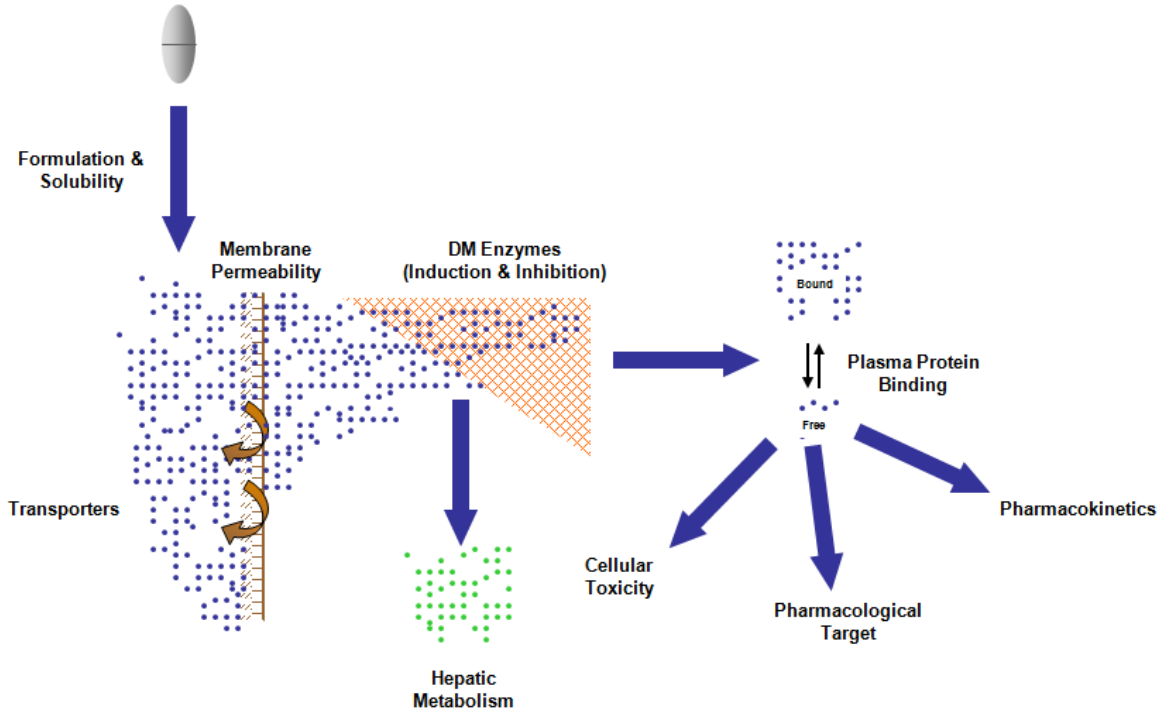


Reason for drug failure: 2010



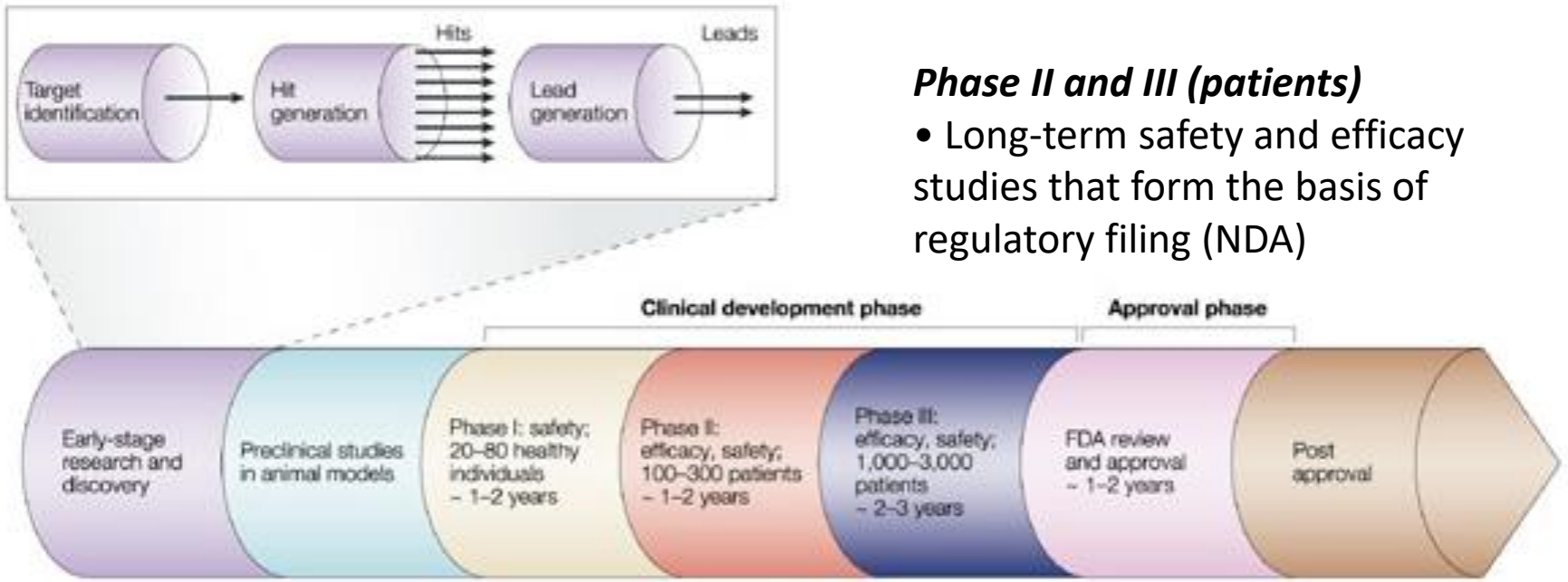
Looking for the best candidates to be taken forward into the Clinical





- **Challenges for the DM Scientist beyond PK issues in Drug Discovery**
  - **Minimizing potential for toxicity (esp. cardiovascular and liver toxicity)**
  - **Minimizing potential drug-drug interaction potential of parent drug and metabolites**
  - **Dealing prospectively with reactive drug metabolite issues**
  - **Developing strategies to respond to stable drug metabolites (“MIST”)**
  - **Characterizing the impact of pharmacologically active drug metabolites**

Basic Scheme of the Drug Development Continuum



Phase II and III (patients)

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

FIH (First In Human) ***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)

Terminology

Investigational New Drug (IND) program is the means by which a pharmaceutical company obtains permission to ship an experimental drug across state lines (usually to clinical investigators) before a marketing application for the drug has been approved.

The IND application must contain information in three broad areas:

- **Animal Pharmacology and Toxicology Studies - Preclinical data to permit an assessment as to whether the product is reasonably safe for initial testing in humans.**

- **Manufacturing Information - Information pertaining to the composition, manufacturer, stability, and controls used for manufacturing the drug substance and the drug product.**

- **Clinical Protocols and Investigator Information - Detailed protocols for proposed clinical studies to assess whether the initial-phase trials will expose subjects to unnecessary risks**

Terminology

New Drug Application (NDA). Since 1938, every new drug has been the subject of an approved NDA before U.S. commercialization. The NDA application is the vehicle through which drug sponsors formally propose that the FDA approve a new pharmaceutical for sale and marketing in the U.S.

The goals of the NDA are to provide enough information to permit FDA reviewer to reach the following key decisions:

- **Whether the drug is safe and effective in its proposed use(s), and whether the benefits of the drug outweigh the risks.**
- **Whether the drug's proposed labeling (package insert) is appropriate, and what it should contain.**
- **Whether the methods used in manufacturing the drug and the controls used to maintain the drug's quality are adequate to preserve the drug's identity, strength, quality, and purity.**

The documentation required in an NDA is supposed to tell the drug's whole story, including what happened during the clinical tests, what the ingredients of the drug are, the results of the animal studies, how the drug behaves in the body, and how it is manufactured, processed and packaged.

Key Regulatory Questions in an IND Review

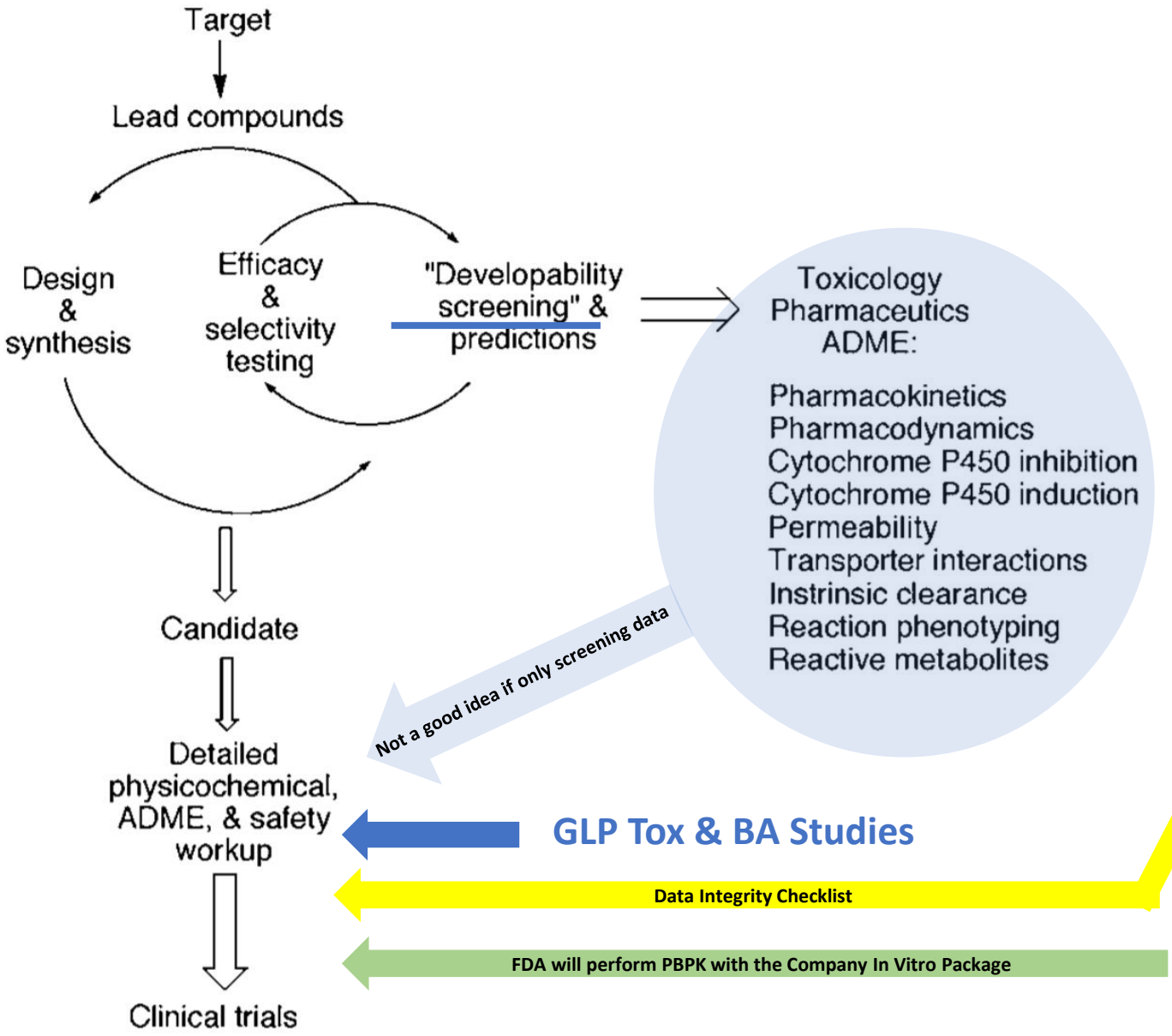
TITLE 21--FOOD AND DRUGS
CHAPTER I--FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH AND HUMAN SERVICES
SUBCHAPTER D--DRUGS FOR HUMAN USE
PART 312 INVESTIGATIONAL NEW DRUG APPLICATION

- **Does the submission contain sufficient information to assess risks to the subjects in the proposed trail?**
- ***Were adequate preclinical studies performed to describe the drug's disposition?***
- ***Is the data submitted in sufficient detail to conduct an independent FDA review?***
- ***Does the design of the proposed clinical trail contain adequate safeguards for subject safety?***

2.4.1	OVERVIEW OF NONCLINICAL TESTING STRATEGY
2.4.2	PHARMACOLOGY
2.4.2.1	Brief Summary
2.4.2.2	Primary Pharmacodynamics
2.4.2.3	Secondary Pharmacodynamics
2.4.2.4	Safety Pharmacology
2.4.2.5	Pharmacodynamic Drug Interactions
2.4.3	PHARMACOKINETICS
2.4.3.1	Brief Summary
2.4.3.2	Methods of Analysis
2.4.3.3	Absorption
2.4.3.4	Distribution
2.4.3.5	Metabolism
2.4.3.6	Excretion
2.4.3.7	Pharmacokinetic Drug Interactions
2.4.3.8	Other Pharmacokinetic Studies
2.4.4	TOXICOLOGY
2.4.4.1	Brief Summary
2.4.4.2	Single-Dose Toxicity
2.4.4.3	Repeat-Dose Toxicity
2.4.4.3.1	Relationship of Findings to Pharmacokinetics
2.4.4.3.2	Target Organ Toxicity
2.4.4.3.2.1.	<TARGET 1>
2.4.4.3.2.2.	<TARGET 2>
2.4.4.3.2.3.	<TARGET 3>
2.4.4.4	Genotoxicity
2.4.4.5	Carcinogenicity
2.4.4.6	Reproductive and Developmental Toxicity
2.4.4.6.1	Fertility and early embryonic development
2.4.4.6.2	Embryo-fetal development
2.4.4.6.3	Prenatal and postnatal development, including maternal function
2.4.4.6.4	Studies in which the offspring (juvenile animals) are dosed and/or further evaluated
2.4.4.7	Local Tolerance
2.4.4.8	Other Toxicity Studies
2.4.4.8.1	Phototoxicity
2.4.4.8.2	Antigenicity
2.4.4.8.3	Immunotoxicity
2.4.4.8.4	Mechanistic Studies
2.4.4.8.5	Dependence
2.4.4.8.6	Studies on Metabolites
2.4.4.8.7	Studies on Impurities
2.4.4.8.8	Other Studies
2.4.5	INTEGRATED OVERVIEW AND CONCLUSIONS
2.4.6	LIST OF LITERATURE REFERENCES

A systematic and integrated presentation of the findings from preclinical studies are required such that an informed and experienced expert would reasonably understand and consider possible signals of human risk.

Transition between Drug Discovery and Candidate Selection



Associated metabolism information is not conducted to GLP standards but over the past few years there has been an increased level of scrutiny regarding the quality and accessibility of the information

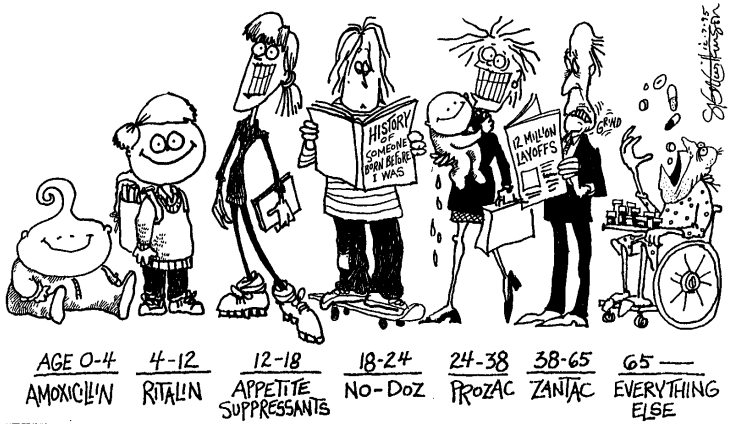
Complete, consistent and accurate data

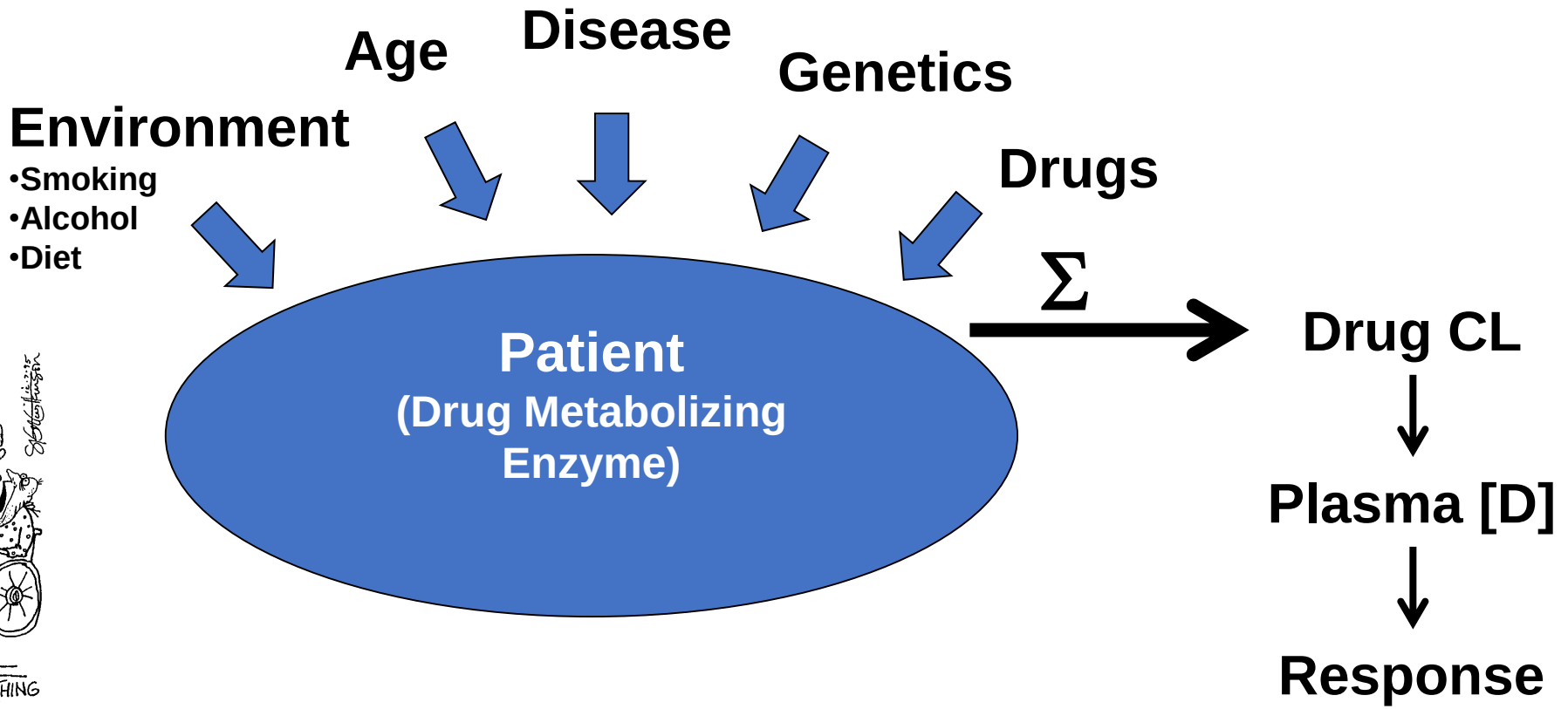
Characteristics	Meaning
Attributable	**Establishes who performed an action and when. Traceable to an individual.**
Legible	**Data must be recorded permanently in a durable medium and be readable by others. Traceable changes.**
Contemporaneous	**Activities must be recorded at the time they occur (when activity is performed or the information is obtained).**
Original	**The information must be the original record (first capture of the data) or a certified true copy. Not the transcribed data.**
Accurate	**Data reflect true information.**

SIMCYP

Integration of Factors Associated with Patient Variability in Drug Metabolism

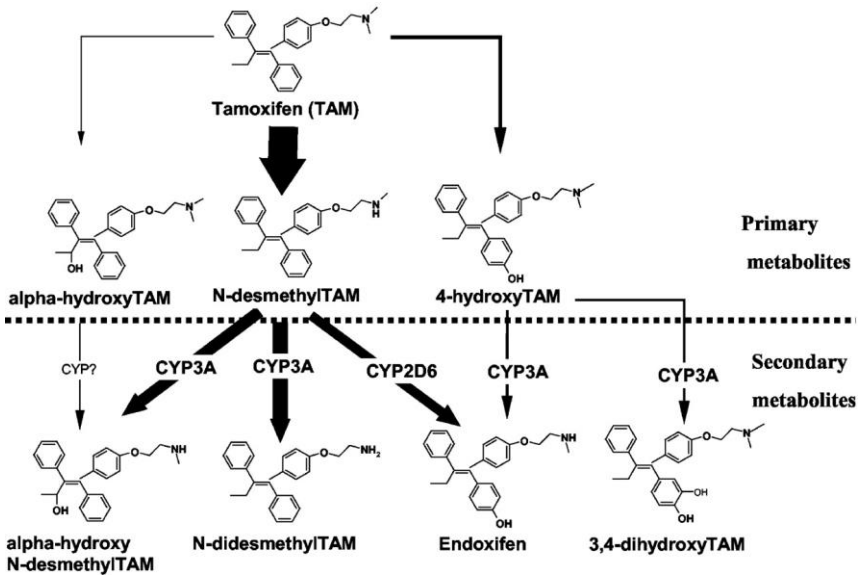
Clinical trials provide evidence of efficacy and safety at usual doses in controlled populations





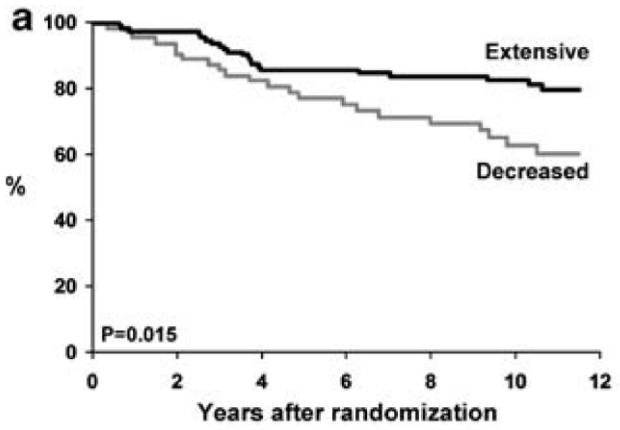
Metabolites with Pharmacological Activity: Tamoxifen > Endoxifen

Tamoxifen is widely used to reduce the risk of breast cancer (BC) recurrence and extend disease-free survival among women with estrogen-sensitive breast cancers. Tamoxifen efficacy is thought to be attributable to its active metabolite, endoxifen and 4-hydroxytamoxifen (4-HT), have been shown to be up to 100 times more potent estrogen receptor (ER) antagonists than the parent compound and are therefore likely to contribute to target inhibition and, thereby, the outcome of therapy.

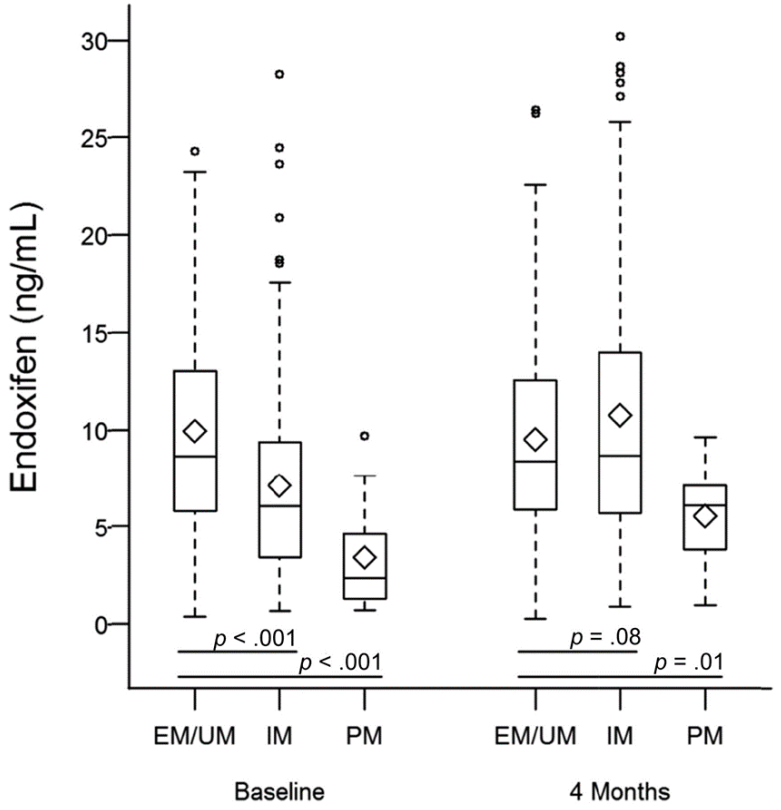


Desta Z, Ward BA, Soukhova NV, Flockhart DA. J Pharmacol Exp Ther. 310(3):1062-75 (2004).

Kaplan–Meier estimates of time to breast cancer recurrence based on CYP2D6 metabolism (extensive vs. decreased)

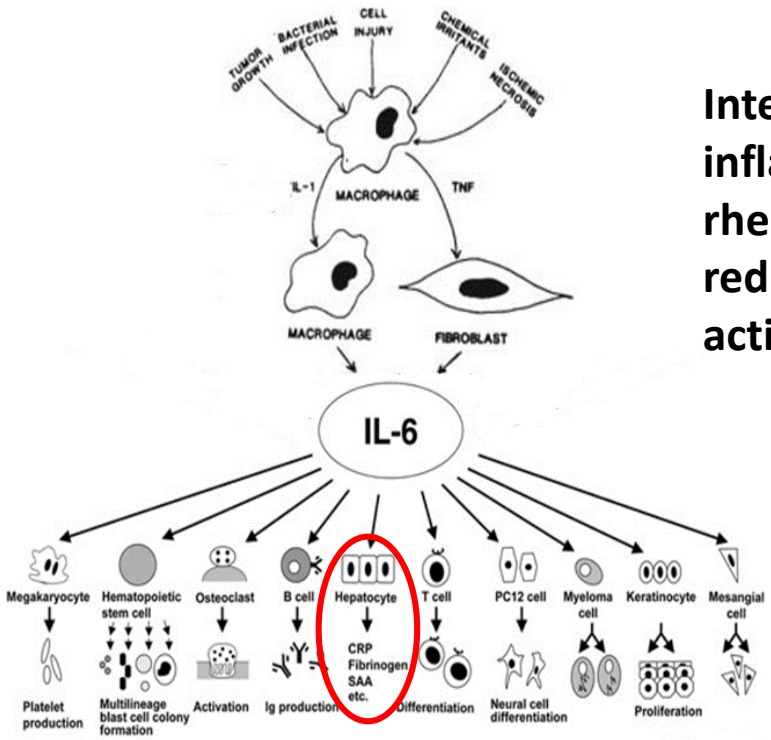


Goetz MP et al., Breast Cancer Res Treat. 101(1):113-21 (2007)

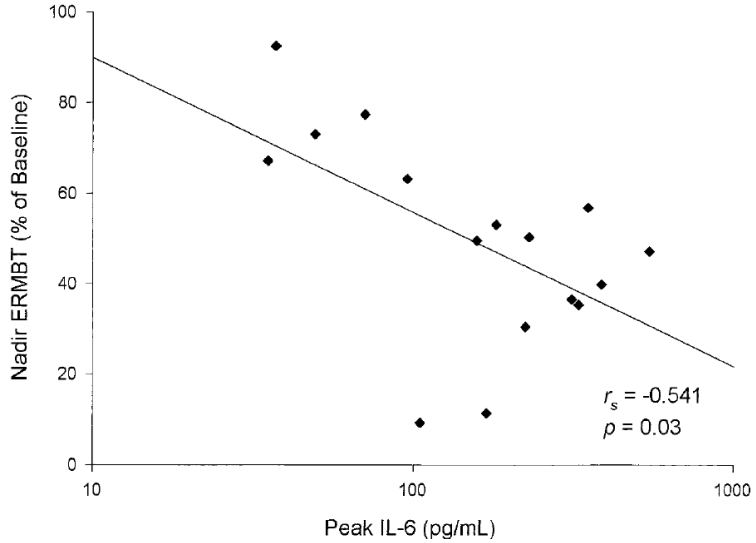


Daniel L. Hertz et al. The Oncologist 2016;21:795-803

Impact of Inflammation on Drug Metabolism

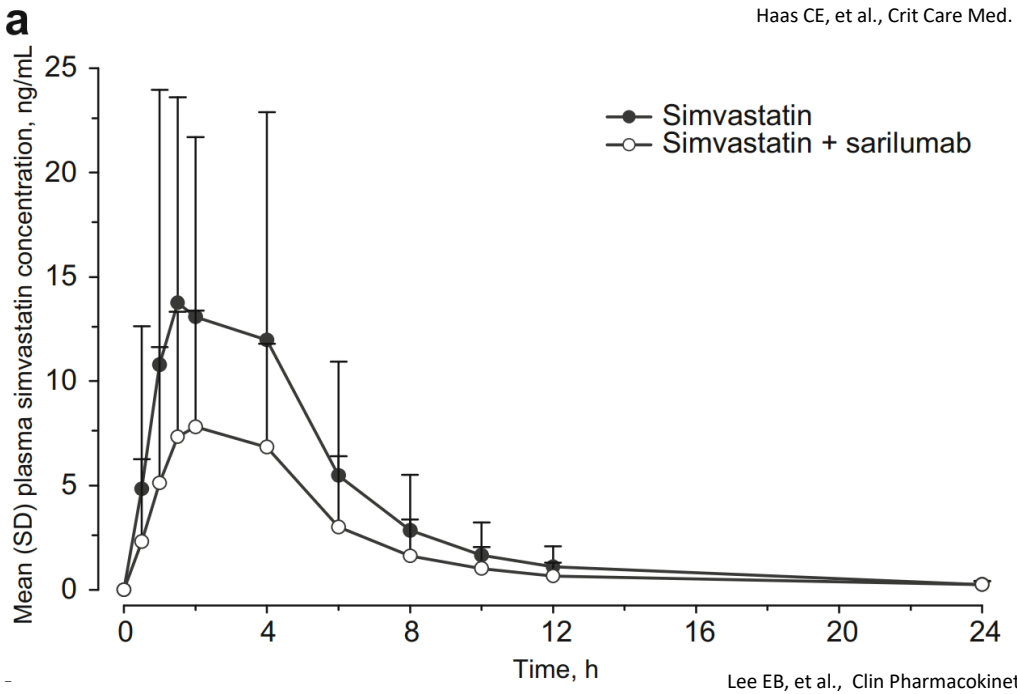


Interleukin (IL)-6 levels are elevated in inflammatory conditions, including rheumatoid arthritis (RA), leading to reduced cytochrome P450 (CYP) 3A4 activity



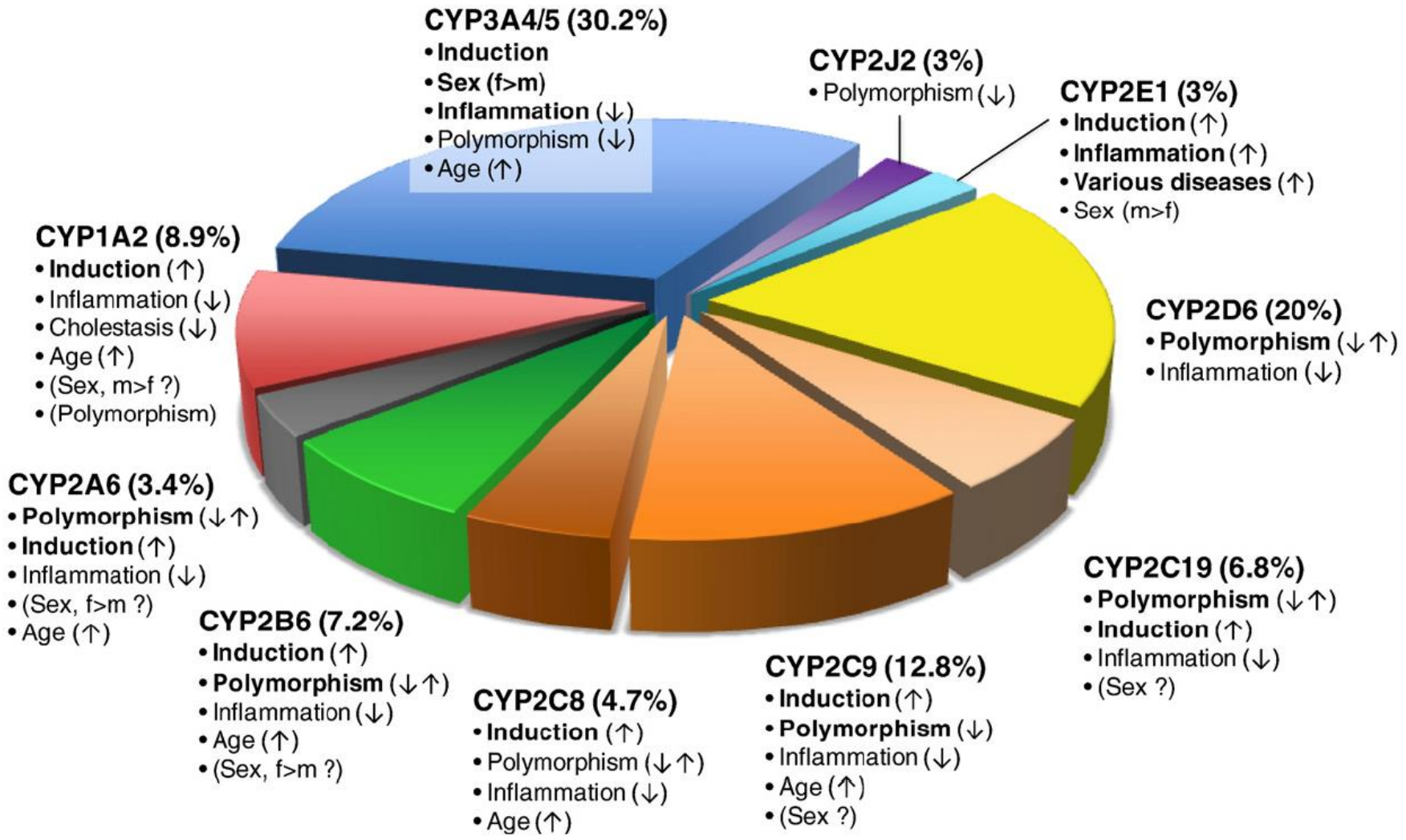
Haas CE, et al., Crit Care Med. 31(5):1338-46 (2003).

Sarilumab, a human monoclonal antibody blocking the IL-6 receptor-a (IL-6Ra), restores CYP3A4 activity, which results in decreased exposure of the sensitive CYP3A4 substrate simvastatin



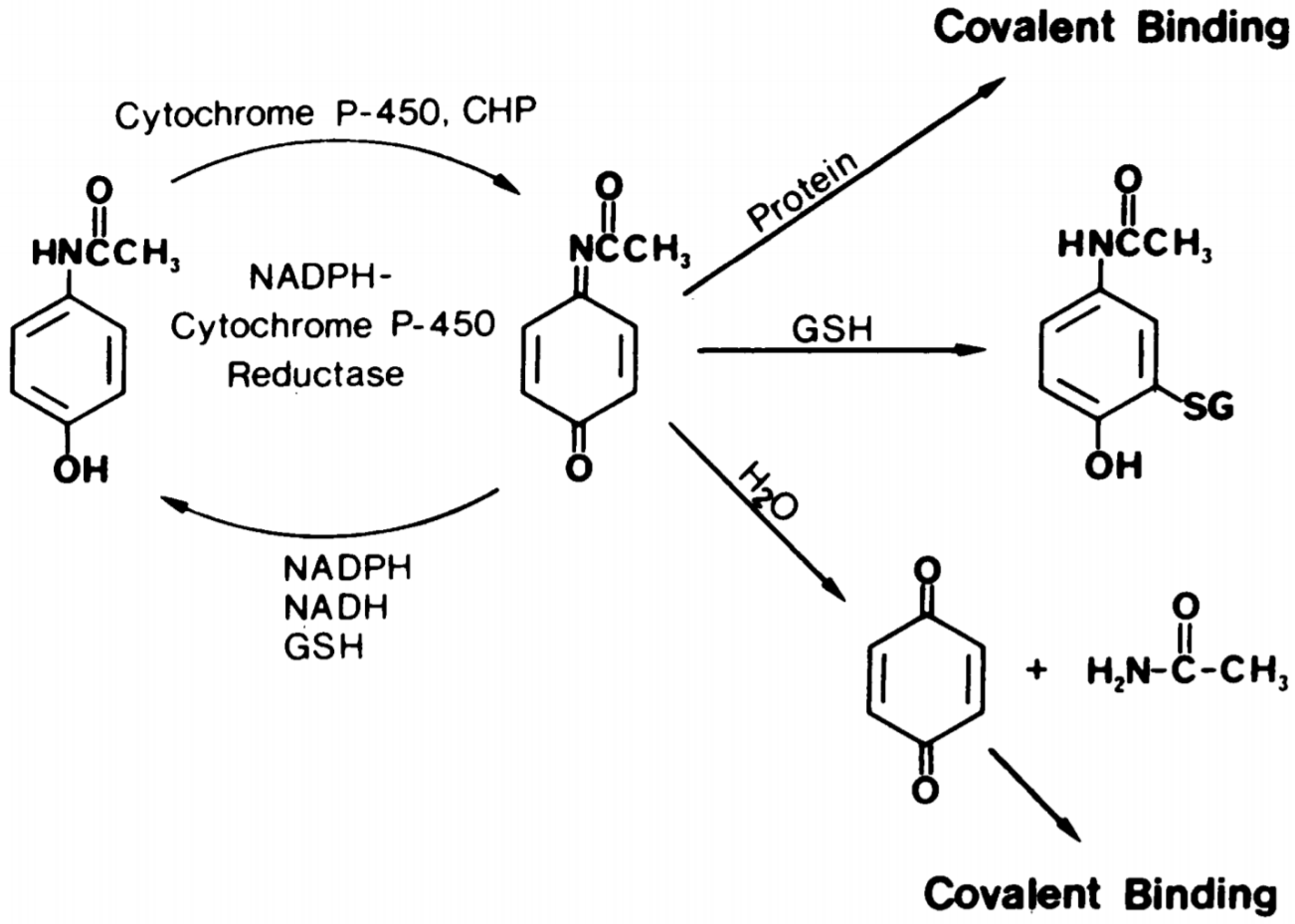
Lee EB, et al., Clin Pharmacokinet. 56(6):607-615 (2017)

Fraction of Clinically used Drugs Metabolized by P450 Isoforms and Factors Influencing Variability

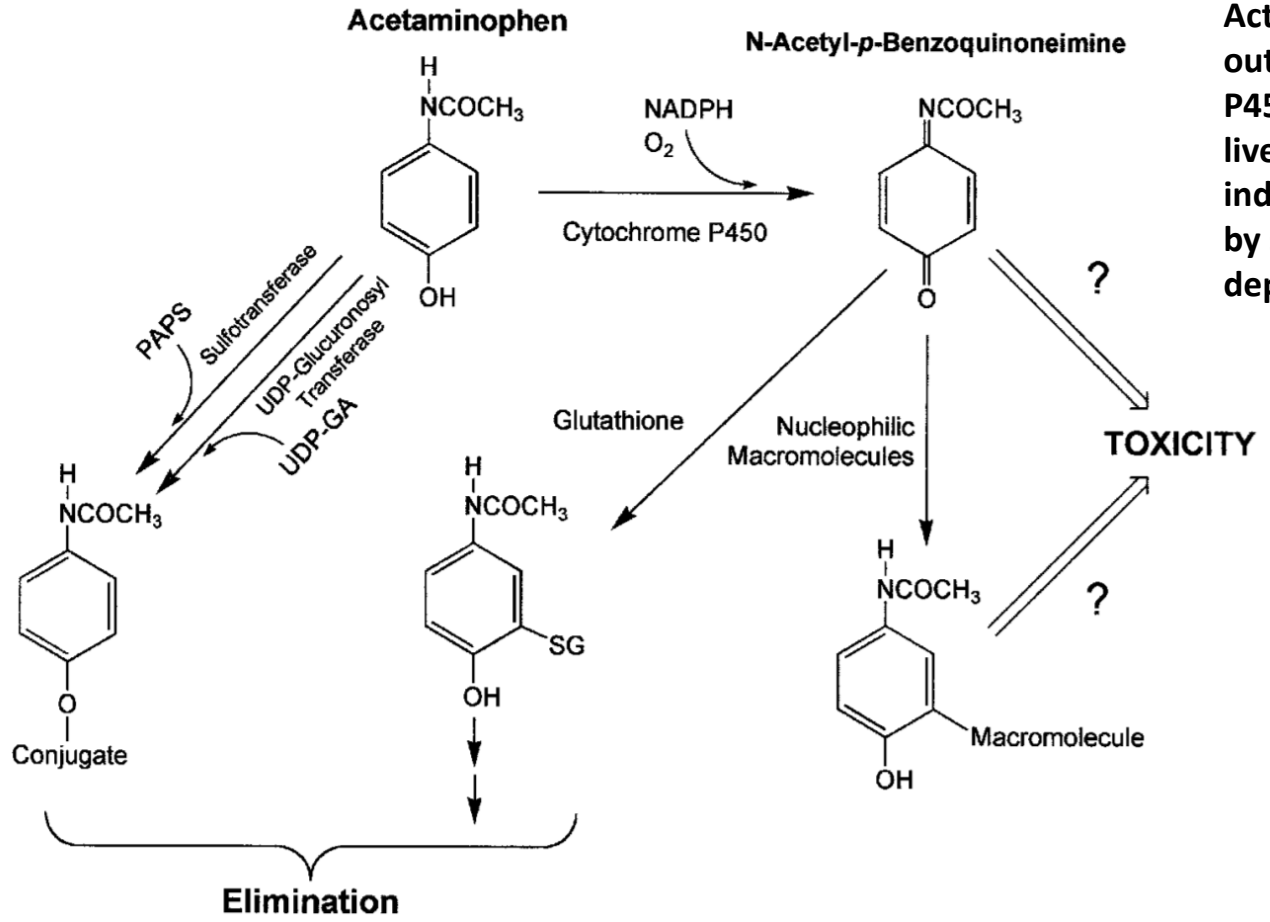


Acetaminophen Metabolism & Toxicity

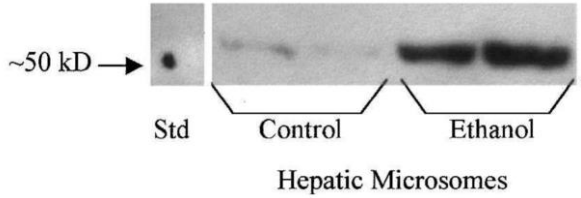
Acetaminophen has been approved for OTC use since 1960 and in most cases the drug is remarkably safe, however toxicity can occur at therapeutic doses.



Acetaminophen Metabolism & Toxicity

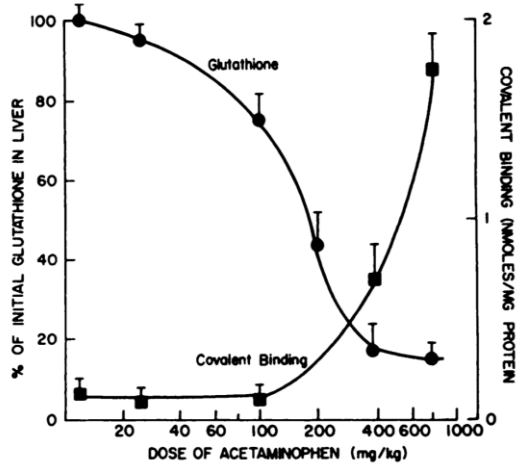


Activation of acetaminophen is primarily carried out by CYP2E1, an ethanol-inducible cytochrome P450. CYP2E1 is developmentally regulated, under liver-specific control, and undergoes substrate-induced protein stabilization. It is also regulated by starvation and diabetes through insulin-dependent mRNA stabilization.



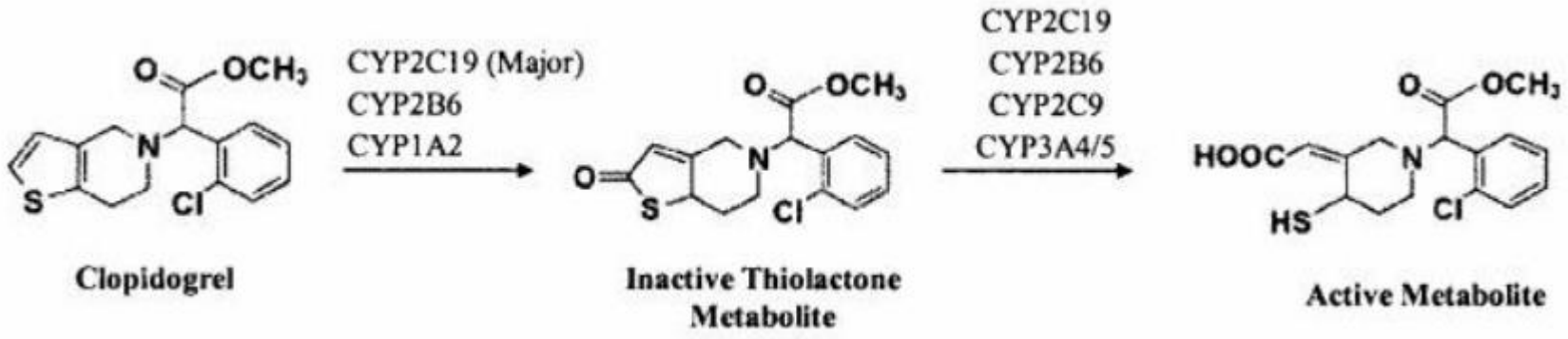
90% of APAP elimination is catalyzed by UDP-glucuronosyltransferases (UGT1A1 and 1A6) and sulfotransferases (SULT1A1, 1A3/4, and 1E1), respectively. As a consequence, Gilbert's Syndrome and other reductions in UGT activity putatively reduce the amount of APAP being cleared via this pathway. Moreover, SULT1A3 and SULT1A4 genotypes possess significant differences in binding affinity and catalytic activity towards analgesic compounds (including acetaminophen).

Glutathione levels in the body may be reduced by a number of factors, including poor nutrition, environmental toxins, and stress. Its levels also decline with age.



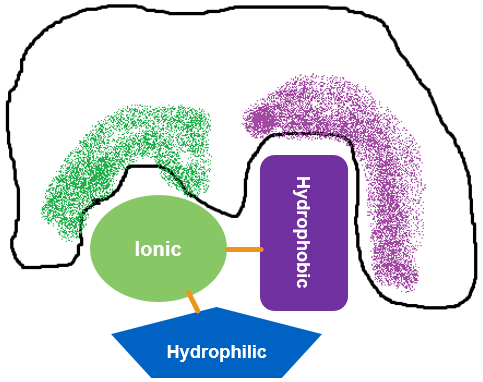
Outline for Today

- *Background*
- **Active Drug Metabolites**
  - **Inhibitory Metabolites**
  - **Pharmacological Active Metabolites**
  - **Cardiovascular toxicity (QTc effects) caused by DDI**

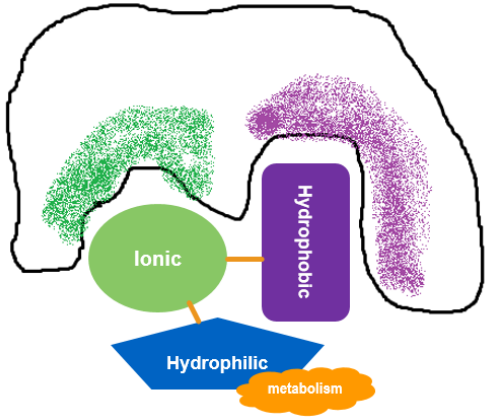


- **Metabolism-dependent Drug Safety Concerns**

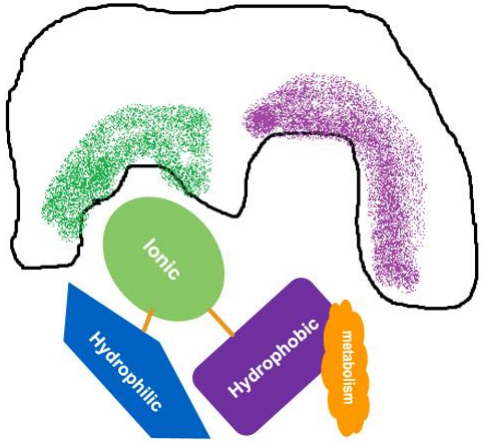
Rationale around Active Metabolites



Parent Drug

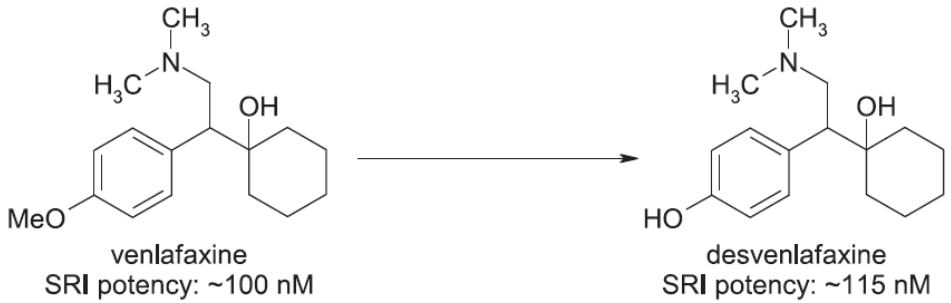


Metabolite 1



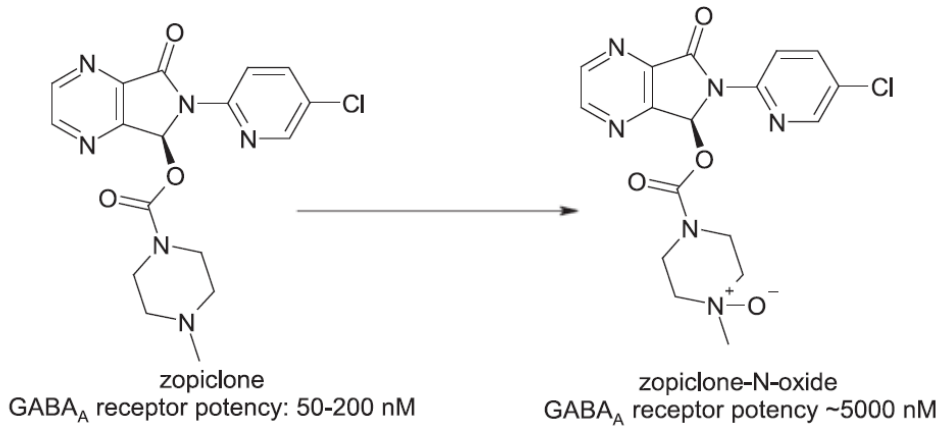
Metabolite 2

For metabolite 1, the biotransformation modification occurs on a position not involved in ligand binding, so the metabolite would be predicted to posses reasonable of activity. However, in the case of metabolite 2 the modification is on a position that disrupts receptor interaction, so the metabolite would be inactive.



venlafaxine
SRI potency: ~100 nM

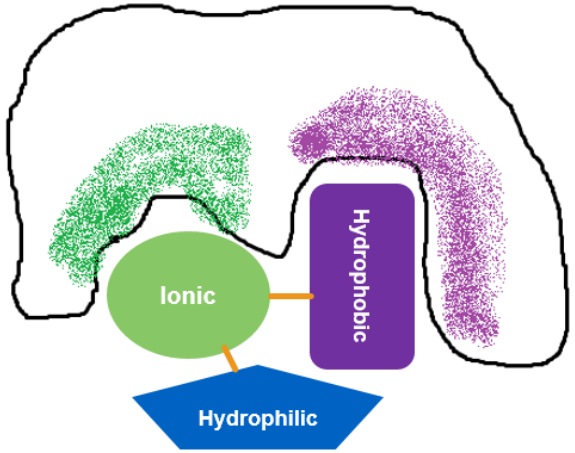
desvenlafaxine
SRI potency: ~115 nM



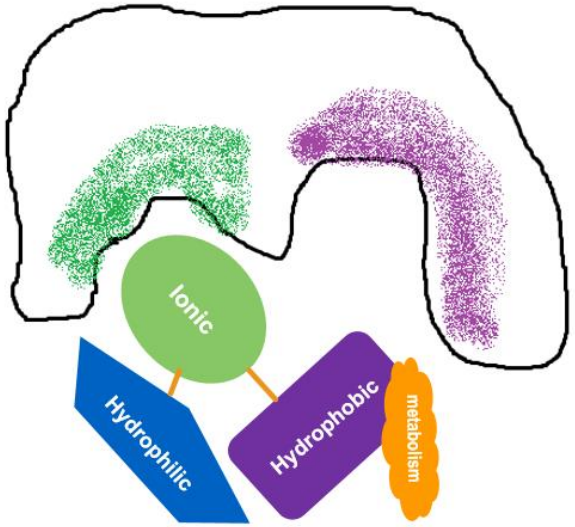
zopiclone
$GABA_A$ receptor potency: 50-200 nM

zopiclone-N-oxide
$GABA_A$ receptor potency ~5000 nM

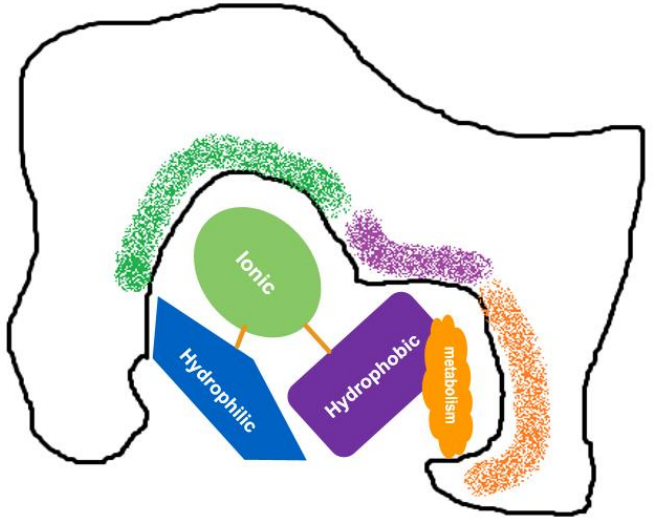
Rationale around Active Metabolites



Parent Drug

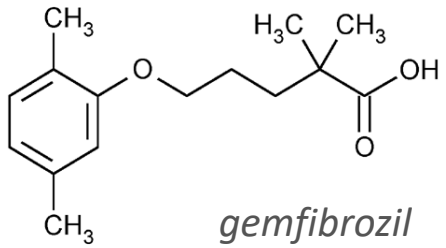


Metabolite 2

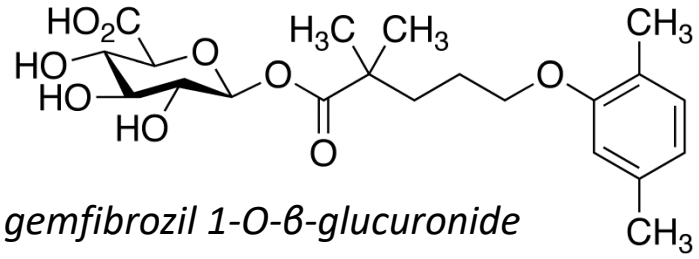


Metabolite 2

That said, while modification on metabolite 2 disrupts receptor interaction, the change may now introduce a binding motif for a different enzyme where metabolite 2 now possesses activity.



gemfibrozil



gemfibrozil 1-O-β-glucuronide

Drug-Drug Interactions

Defined: A modification of the effect of a drug when co-administered with another drug. The effect may be an increase or a decrease in the action of either substance, or it may be an adverse effect that is not normally associated with either drug.

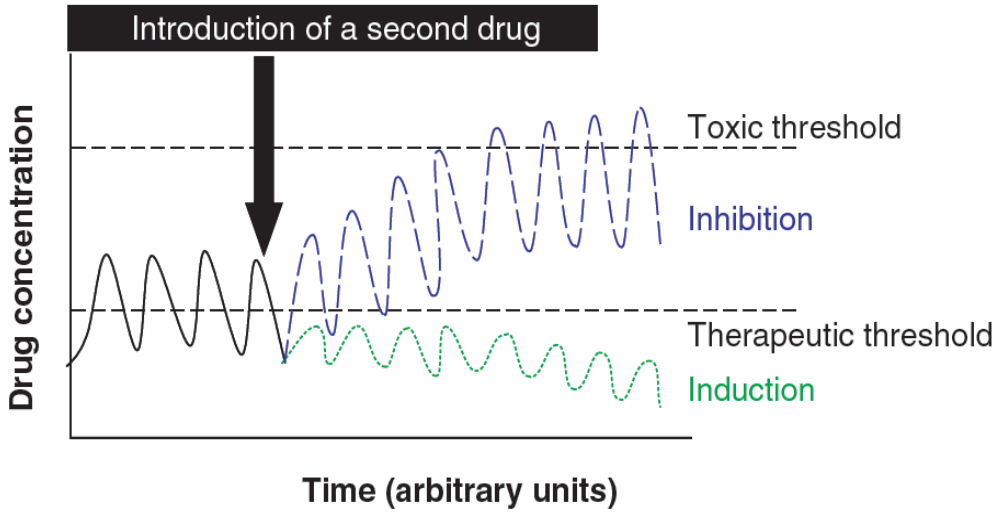
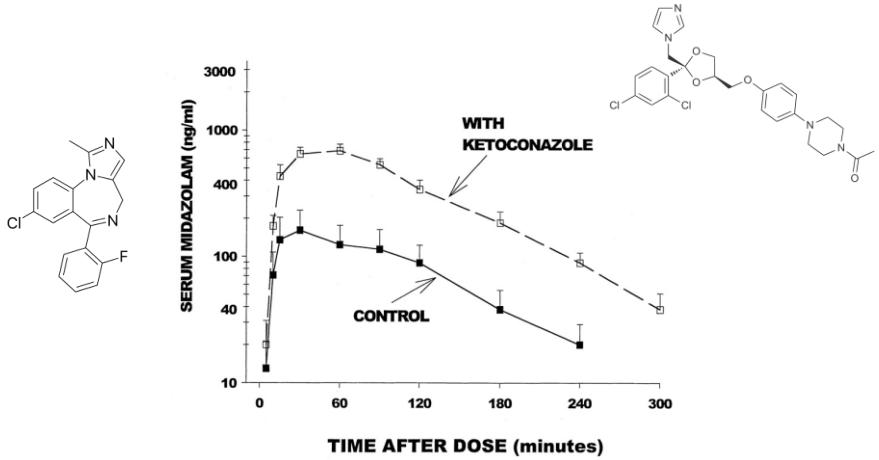


Table II: Molecular mechanisms of drug-drug interactions.

General mechanism	Pharmacokinetic change	Theoretical change in exposure to victim drug
Competitive inhibition	Increased systemic exposure	$\frac{AUC_i}{AUC} = 1 + \left(\frac{[I]}{K_i}\right)^*$
Noncompetitive inhibition	Increased systemic exposure	$\frac{AUC_i}{AUC} = 1 + \left(\frac{[I]}{K_i}\right)$
Uncompetitive inhibition	Increased systemic exposure	$\frac{AUC_i}{AUC} = 1 + \left(\frac{[I]}{K_i}\right)\left(\frac{[S]}{[S]+K_M}\right)$
Irreversible inactivation	Increased systemic exposure (with potential hysteresis of effect)	$\frac{AUC_i}{AUC} = \frac{V_{max}}{V_{max\ (inactivated)}}$
Induction	Decreased systemic exposure (with potential hysteresis of effect)	$\frac{AUC_i}{AUC} = \frac{V_{max}}{V_{max\ (induced)}}$

*Assumes $[S] << K_M$. As the dose increases (hence [S] increases), the extent of interaction will decrease when compared to control. AUC_i refers to the exposure occurring with coadministration of a perpetrator drug.



- **For the most part, our thinking is directly linked towards the interplay between the candidate molecule and the enzyme.**

PRESCRIBING INFORMATION

TAGAMET®

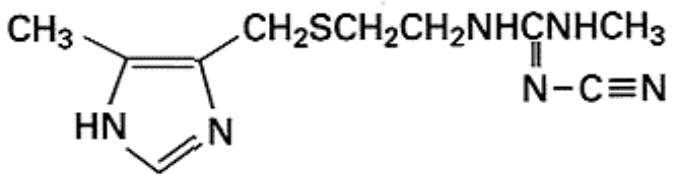
brand of

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-

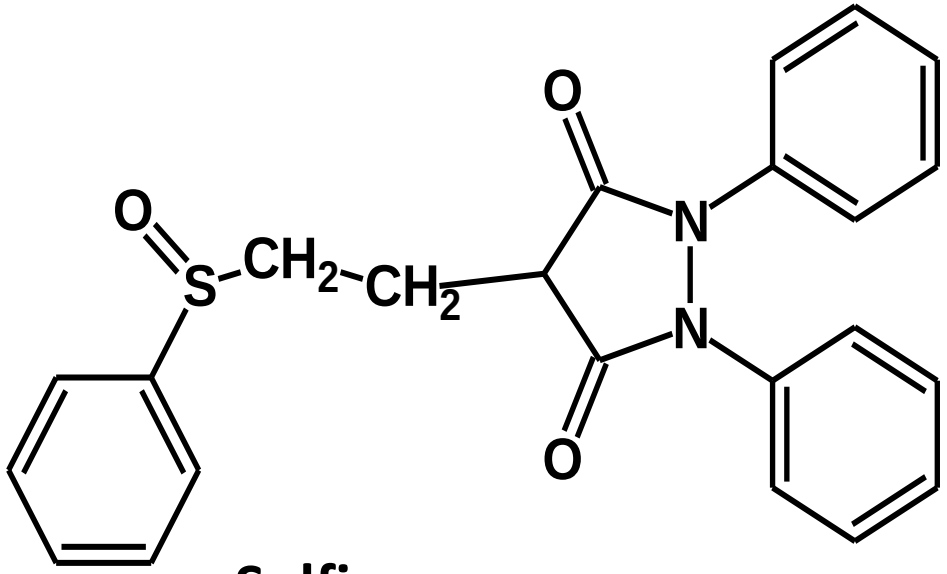
Drug Interactions: *Tagamet*, apparently through an effect on certain microsomal enzyme systems, has been reported to reduce the hepatic metabolism of warfarin-type anticoagulants, phenytoin, propranolol, nifedipine, chlordiazepoxide, diazepam, certain tricyclic antidepressants, lidocaine, theophylline 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.

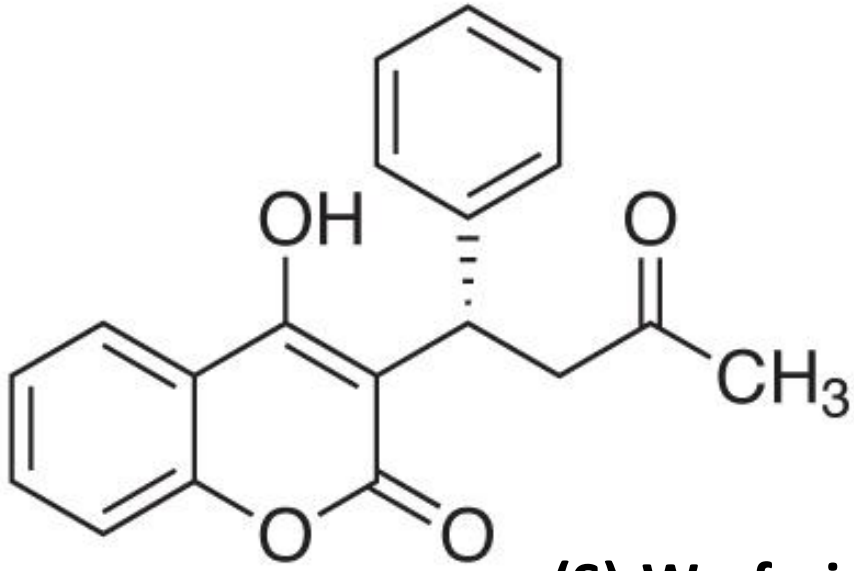
300 mg q.i.d. or 800 mg h.s. concomitantly with a 300 mg b.i.d. dosage of theophylline (Theo-Dur®, Key Pharmaceuticals, Inc.) demonstrated less alteration in steady-state theophylline peak serum levels with the 800 mg h.s. regimen, particularly in subjects aged 54 years and older. Data beyond 10 days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

Metabolites Contributing to Clinical DDI:

Sulfinpyrazone and (S)-Warfarin Clinical Drug Interaction



Sulfinpyrazone

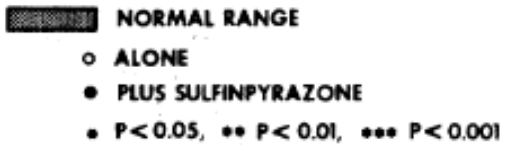


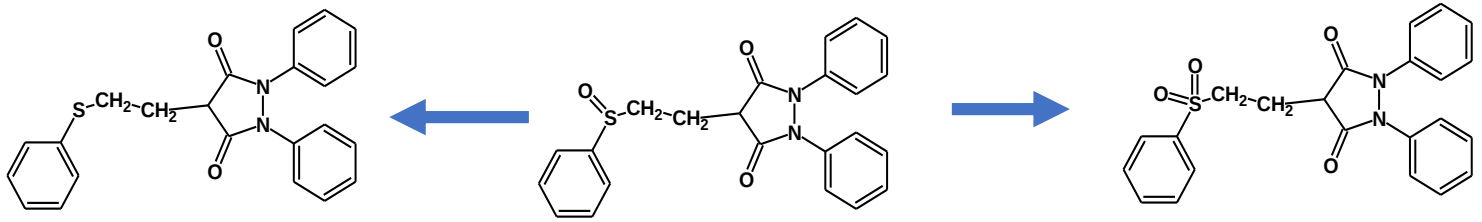
(S)-Warfarin

- (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 Inhibits (S)-Warfarin Clearance

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.

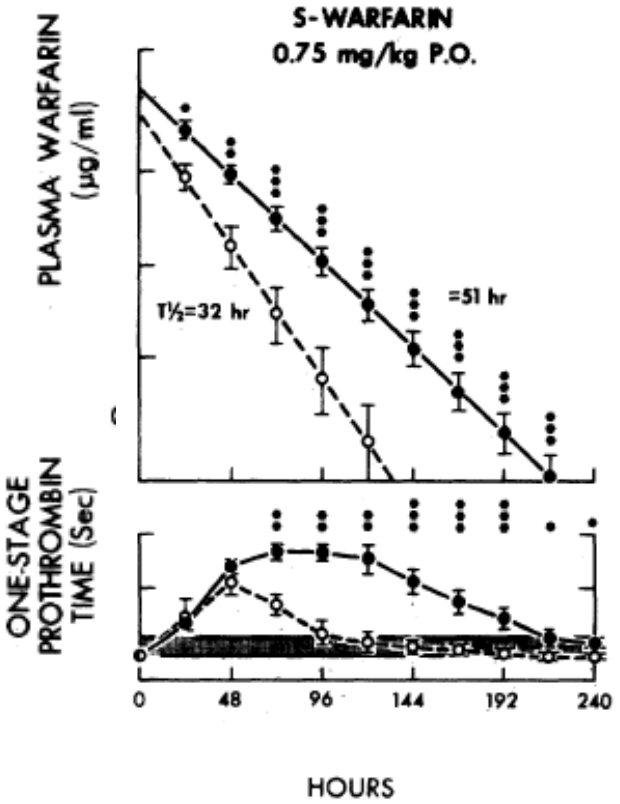




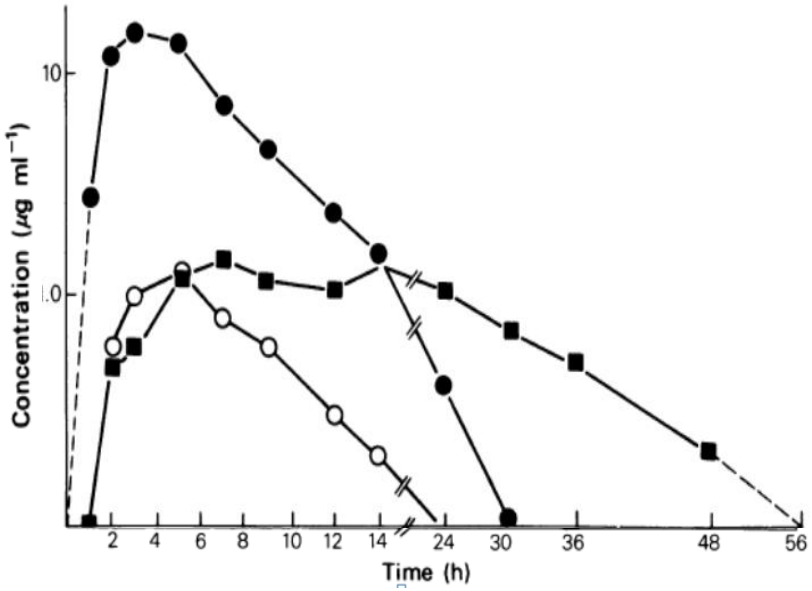
Sulfinpyrazone sulfone
CYP2C9 Ki = 73 μM

Sulfinpyrazone
CYP2C9 Ki = 230 μM

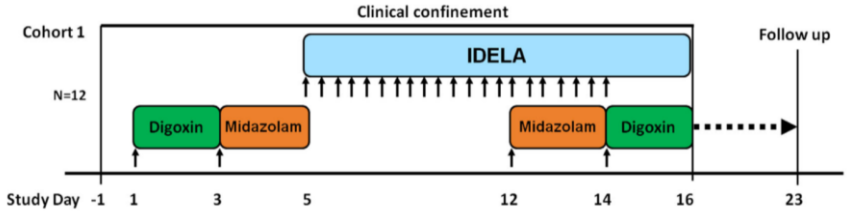
Sulfinpyrazone sulfide
CYP2C9 Ki = 17 μM

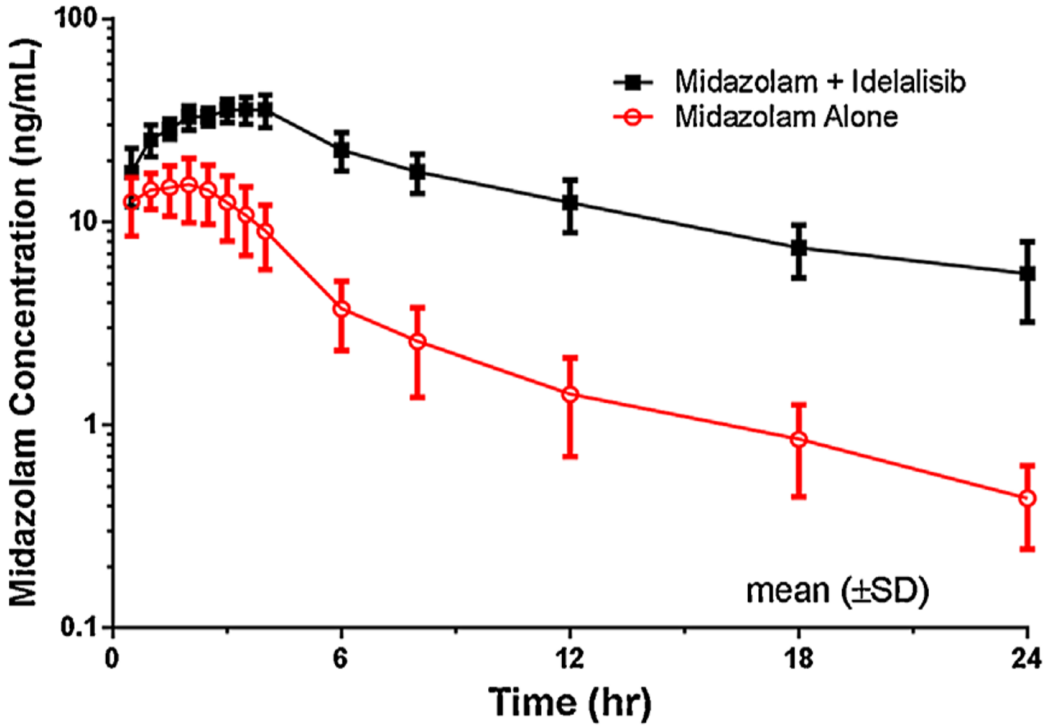


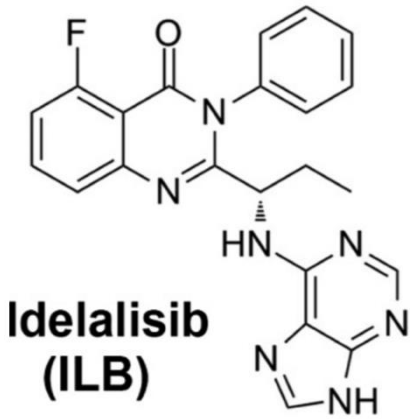
Plasma concentration-time profiles for sulphinpyrazone (●), sulphide (■) and sulphone (○) after receiving 200 mg oral dose.



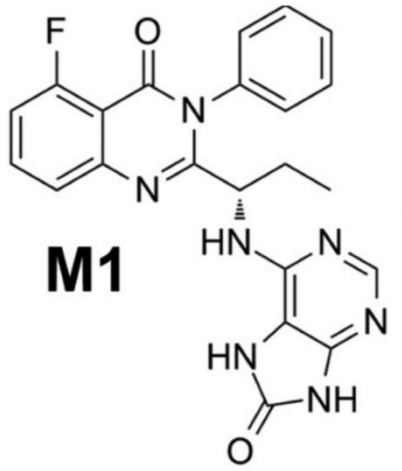
Metabolites Contributing to Clinical DDI: Idelalisib







Idelalisib (ILB)



M1

Because idelalisib itself is not a clinically relevant inhibitor of CYP3A (IC50, ~44μM), these data suggest that, M1 (GS-563117) is a time-dependent inhibitor of CYP3A (Ki = 0.2μM; and rate of enzyme inactivation [kinact], 0.033 min^{-1}) and is responsible for the observed clinical DDI.

Idelalisib or its metabolite inhibited CYP3A, CYP2C19, P-glycoprotein (P-gp), OATP1B1 and OATP1B3 in vitro. Idelalisib increased midazolam AUC by 5.4-fold; therefore, idelalisib should not be coadministered with sensitive CYP3A substrates. No changes in exposure to rosuvastatin (OAT1B1 and OATP1B3) or digoxin (P-gp) were observed.

Spectrum of the Consequences of Drug Metabolism

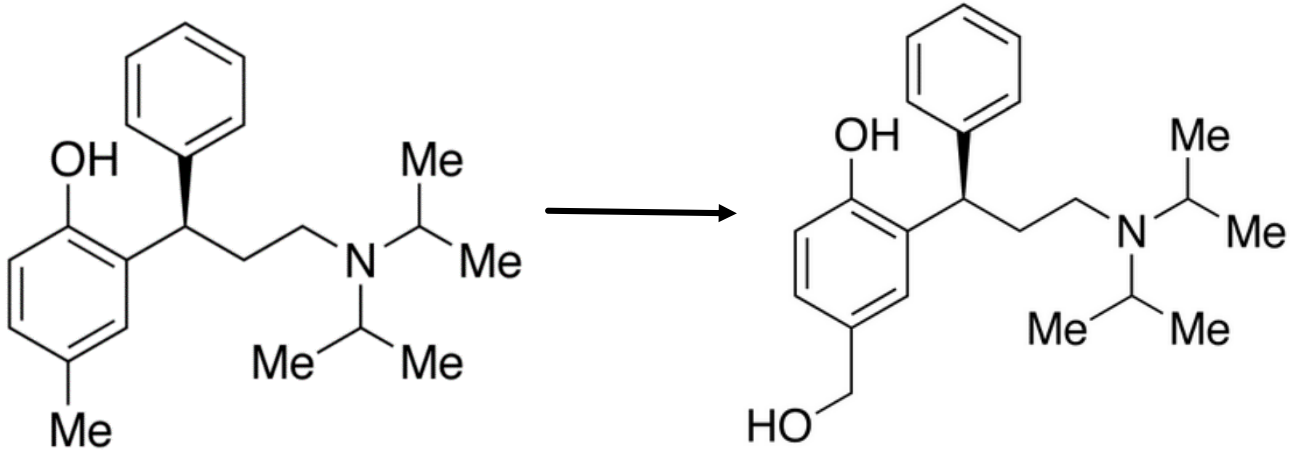
- *Inactive Products*
- ✓ *Active metabolites similar to parent*
- ✓ *Metabolites more active than parent*
- *Metabolites with completely new activity*
- *Formation of metabolites which are toxic*

Active metabolites developed as drugs

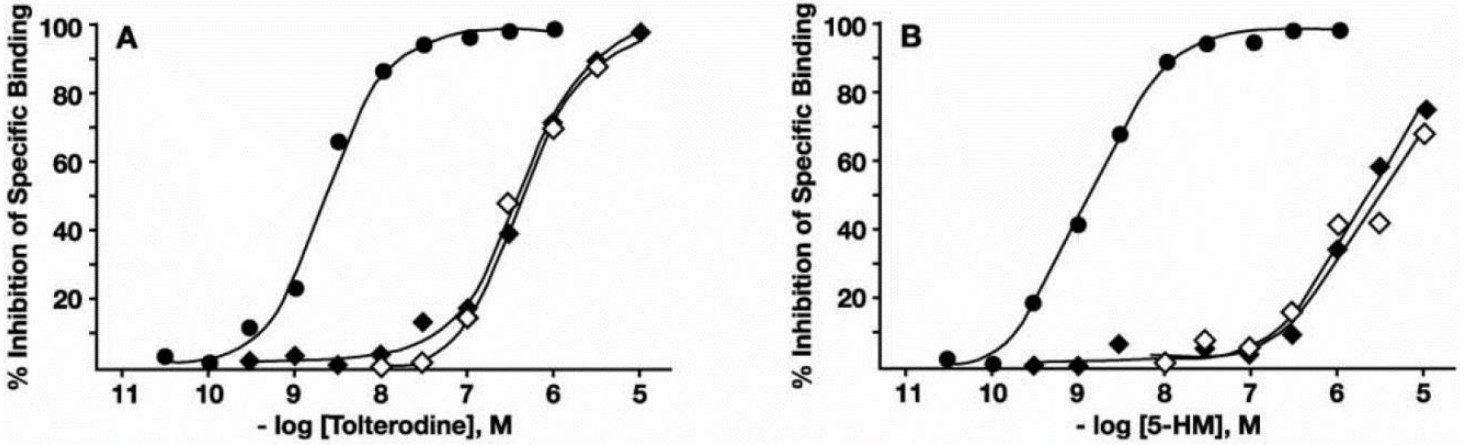
Metabolite drugs	Parent drugs
Acetaminophen	Phenacetin
Cetirizine	Hydroxyzine
Desimipramine	Imipramine
Desloratadine	Loratadine
Digoxin	β-methyldigoxin
Fexofenadine	Terfenadine
Mesoridazone	Thioridazone
Morphine	Codeine
Nortriptyline	Amitriptyline
Oxazepam	Diazepam
Phenobarabital	Primidone

Fura A. Role of pharmacologically active metabolites in drug discovery and development. Drug Discov Today. 11(3-4):133-42. (2006)

Additive Effects of an Active Metabolite (Tolterodine & CYP2D6)

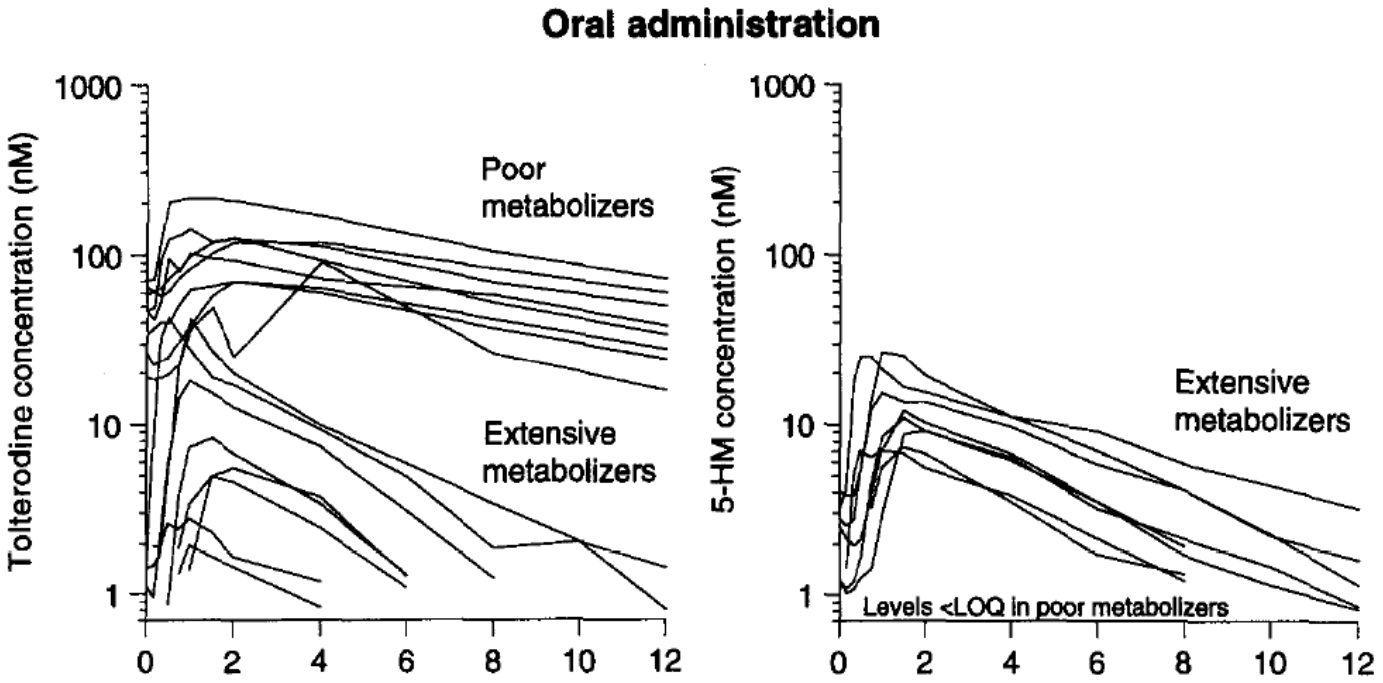


The primary metabolic route for tolterodine is via oxidation of the 5-methyl group and is mediated by the CYP2D6 and leads to the formation of a pharmacologically active 5-hydroxymethyl metabolite.



Additive Effects of an Active Metabolite (Tolterodine & CYP2D6)

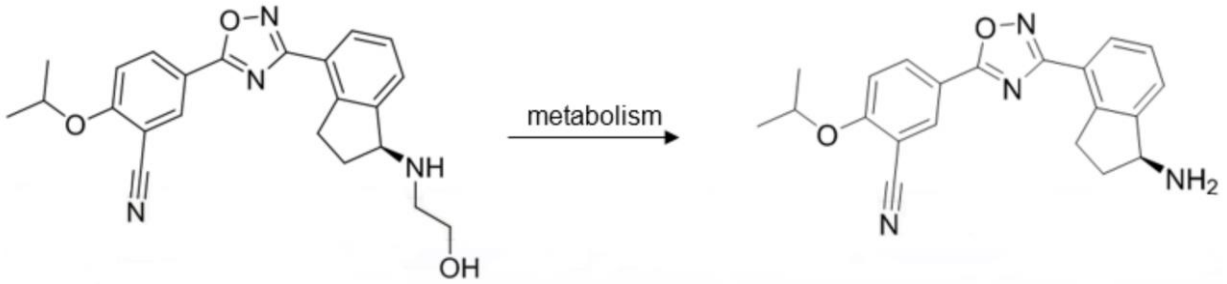
- **Tolterodine is cleared at a slower rate in poor metabolizers than in extensive metabolizers; this results in significantly higher serum concentrations of tolterodine and in negligible concentrations of the 5-hydroxymethyl metabolite.**



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.

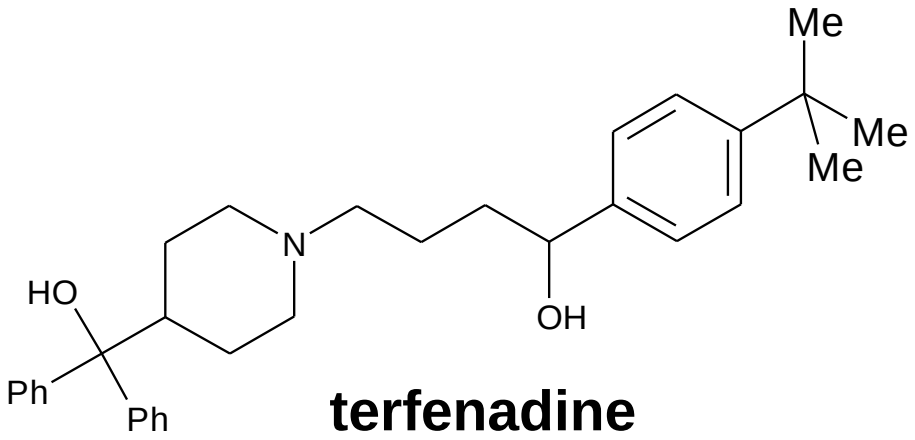
Metabolites with Pharmacological Activity: Ozanimod

- In 2015, Celgene acquired Receptos for $7.2 billion as a means to expand the immunology franchise. The primary driver for the acquisition was for the rights to the drug, Ozanimod, then, described as a potential best-in-class, oral, once-daily, selective sphingosine 1-phosphate 1 and 5 receptor modulator and was expected to bring in 4-6 billion in peak sales.
- Ozanimod is metabolized in humans to form one major (active) metabolite CC-112273:
  - Structurally similar to Ozanimod and possessed similar potency and selectivity to S1P1 and S1P5 as Ozanimod
  - The metabolite possesses a Tmax of 6-10 hours with a Half-life of 10-13 days, as a consequence, it accounts for the majority of the pharmacological activity observed in humans
  - While Receptos conducted safety studies of ozanimod in rodents for six months and in primates for nine months, it was observed that CC-112273 was a minor metabolite in preclinical safety species

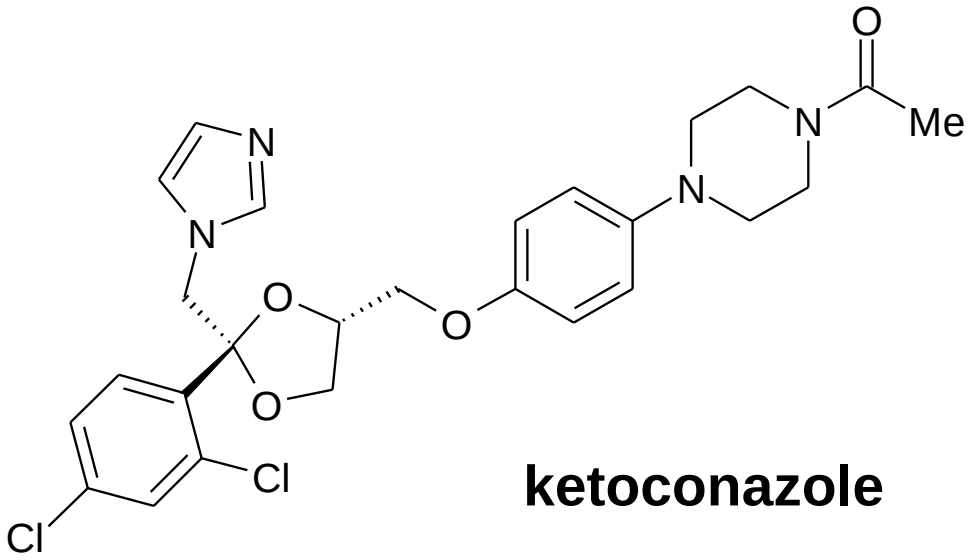


- In February 2018, a Refusal to File (RTF) letter was sent to Celgene. The Refuse-to-file communications was based around deficiencies identified in a drug application (note: the notification gives a company the chance to address the deficiencies before a "complete response letter," which amounts to an FDA rejection). In brief, the FDA found that the non-clinical and clinical pharmacology sections of its application were "insufficient to permit a complete review. The RTF notice was based on Celgene's omission of preclinical and clinical pharmacology information on the oral pill, which meant a complete review of ozanimod could not take place. Basically the NDA lacked any meaningful information regarding the characterization of disposition and tox coverage for CC112273.

Terfenadine / Ketoconazole DDI

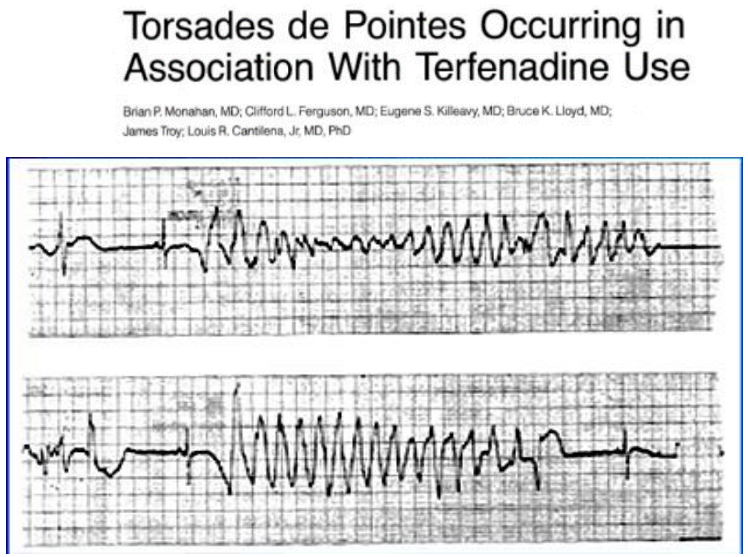


terfenadine



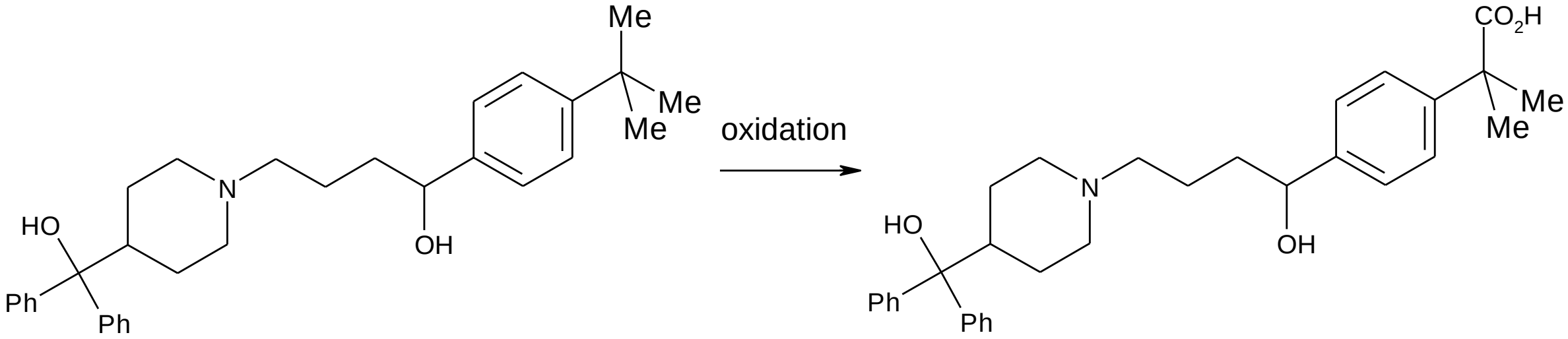
ketoconazole

- **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) via QTc prolongation.**
- **Caused by inhibition of hepatic P_{450} enzymes, specifically CYP3A4.**



Terfenadine / Ketoconazole DDI

Found that the major metabolite of terfenadine, caused by oxidation of the *tert*-butyl group, is the pharmacologically active species.



Moreover, the major metabolite, fexofenadine, has little hERG activity as it is a zwitterion, and was developed as a medicine.

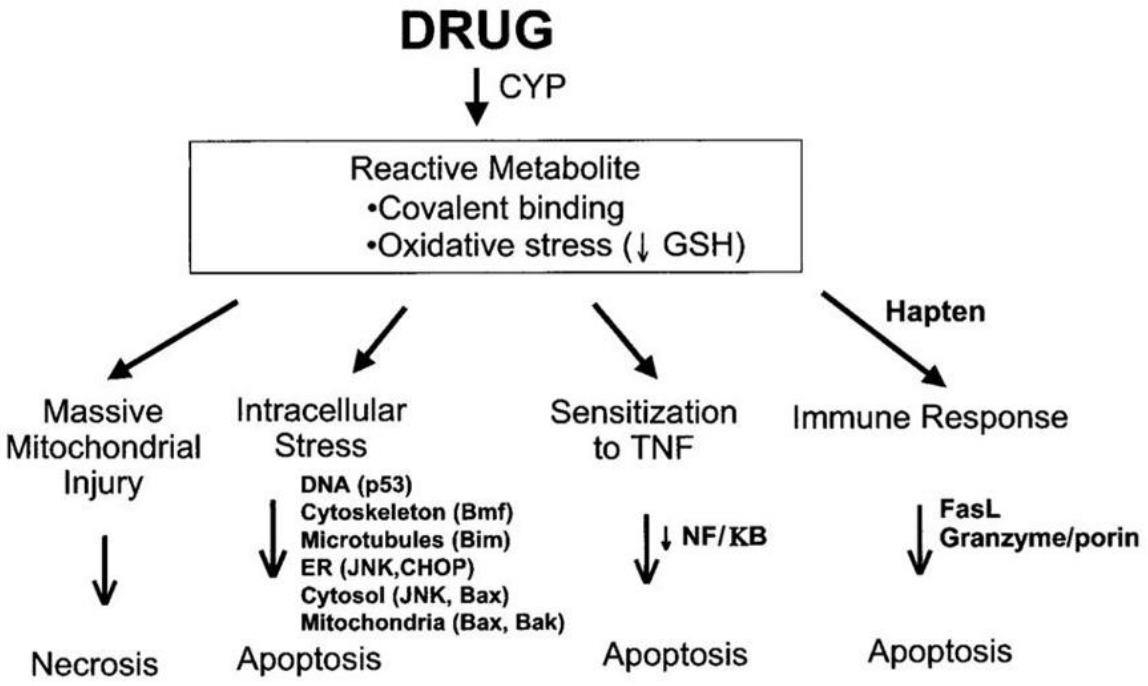
Bottom-line:

To characterize an active and/or inhibitory metabolite of significant exposure you essentially need a set of information that looks the same as the set assembled to understand the pharmacology/ADME of the parent drug.

- ✓ ***Target binding potency, as well as a readout of functional activity (i.e., antagonist, agonist, partial agonist, inhibitor, activator, etc.).***
- ✓ ***Plasma protein binding in humans and laboratory animal species.***
- ✓ ***In some cases, target tissue(s) concentrations and prediction of the free tissue–to–free plasma concentration ratio (i.e., Kp,uu).***
- ✓ ***Pharmacokinetics in humans and laboratory animal species, with some level of understanding of underlying clearance pathways for the active metabolite in humans.***

Outline for Today

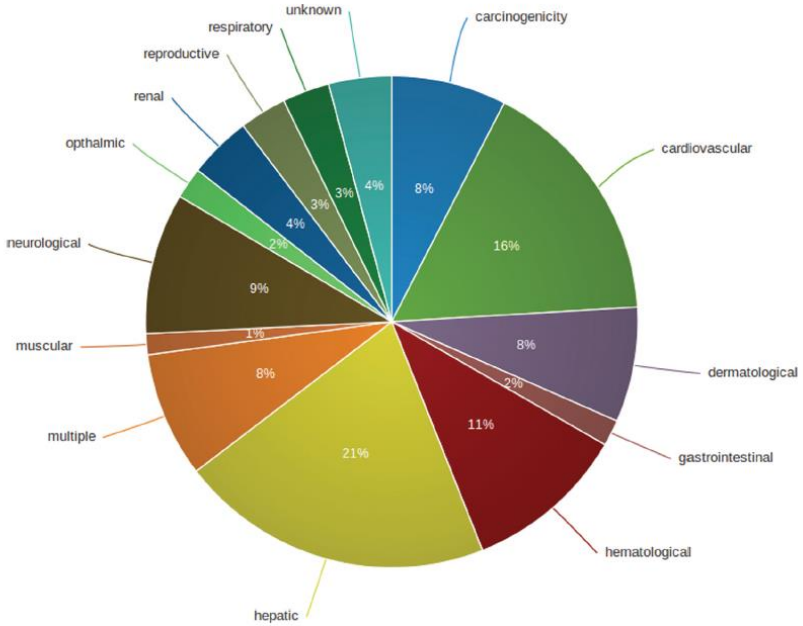
- *Background*
- *Active Drug Metabolites*
- **Metabolism-dependent Drug Safety Concerns**
  - **Liver injury (chemically reactive drug metabolites)**
  - **Species differences in drug metabolism & toxicity**
  - **FDA and ICH* "MIST" Guidance**

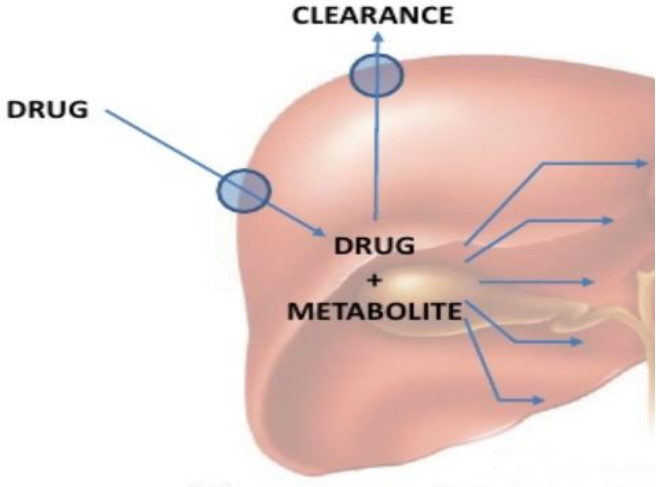


Toxicity types Associated with Drug Withdrawal or Restricted Use

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**

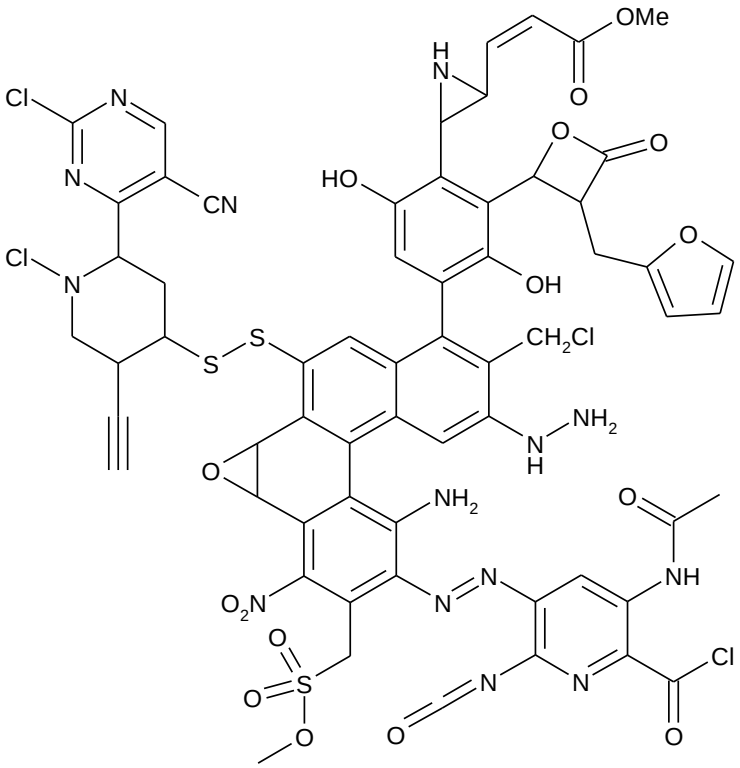




- **Hepatocellular injury, immunoallergic**: phenytoin, sulfonamides, allopurinol, halothane, diclofenac, quinolones, telithromycin,
- **Hepatocellular injury, metabolic**: INH, troglitazone, ximelagatran, bromfenac
- **Cholestatic**: estrogens, 17a androgens, chorpromazine, clavulinic acid, piroxicam
- **Bile duct injury**: carbamazepine, chorpromazine, chlorpropramide, cyproheptadine, thiabendazole, haloperidol
- **Microvescicular steatosis**: valproate, tetracycline, didanosine
- **Phospholipidosis & pseudoalcoholic hepatitis**: amiodarone, perhexiline maleate
- **Chronic autoimmune-like hepatitis**: dantrolene, methyldopa, nifurantoin, oxyphenisatin, propylthiouracil, tienilic acid
  - fibrosis
  - cholestasis

Anticipating Drug Activation & Toxicity

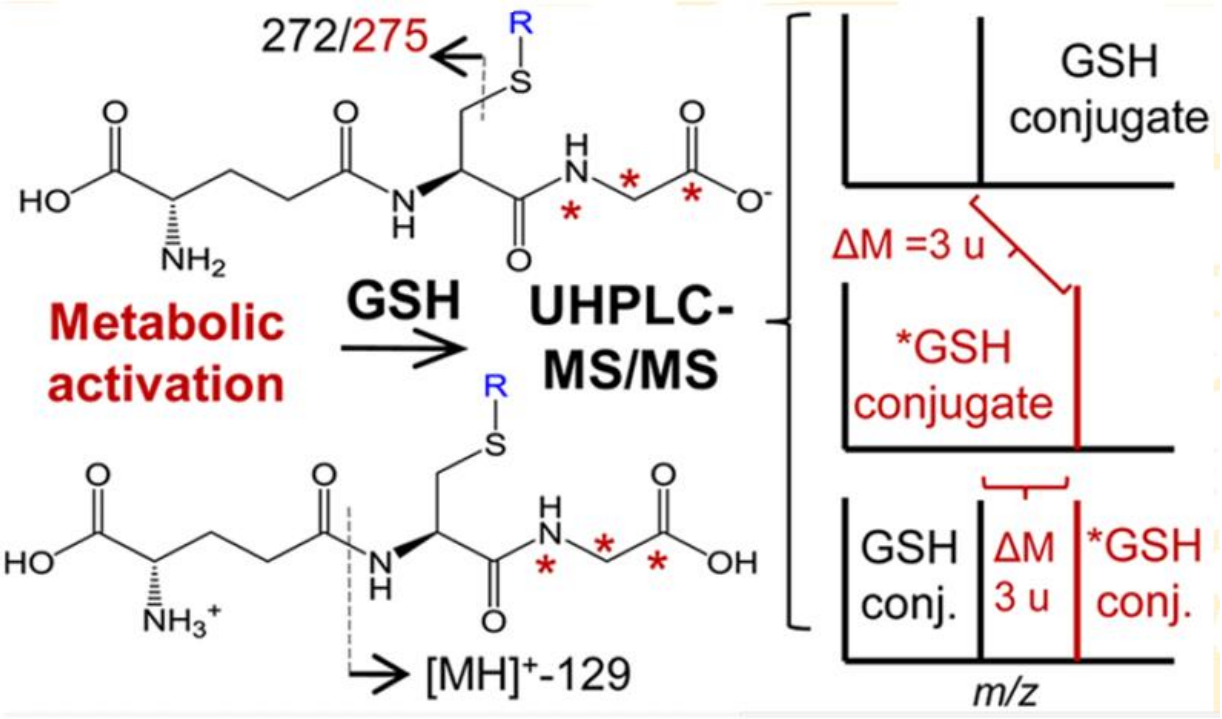
One means to minimize metabolism related toxicity is to avoid the formation of reactive metabolites is to identify functional groups that are known to form reactive metabolites and avoid these functional groups in the structure of drug candidates.



So regarding the question around whether a drug candidates that can form reactive metabolites must be totally avoided. There really is no simple answer to this question. That said, two major factors seem to be important: the dose of the drug and the fraction of the metabolic pathways that lead to covalent binding. As mentioned previously, drug that is taken at a total dose of 10 mg/day or less is unlikely to be associated with a high incidence of idiosyncratic drug reactions in humans.

Screening for Metabolic Activation via GSH Trapping

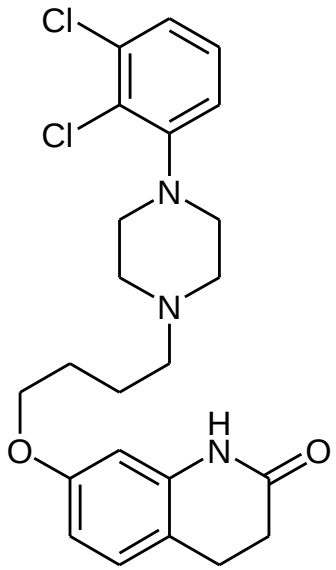
One of the methods to detect the formation of electrophilic intermediates is to look for GSH conjugates of the drug. Basically, formation of GSH conjugates can be detected by mass spectrometry, which, in turn, provides insight into the reactive metabolite structure.



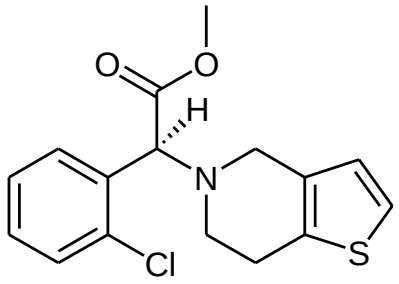
It is noteworthy to point out that not all reactive metabolites can be trapped with GSH. Hard electrophiles including DNA-reactive metabolites (e.g., electrophilic carbonyl compounds) will preferentially react with hard nucleophiles such as amines (e.g., semicarbazide and methoxylamine), amino acids (e.g., lysine), and DNA bases (e.g., guanine and cytosine) affording the corresponding Schiff bases.

Anticipating Metabolism Based Drug Toxicity

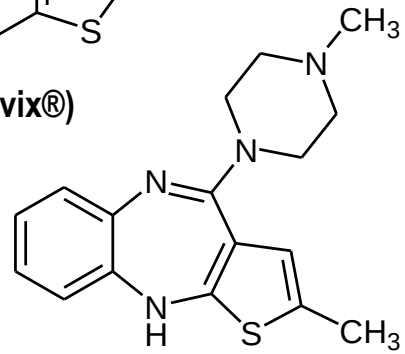
There are ways to minimize the metabolic liability associated with reactive metabolite formation: Substituting the structural alert with substituents that are resistant to metabolism; (b) Incorporating moieties which change the electron withdrawing characteristics of the structure alert; (c) Incorporating a bulky substituent close to the site of metabolism to block the site of metabolic activation.



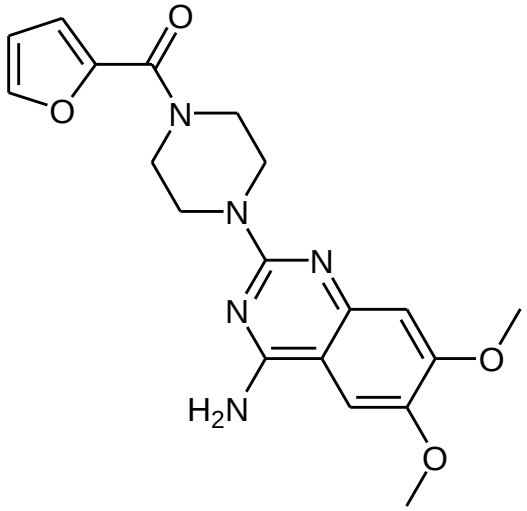
Aripiprazole (Abilify®)



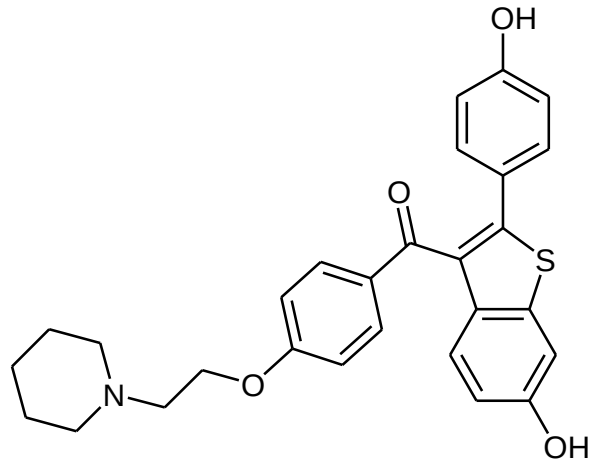
Clopidogrel (Plavix®)



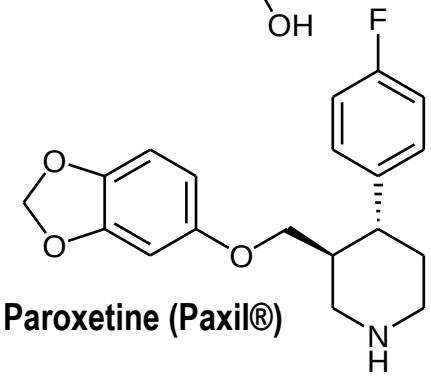
Olanzapine (Zyprexa®)



Prazosin (Minipress®)



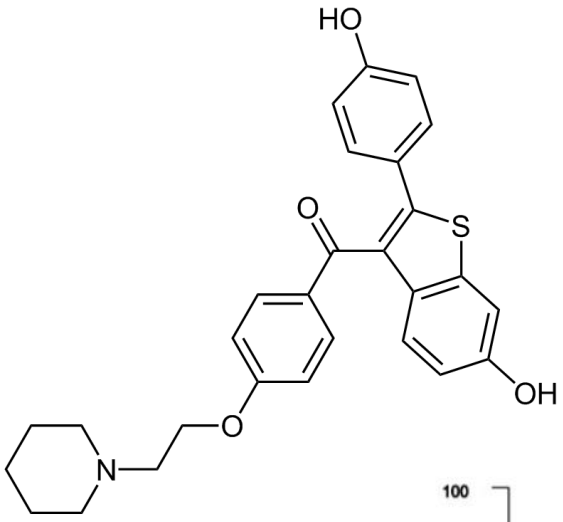
Raloxifene (Evista®)



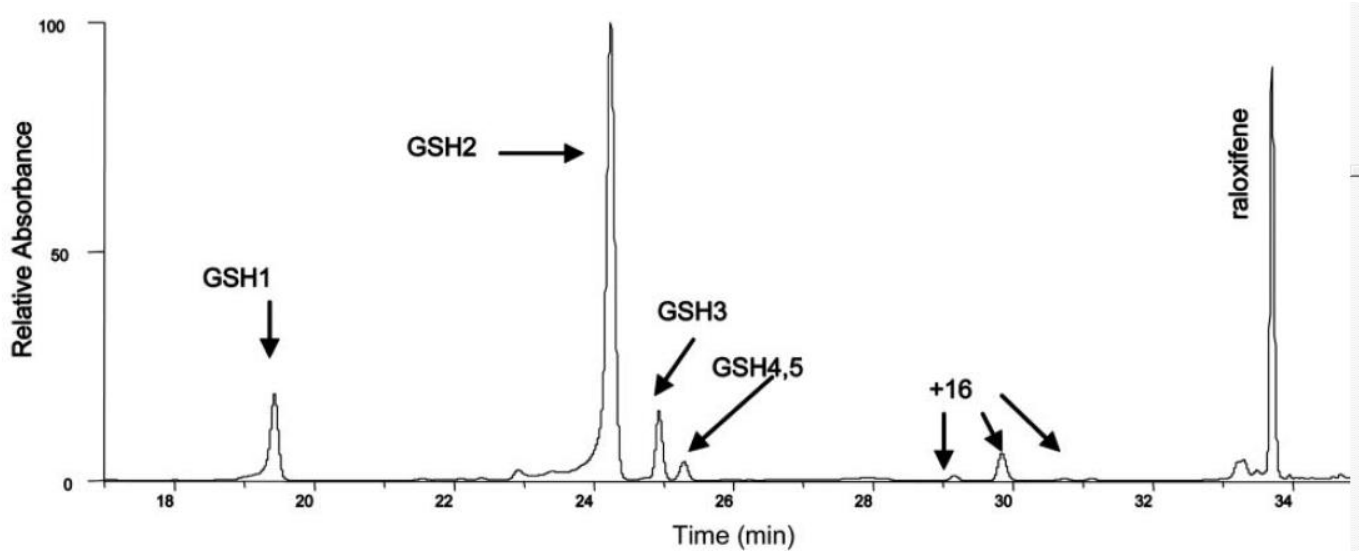
Paroxetine (Paxil®)

Of course elimination of reactive metabolite formation will be of no benefit if it also eliminates the therapeutic effects of the drug and, therefore, it is essential that the pharmacological effects of drug candidates be tested at each step in the optimization of the structure.

Raloxifene Oxidation to Reactive Intermediates

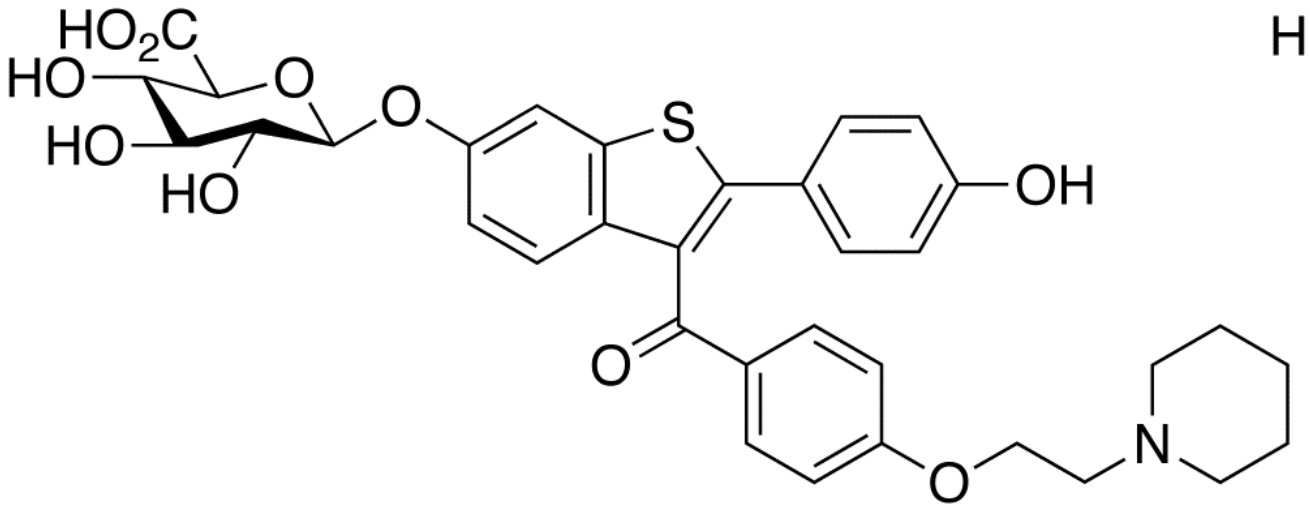


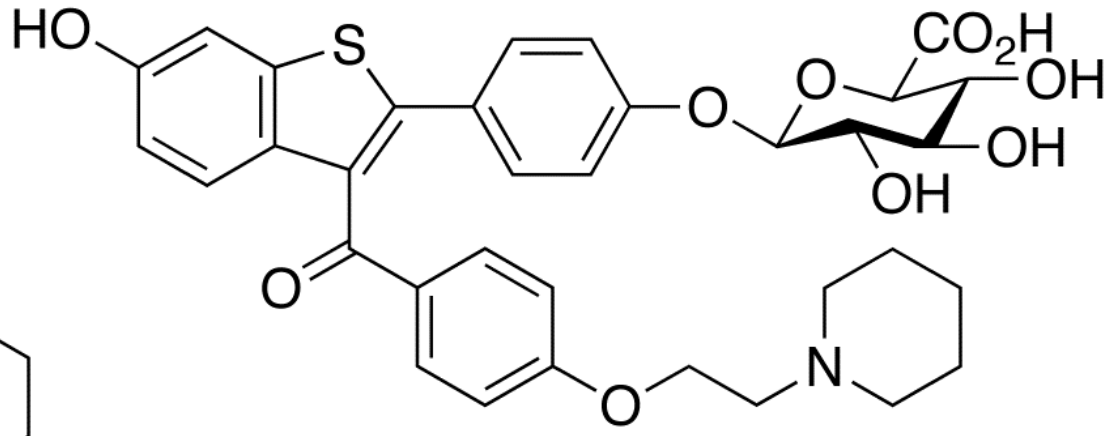
Raloxifene (Evista) is selective estrogen receptor modulator used in the treatment of osteoporosis and for chemoprevention of breast cancer. In vitro raloxifene is bioactivated to reactive intermediates, which covalently bind to proteins and form GSH conjugates upon incubation with NADPH and GSH-supplemented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



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

Raloxifene Metabolism via Glucuronidation vs Oxidation Pathways



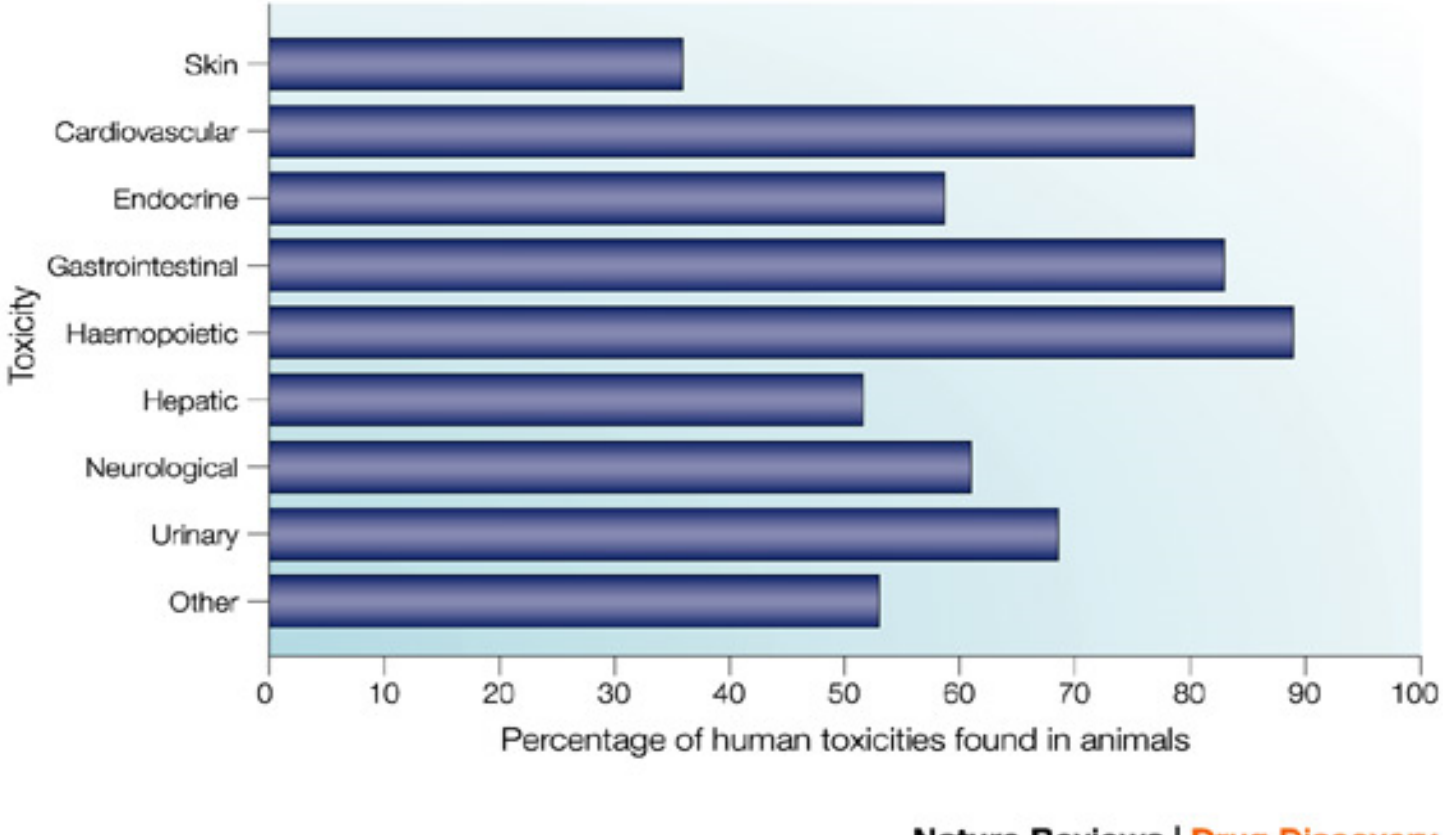


- **UGT1A1 and 1A8 were found to catalyze the formation of both the 6-beta- and 4'-beta-glucuronides.**
- **Raloxifene is rapidly absorbed from the gastrointestinal tract and undergoes extensive first-pass glucuronidation.**
  - **Approximately 60% of an oral dose is absorbed; however, because of extensive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

Rationale for Conducting Preclinical Safety Studies

Safety or secondary pharmacology studies are generally more standardized animal studies using mainly physiological monitoring of vital organs or organ systems.

Toxicology studies have been standardized by GLP guidelines that embody daily dosing of animals, general clinical examination and monitoring, and clinical pathology testing of blood and urine, followed by extensive histopathological examination of tissues after detailed NECROPSY.



- **Serves as basis for calculating a safe starting for clinical studies**
- **Provides understanding the potential for reversibility of toxic effects**

Bottom-line Question: “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

The new FDA guidance published in 2016 states that the "discovery of disproportionate drug metabolites late in drug development can potentially cause development and marketing delays" and that "human metabolites that can raise a safety concern are those formed at greater than 10 percent of total drug-related exposure at steady state."

Possible Decision Tree for Drug Metabolites

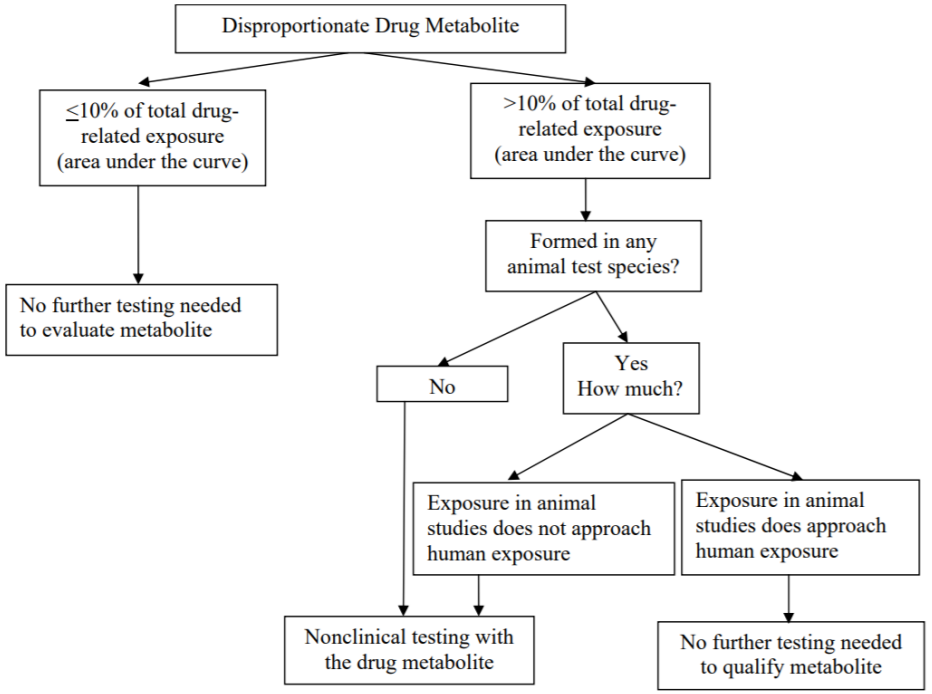
Preliminary studies on circulating human metabolites need to be conducted during early clinical development (Phase I / II) such that "disproportionate" metabolites can be identified and addressed preclinically.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

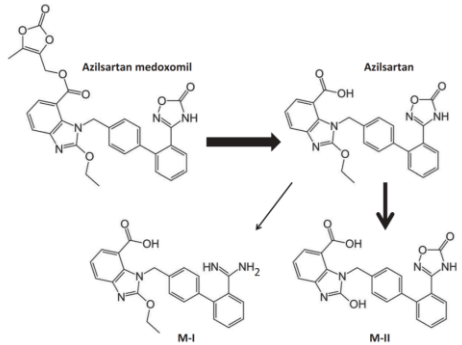
November 2016
Pharmacology/Toxicology

Revision 1

APPENDIX A:
DECISION TREE FLOW DIAGRAM



It does happen: the prodrug azilsartan medoxomil (Edarbi®) was approved for treatment of hypertension. Azilsartan is metabolized to two primary metabolites. The major metabolite in plasma (M-II) is formed by O-dealkylation primarily via CYP2C9 and the minor metabolite (M-I) is formed by decarboxylation primarily via CYP2C8. Systemic exposures to these metabolites in humans were approximately 50% and respectively.

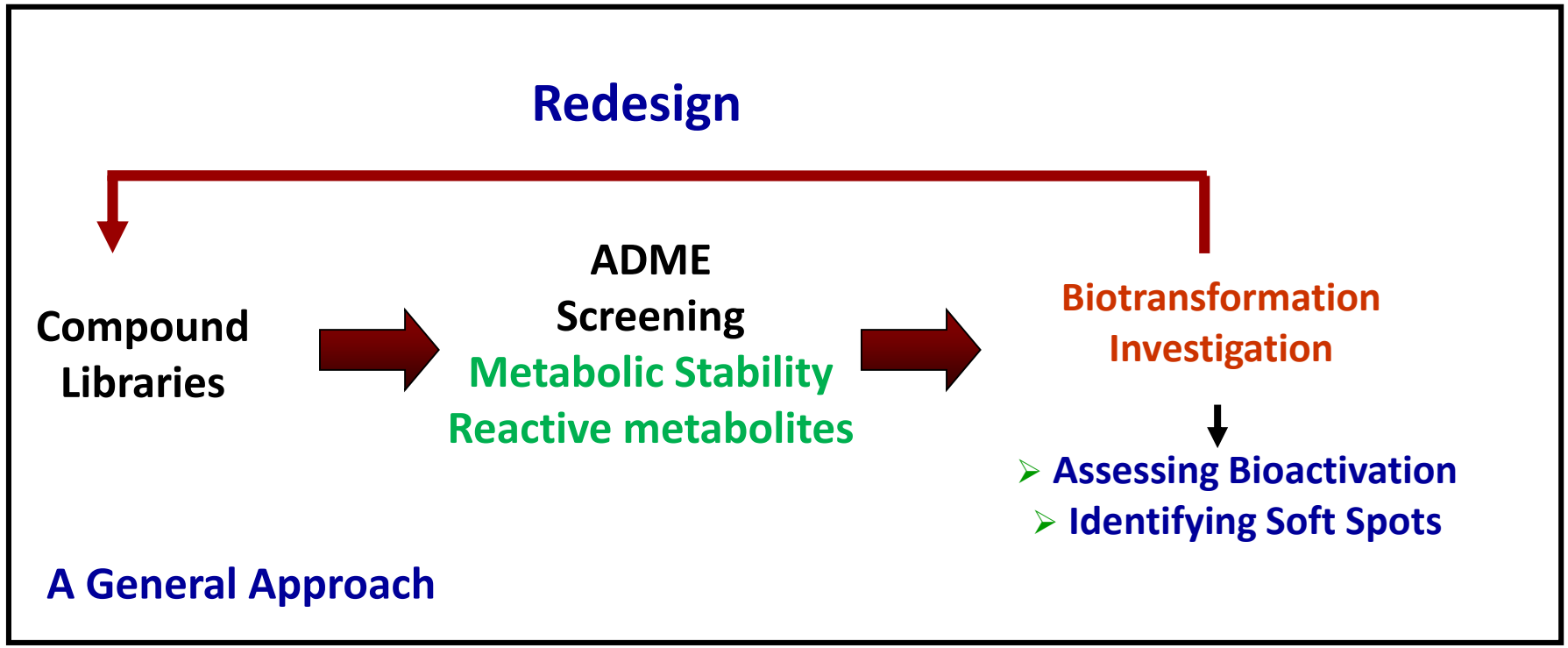


M-I and M-II do not contribute to the pharmacologic activity of the drug. Metabolite M-II was studied in 13-week rat and dog repeat-dose toxicity studies and in reproduction/developmental studies. H

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. Clearly, adopting a proactive approach to the demonstration of safety coverage of human metabolites is the key to enabling a smooth regulatory path to US submission and approval.

Metabolism and Lead Optimization

- Drug metabolism plays a defining role in safety and efficacy
- Optimizing metabolism has become a routine in drug discovery
  - Addresses drug metabolism issues early on



The objective is to attenuate CYP-mediated clearance

ADME Properties and the Expected Effects of Increasing MW or clogP

30,000 compounds were analyzed based upon physicochemical properties and their impact on in vitro ADME

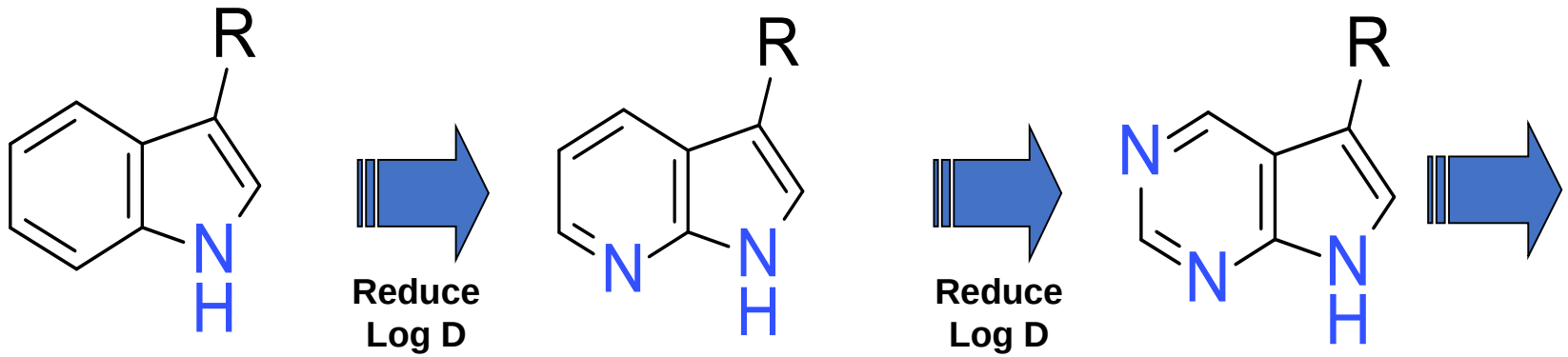
Assay	Neutral Molecules		Basic Molecules		Acidic Molecules	
	MW < 400 and clogP < 4	MW > 400 and clogP > 4	MW < 400 and clogP < 4	MW > 400 and clogP > 4	MW < 400 and clogP < 4	MW > 400 and clogP > 4
Solubility	Average	Lower	Higher	Lower	Higher	Average
Permeability	Higher	Average	Higher	Average	Lower	Average
Plasma Protein Binding	Average	Higher	Lower	Average	Average	Higher
P-gp Efflux	Average	Higher	Average	Higher	Lower	Lower
Clearance	Average	Average	Average	Higher	Lower	Average
hERG Inhibition	Lower	Lower	Average	Higher	Lower	Lower
P450 Inhibition	Lower	Higher CYP3A	Average	Higher CYP2D6 & CYP3A4	Lower	Average
		Lower CYP1A2	Higher CYP2D6			Higher CYP2C9

Gleeson, M.P. *J Med Chem* **51** (2008) 817-834

Depending on the program needs will determine whether a property should be "built in" or "built out"

Reducing CYP-mediated Clearance has helped Improve PK but has also Introduced New Problems

- **Strategy used:**
  - **Reduce the Log P of new candidates**
  - ✓ **Introduce azaheterocycles in the molecule**

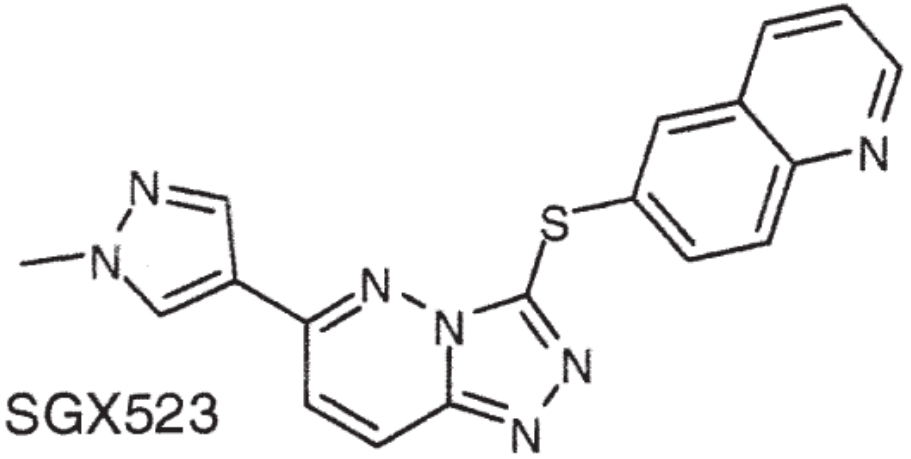


Addition of "N" Minimizes P450-mediated metabolism

- **Enables advancement of compounds with minimal P450 metabolism**
- **Has steered new compounds into non-CYP space for clearance**

Selecting the Most Relevant Preclinical Species for 30 day Toxi Studies

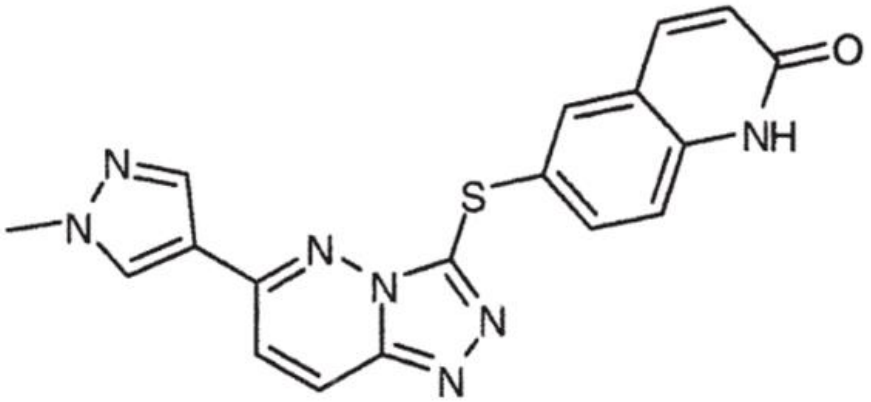
Background: (SGX523) was an orally bioavailable, potent, and selective small molecule inhibitor of c-MET, and was one of the first selective c-MET inhibitors to be evaluated in patients. Because the microsomal metabolism profile of SGX523 was similar among preclinical species and human, investigational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



SGX523

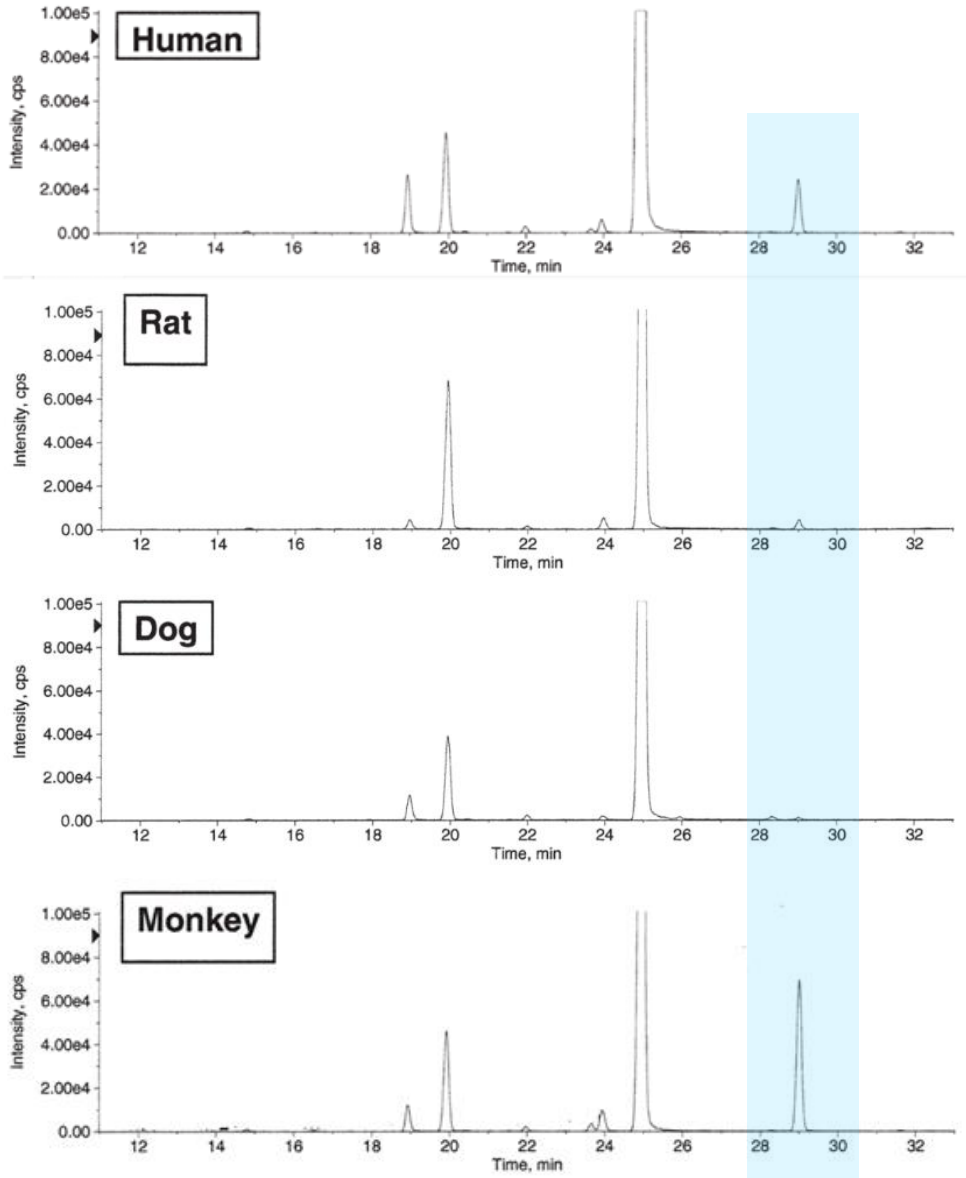
Problem: The SGX523 Phase I study was started at a dose of 40 mg in patients. After escalating to doses 80 mg of SGX523 in patients, acute renal failure was observed as evidenced by increased serum creatinine. The analysis of samples from the discontinued clinical trial revealed a metabolism profile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

Selecting the Best Preclinical Species for Toxicological Evaluation



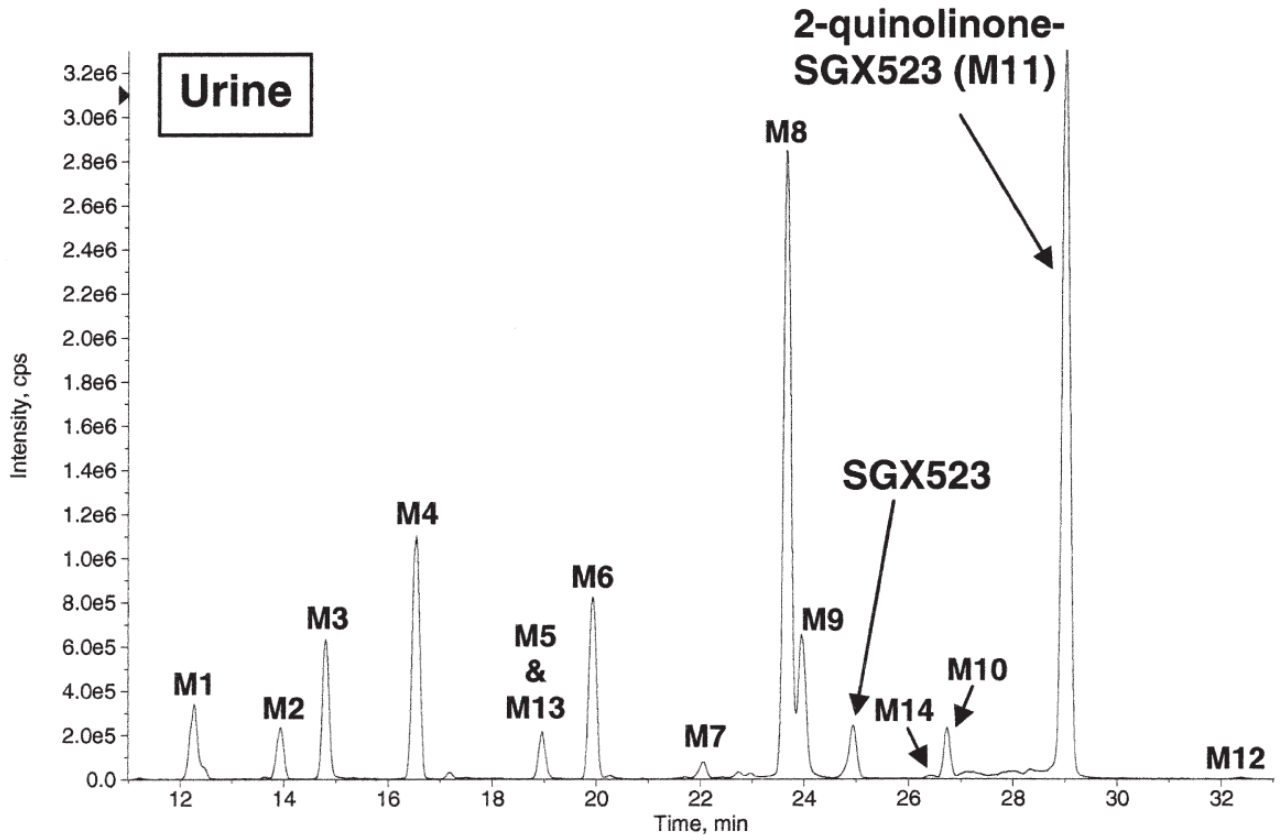
M11 (2-quinolinone-SGX523)

The selection of an appropriate safety species for a molecule which is a substrate of AO, is difficult as the relative order of AO activity in animal species relative to human is highly dependent on the substrate. That said the general dogma regarding species differences is that AO activity is high in monkeys and humans, low in rats and deficient in dogs, such that dog is not an appropriate species to evaluate the safety of an AO-derived metabolite.



Selecting the Correct Preclinical 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, M11 which was likely involved in the observed obstructive nephropathy reported in clinical studies.

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. So in all cases features which we need to keep as part of the project conversation include:

- **Is the drug intended to address a previously unmet medical need or a life threatening disease?**
- **Is the drug candidate intended to provide proof-of-mechanism for a novel target (first in class)?**
- **Is the drug intended for acute or chronic use?**
- **Is the clinical dose predicted to be low?**
- **What is the intended patient population (e.g., would it be given to immunecomprised patients or patients with impaired liver functions)?**
- **Are there alternate chemical series with comparable pharmacologic and pharmacokinetic attributes, wherein bioactivation liability is minimized or eliminated?**
- **Is there an alternative (higher affinity but innocuous) route of metabolism within the drug candidate that minimizes bioactivation liability associated with the compound?**
- **Is metabolism the exclusive route of elimination? What is the likelihood of nonmetabolic elimination processes (e.g., renal and/or biliary excretion of unchanged parent) in humans?**

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

Questions?