Advanced Drug Metabolism

MEDCH 527

Winter Quarter 2013

"Safety Considerations in Drug Development"

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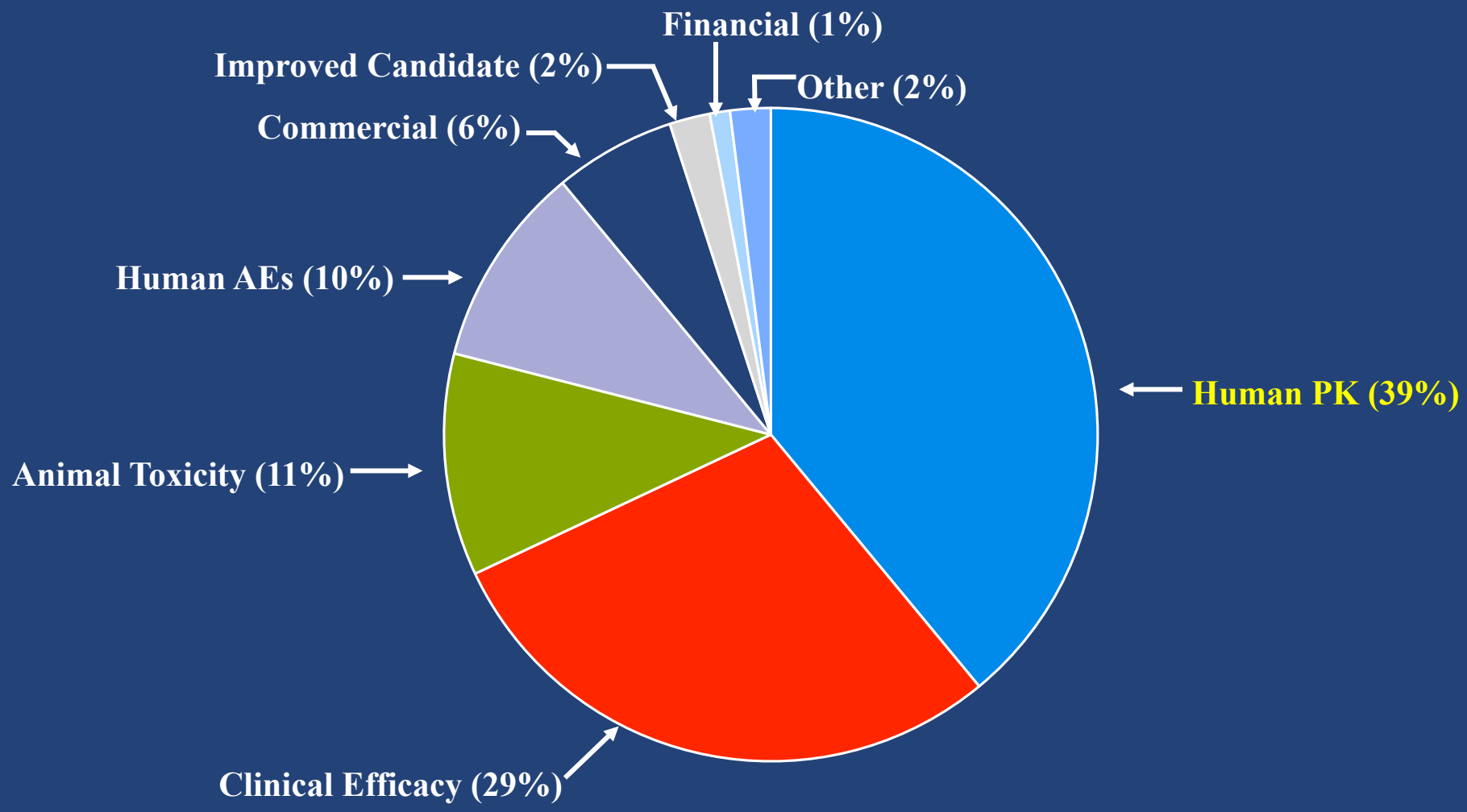
Outline

- *Background*
  - Toxicity as a major source of attrition in drug development
  - Toxicity-related drug withdrawals
  - Toxicity studies in drug development
- *Metabolism-dependent drug toxicities*
  - Cardiovascular toxicity (QT_c effects) caused by drug interactions
  - Liver injury (chemically reactive drug metabolites)
- *Stable drug metabolites ("MIST")*
  - FDA and ICH* Guidances
  - Species differences in drug metabolism & toxicity

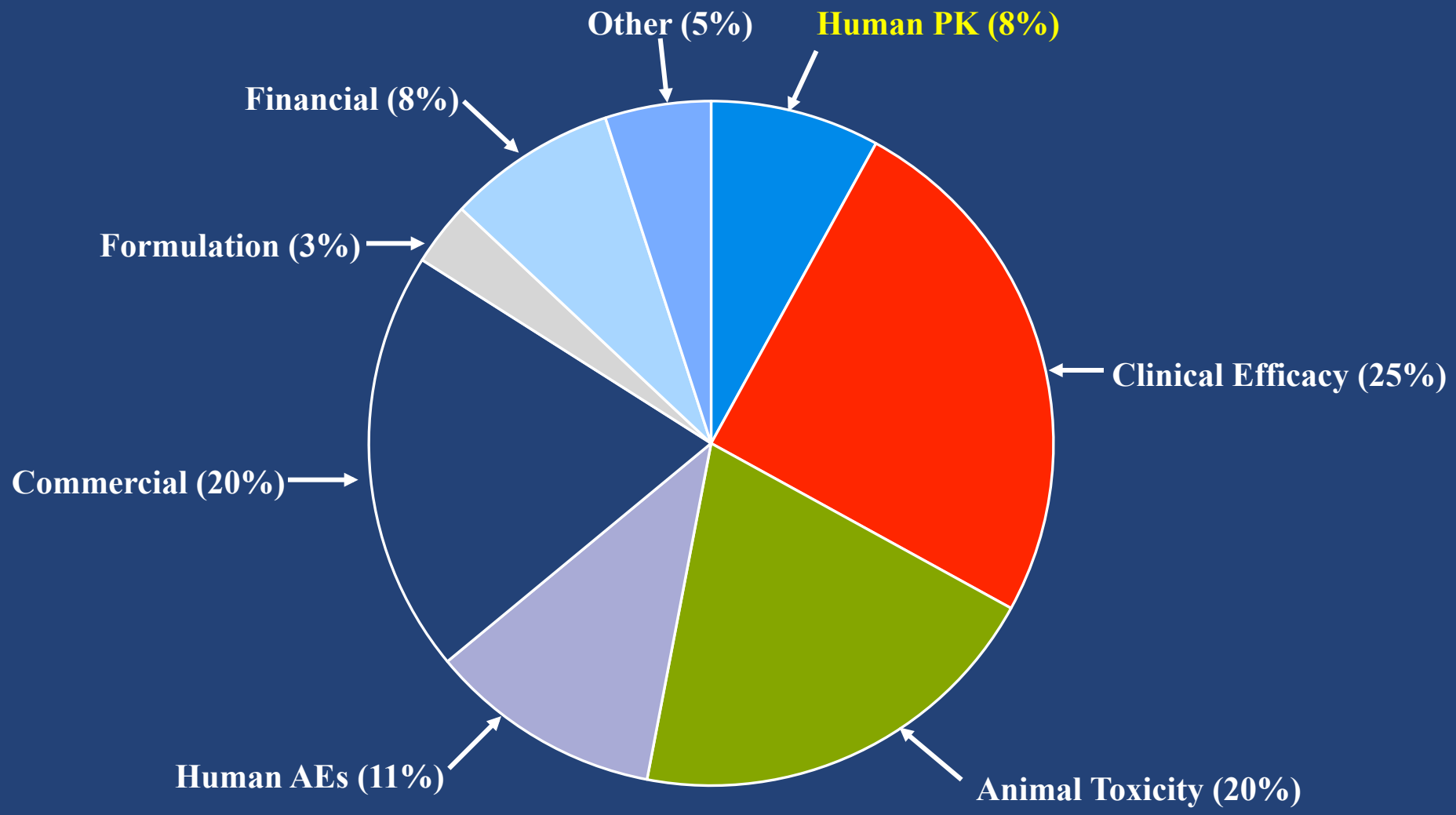
*ICH = International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use

Reasons for Termination of Drug Candidates in Development (1964 - 1985)



R.A. Prentis, Y. Lis and S.R. Walker, *Br. J. Clin. Pharmacol.*, 25, 387 (1988)

Reasons for Termination of Drug Candidates in Development (2000)



I. Kola and J. Landis, *Nature Reviews / Drug Discovery*, 3, 711-715 (2004)

Target Organ Contributions to Drug Withdrawals

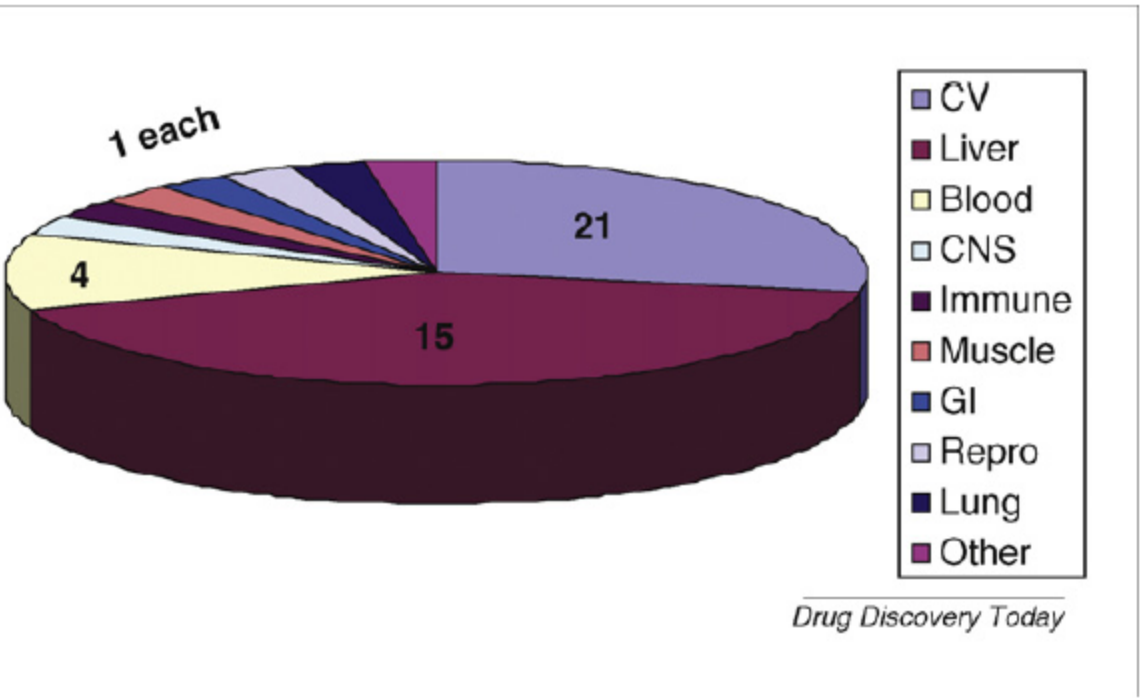


FIGURE 1

Summary of target organ contributions to drug withdrawals 1975–2007. Data were compiled from reviews [8–10] and CDER Reports to the Nation [11] as well as the CDER website. Target organs were identified on the basis of the reasons for withdrawal as noted in the references.

J. L. Stevens and T. K. Baker, *Drug Discovery Today,* **14**, 162-167 (2008)

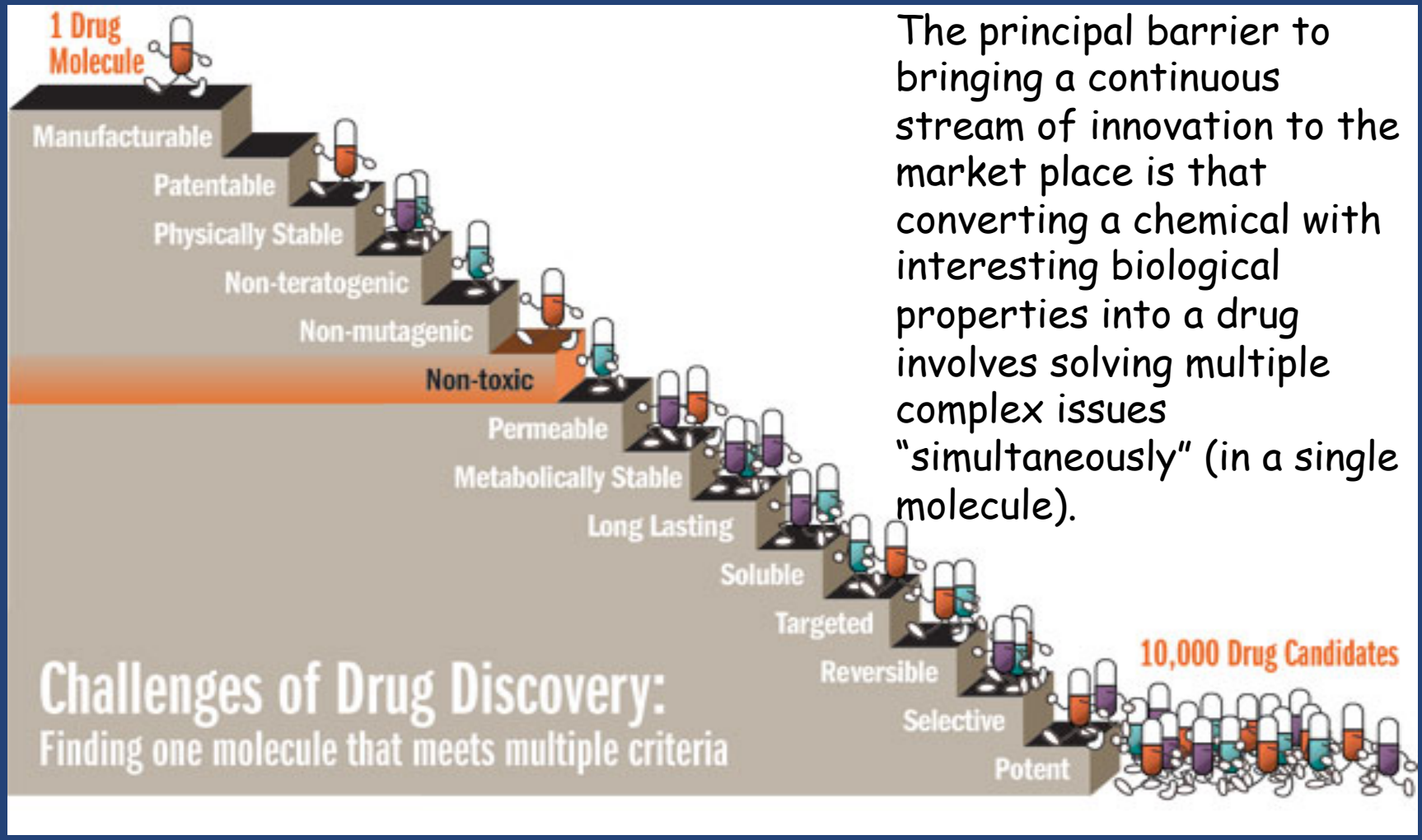
Widely Accepted That Attrition Must Occur Earlier!

- 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)
  - "Dialing-out" drug-drug interaction potential
  - Dealing prospectively with reactive drug metabolite issues
  - Addressing potential issues with stable drug metabolites ("MIST")

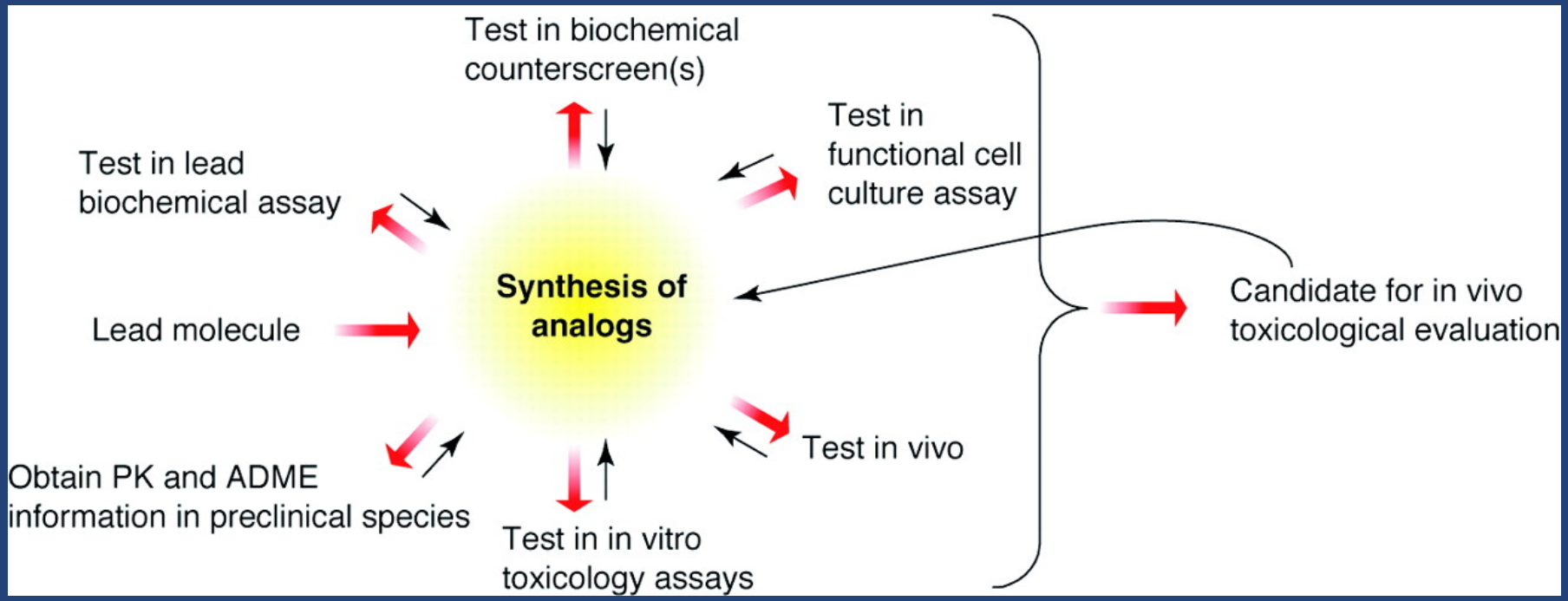
The Pharmaceutical Industry Challenge is: Solving Multiple Issues Simultaneously



The principal barrier to bringing a continuous stream of innovation to the market place is that converting a chemical with interesting biological properties into a drug involves solving multiple complex issues "simultaneously" (in a single molecule).

Figure modified from: *Drug Discovery and Development,* July 2004

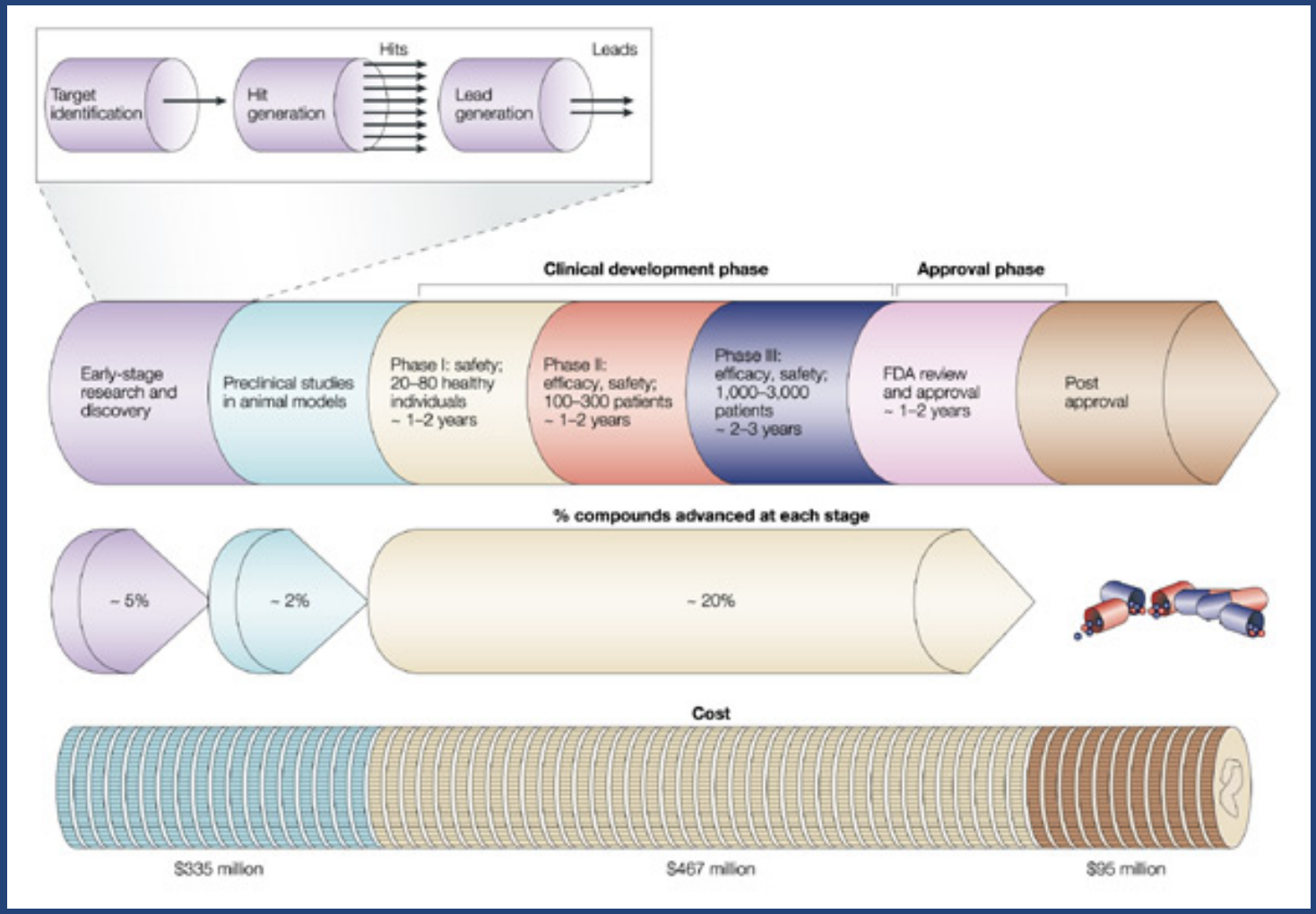
From Sequential to Parallel Data Acquisition
A Paradigm Shift in Drug Discovery



ADME = Absorption, Distribution, Metabolism, and Excretion

M. MacCoss and T. A. Baillie, *Science,* **303**, 1810-1813 (2004)

The Drug Discovery / Development Pipeline



Preclinical Toxicity Studies in Support of Drug Development

- Mutagenicity - Ames, chromosomal aberration
- hERG Binding - indicative of QTc prolongation
- General Toxicology - rodent (rat or mouse) and non-rodent (dog or monkey)
  - Single doses to evaluate effects of high acute exposure (significant tox or lethality) and maximum tolerated dose (MTD)
  - Multiple doses at sub-lethal levels to assess sub-acute tox profile and define “no adverse effect level” (NOAEL)
  - >2-Week multiple dose data needed to support clinical trials up to 2 weeks in duration
  - *In vivo* studies incorporate toxicokinetic (TK) assays to assess exposure (C_{max} and AUC) to parent (and abundant circulating metabolites)

http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/default.htm

Preclinical Toxicity Studies in Support of Drug Development (Cont'd)

- Safety Pharmacology
  - *In vitro:* panel of receptor binding assays
  - *In vivo:* battery of studies to assess effects on CV, CNS, GI, and respiratory systems
- Reproductive Toxicity
  - Needed prior to enrollment of women of child-bearing potential
- Carcinogenicity
  - 2-Year bioassay in rats and mice (starts ~4 years prior to anticipated filing date)

http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/default.htm

Clinical Safety Studies in Support of Drug Development

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

Phase II and III (patients)

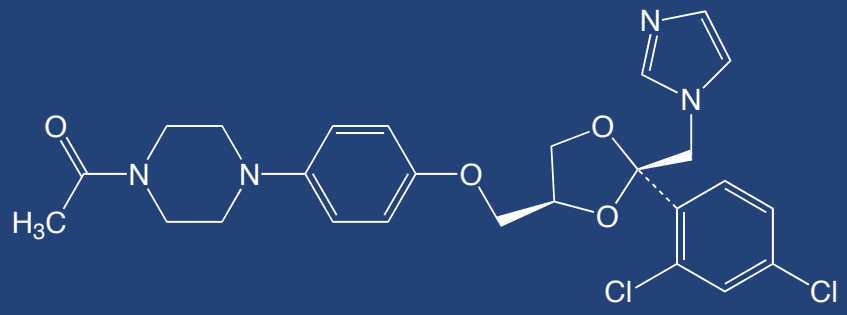
- Long-term safety and efficacy studies that form the basis of regulatory filing (NDA)

Postmarketing

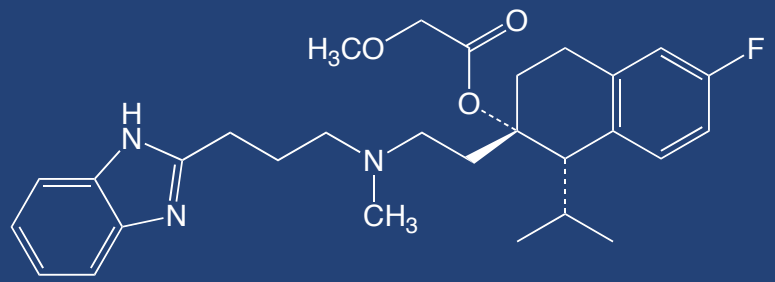
- Pharmacovigilence (adverse event reporting)

Toxicity Due to Drug-Drug Interactions at the Level of P450 Inhibition

P450 Inhibitors that Have Caused Life-Threatening DDIs



Ketoconazole
Competitive IC_{50} <0.1μM for CYP3A
Warnings for serious DDIs



Mibefradil
Withdrawn in 1998 due to DDIs (QTc prolongation)
Competitive IC_{50} for CYP3A <1μM
Mechanism-based CYP3A4/5 inhibitor (partition ratio 1.7)

http://www.fda.gov/cder/drug/druginteractions

T. D. Bjornsson *et al., J. Clin. Pharmacol.,* **43**, 443-469 (2003)

T. Prueksaritanont *et al., Br. J. Clin. Pharmacol.,* **47**, 291-298 (1999)

Metabolism by P450 Enzymes – A Key Determinant of Drug Clearance

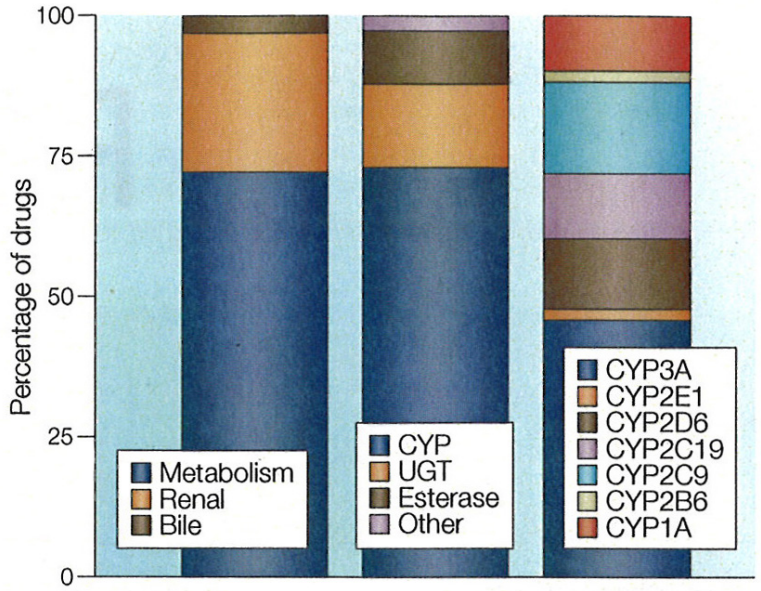
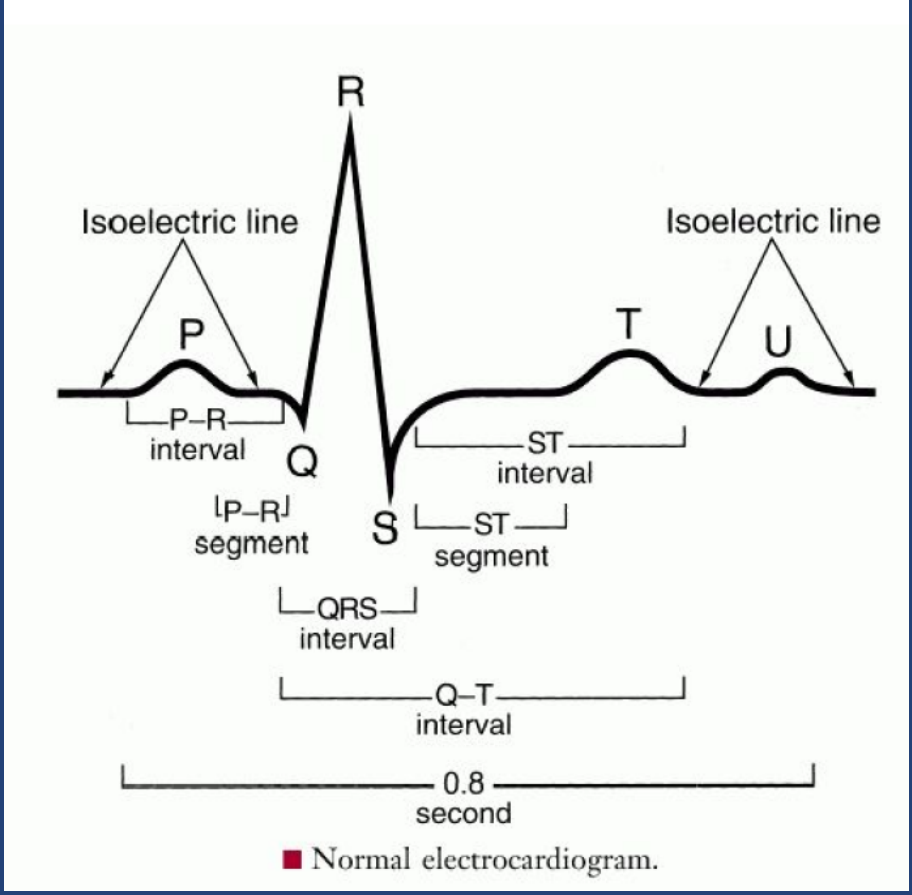


Figure 1 | **Routes of elimination of the top 200 most prescribed drugs in 2002.** Metabolism represents the listed clearance mechanism for ~73% of the top 200 drugs. Of the drugs cleared via metabolism, about three-quarters are metabolized by members of the cytochrome P450 (CYP) superfamily. For the CYP-mediated clearance mechanisms, the majority of drug oxidations (46%) were carried out by members of the CYP3A family; followed by 16% by CYP2C9; 12% for both CYP2C19 and CYP2D6; 9% for members of the CYP1A family; and 2% for both CYP2B6 and CYP2E1 (REF. 9). UGT, uridine diphosphate glucuronyl transferase.

L. C. Wienkers and T. G. Heath, *Nat. Rev. Drug Discov.*, **4**, 825-833 (2005)

Cardiovascular Toxicity: Drug-Drug Interactions Leading to QTc Prolongation



■ Normal electrocardiogram.

Inhibition of I_{Kr} channel due to drug binding to hERG (α-subunit) can produce delayed ventricular polarization (QTc interval prolongation)

In extreme cases, QTc prolongation leads to life-threatening ventricular fibrillation (Torsades de Pointes)

hERG Binding and QTc Prolongation

- Adverse cardiovascular effects, often associated with inhibition of hERG (α-subunit of the I_{Kr} potassium channel) leading to QTc prolongation, represent a major source of attrition in drug discovery

- 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)

- While the parent compound normally is the culprit, drug-drug interactions (DDIs) that result in ***reduced drug clearance*** may greatly exacerbate this type of toxicity

- *Potent inhibitors of CYP3A4* have been most frequently implicated in these DDIs, e.g. ketoconazole (warning label), mibefradil (withdrawn)

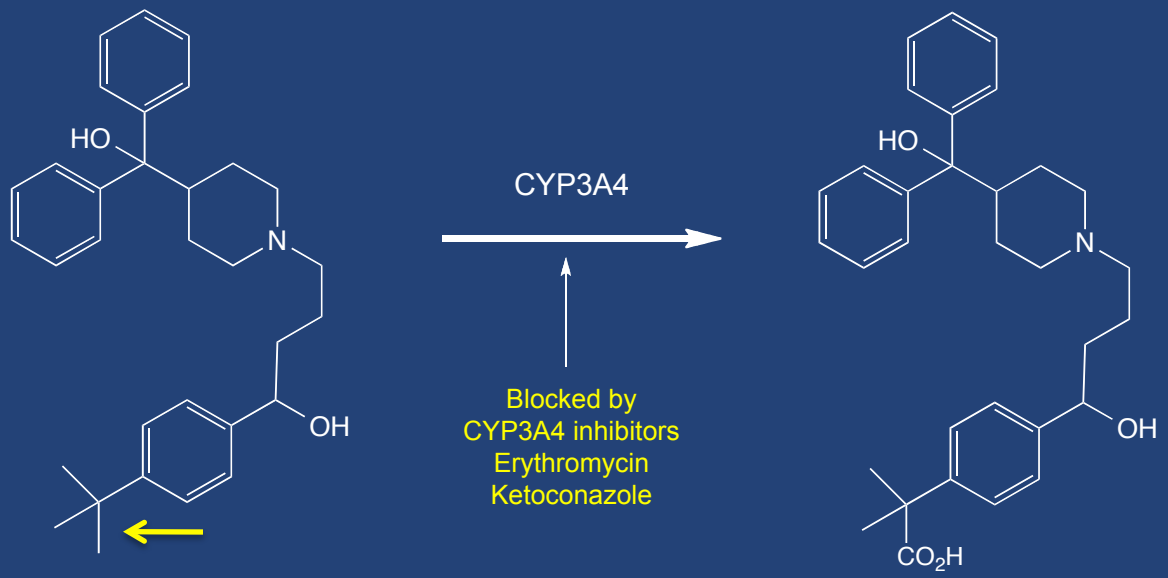
Torsades de Pointes Occurring in Association With Terfenadine Use

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

Torsades de pointes is a form of polymorphic ventricular tachycardia that is associated with prolongation of the QT interval. Although found in many clinical settings, torsades de pointes is most often drug induced. This report describes the first association (exclusive of drug overdose) of symptomatic torsades de pointes occurring with the use of terfenadine in a patient who was taking the recommended prescribed dose of this drug in addition to cefaclor, ketoconazole, and medroxyprogesterone. Measured serum concentrations of terfenadine and its main metabolite showed excessive levels of parent terfenadine and proportionately reduced concentrations of metabolite, suggesting inhibition of terfenadine metabolism. We believe that a drug interaction between terfenadine and ketoconazole resulted in the elevated terfenadine levels in plasma and in the cardiotoxicity previously seen only in cases of terfenadine overdose.

(*JAMA*. 1990;264:2788-2790)

B. P. Monahan *et al., J. Amer. Med. Assoc.,* **264**, 2788-2790 (1990)



Terfenadine
IC_{50} H1 = 1 nM
IC_{50} hERG = 56 nM

Fexofenadine
IC_{50} H1 = 15 nM
IC_{50} hERG = 23000 nM

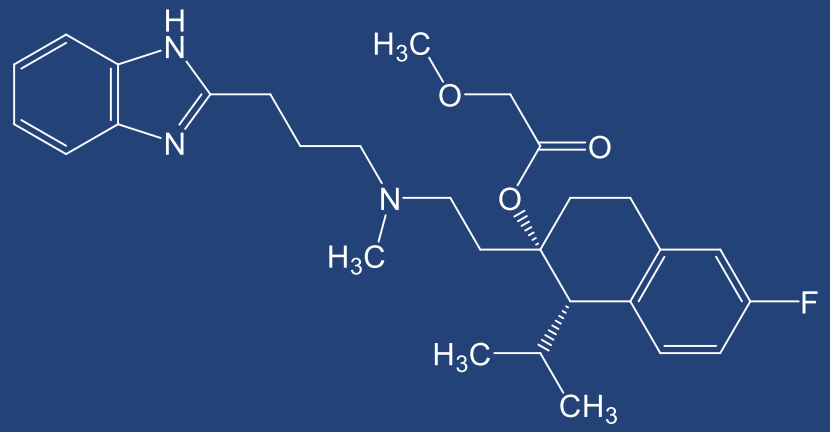
Terfenadine:

- Potent inhibitor of hERG channel
- CYP3A4 inhibitors raise terfenadine levels and cause QTc prolongation
- Introduced as *Seldane* (Marion Merrell Dow) in 1985, withdrawn in 1997

Fexofenadine:

- An active metabolite of terfenadine
- Contributes to H1 antagonism, but not to hERG inhibition
- Not subject to metabolism by CYP3A4 inhibitors (minimizes DDI potential)
- Marketed as *Allegra* (Aventis) in 1996

Paying Attention to Drug-P450 Interaction Potential
Mibefradil (Posicor®)



Mibefradil - Calcium channel blocker, approved as an antihypertensive in 1997

- Exhibited non-linear PK due to self-inactivation of CYP3A4
- Highly potent CYP3A4 inactivator *in vitro* (k_{inact} = 0.5 min^{-1}, K_I = 2 μM)
- Caused serious clinical AEs when dosed with CYP3A4 substrates (eg simvastatin, atorvastatin)
- Fatal cases of DDIs leading to rhabdomyolysis (statins) and QTc prolongation
- Voluntarily withdrawn by the manufacturer in 1998

T. Prueksaritanont *et al., Br. J. Clin. Pharmacol.,* **47**, 291-298 (1999)

hERG Binding – Current Status

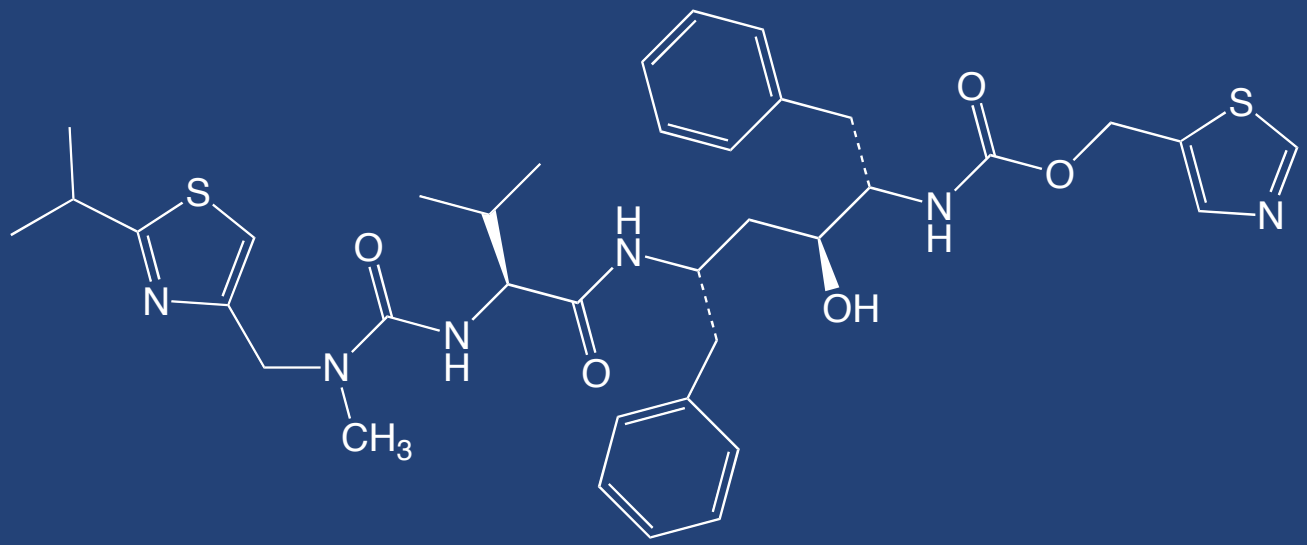
- *In vitro* and *in vivo* screens for CV effects established
- Regulatory guidance for nonclinical (ICH S7B) and clinical (ICH E14) testing strategies published in 2005
- Safety margin based on ratio of:

  (hERG IC_{50} / C_{max}) or (NOAEL / C_{max})

  at expected top dose should be >30, and preferably >100
- Med Chem strategies to minimize QTc effects include:
  - Formation of zwitterions (e.g. terfenedine to fexofenadine)
  - Modulation of LogP
  - Attenuation of pKa
  - Computational (QSAR models)

Curr. Topics Med Chem., **8**(13), 2008

A P450 Inhibitor that is Therapeutically Valuable



Ritonavir (HIV Protease Inhibitor)

Potent mechanism-based CYP3A4 inhibitor
Mechanism of CYP inhibition likely associated with thiazole ring(s)
Used clinically to "boost" exposure of other anti-HIV agents

Other HIV protease inhibitors that lack a thiazole functionality do not serve as mechanism-based inhibitors of CYP3A4

T. Koudriakova *et al., Drug Metab. Dispos.*, **26**, 552-561 (1998)

Drug-Induced Liver Toxicity

Drug-Induced Liver Toxicity

Several forms, including:

- Predictable, dose-dependent toxicities (animal model, clear dose-response relationship, etc)
- "Idiosyncratic" toxicities (rare, not predictable, no animal model)
- Occur only after prolonged dosing (carcinogenicity, teratology)

- *Evidence suggests that, in some cases, reactive metabolites may play a causative role in each of the above forms of liver toxicity*
- *Idiosyncratic toxicities of greatest concern in drug development*

B. K. Park, M. Pirmohamed, and N. R. Ketteringham, *Chem. Res. Toxicol.,* **11**, 969-988 (1998)
D. A. Smith and R. S. Obach, *Chem. Res. Toxicol.,* **22**, 267-279 (2009)
J. Uetrecht, *Annu. Rev. Pharmacol. Toxicol.,* **47**, 513-539 (2007), and *Chem. Res. Toxicol.,* **21**, 84-92 (2008)

Bioactivation and Liver Toxicity

- A wide range of therapeutic agents have been withdrawn from use due to an unacceptably high incidence of hepatotoxicity:

  Aclofenac, alpidem, amodiaquine, amineptine, benoxaprofen, bromfenac, ibufenac, iproniazid, nefazodone, nomifensine, sudoxicam, tienilic acid, tolrestat, troglitazone, trovafloxacin, zileuton, zomepirac

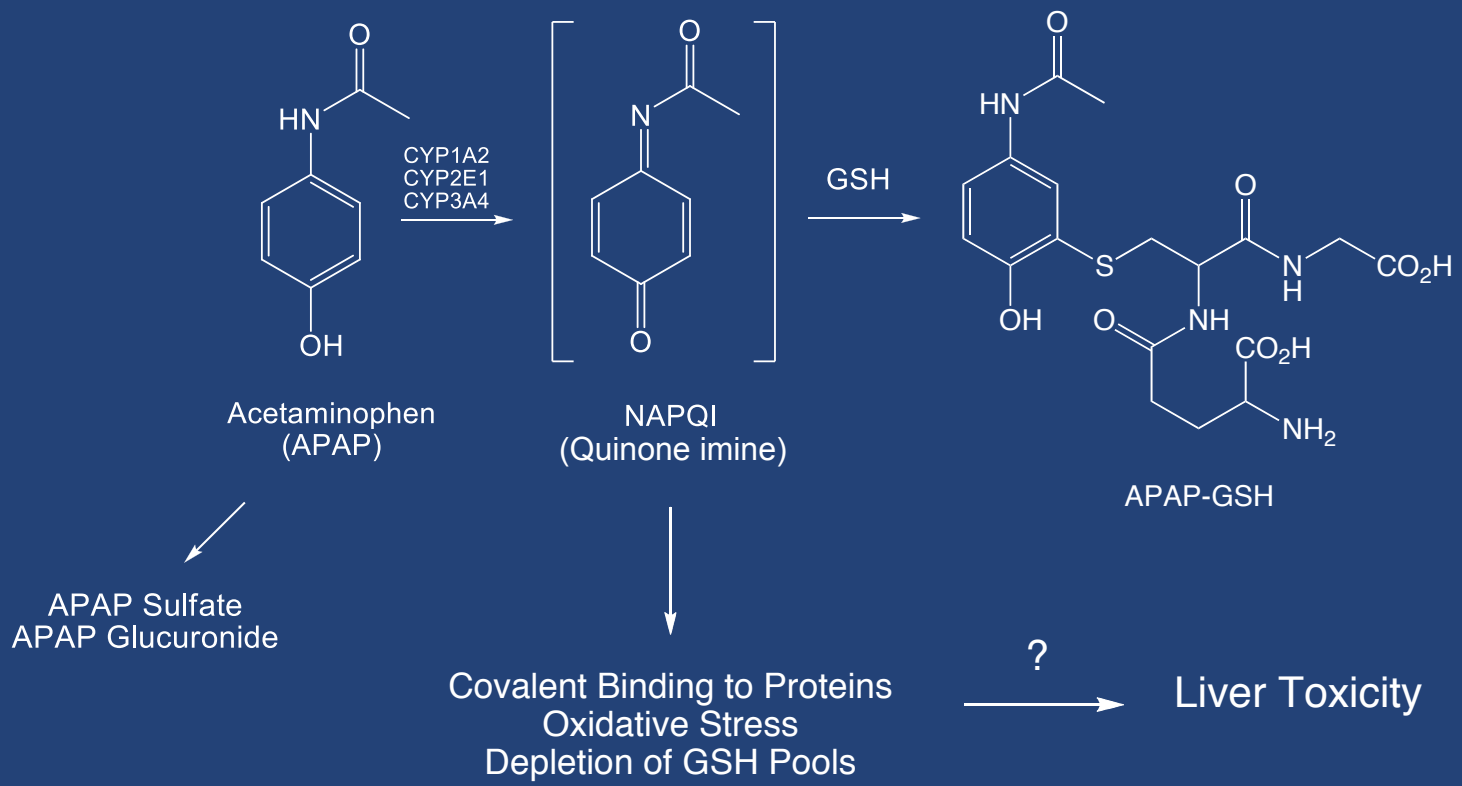
- Many other marketed drugs have warnings for a risk of liver toxicity, or severe restrictions in their use

- For most of these agents, *bioactivation* to reactive metabolites has been demonstrated to occur either *in vitro* (human hepatic tissue) or *in vivo* (characterization of downstream stable metabolites)

- *High dose drugs* (>100mg/day) tend to be the ones which most frequently cause liver toxicity

D. A. Smith and E. F. Schmid, *Curr. Opin. Drug Discov. Develop.*, **9**: 38-46 (2006)

Bioactivation and Liver Toxicity
Acetaminophen



J. R. Mitchell *et al., J. Pharmacol. Exp. Ther.,* **187**, 185-194 (1973)
I. M. Copple *et al., Hepatology,* **48**, 1292-1301 (2008)

Chemical Research in Toxicology

Chem. Res. Toxicol., **25**: 1616-1632 (2012)

Article

pubs.acs.org/crt

In Vitro Approach to Assess the Potential for Risk of Idiosyncratic Adverse Reactions Caused by Candidate Drugs

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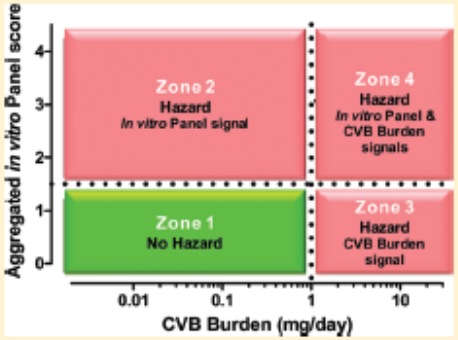
Supporting Information

ABSTRACT: Idiosyncratic adverse drug reactions (IADRs) in humans can result in a broad range of clinically significant toxicities leading to attrition during drug development as well as postlicensing withdrawal or labeling. IADRs arise from both drug and patient related mechanisms and risk factors. Drug related risk factors, resulting from parent compound or metabolites, may involve multiple contributory mechanisms including organelle toxicity, effects related to compound disposition, and/or immune activation. In the current study, we evaluate an *in vitro* approach, which explored both cellular effects and covalent binding (CVB) to assess IADR risks for drug candidates using 36 drugs which caused different patterns and severities of IADRs in humans. The cellular effects were tested in an *in vitro* Panel of five assays which quantified (1) toxicity to THLE cells (SV40 T-antigen-immortalized human liver epithelial cells), which do not express P450s, (2) toxicity to a THLE cell line which selectively expresses P450 3A4, (3) cytotoxicity in HepG2 cells in glucose and galactose media, which is indicative of mitochondrial injury, (4) inhibition of the human bile salt export pump, BSEP, and (5) inhibition of the rat multidrug resistance associated protein 2, Mrp2. In addition, the CVB Burden was estimated by determining the CVB of radiolabeled compound to human hepatocytes and factoring in both the maximum prescribed daily dose and the fraction of metabolism leading to CVB. Combining the aggregated results from the *in vitro* Panel assays with the CVB Burden data discriminated, with high specificity (78%) and sensitivity (100%), between 27 drugs, which had severe or marked IADR concern, and 9 drugs, which had low IADR concern, we propose that this integrated approach has the potential to enable selection of drug candidates with reduced propensity to cause IADRs in humans.



Safety Evaluation of Stable Drug Metabolites

Metabolites in Safety Testing ("MIST")

Metabolites in Safety Testing ("MIST")

- Central Question

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

- PhRMA "White Paper" on best practices published in 2002

  T. A. Baillie *et al., Toxicol. Appl. Pharmacol.,* **182**, 188-196 (2002)

- Final FDA Guidance on "Safety Testing of Drug Metabolites" published in 2008

  http://www.fda.gov/cder/guidance/index.htm

- Numerous commentaries on MIST published during past 10 years

  eg special issue of *Chem. Res. Toxicol.,* **22(2)**, February, 2009, and references therein

Key concern for industry: Resource and time implications for development

FDA Guidance on "Safety Testing of Drug Metabolites"

- Applies only to small molecule non-biologic drug products

- Excludes:
  - anti-cancer agents
  - drug conjugates (other than acylglucuronides)
  - reactive intermediates

- Focuses on:
  - *stable* metabolites circulating in human plasma
  - unique or "disproportionate" metabolites in humans

- Key recommendations:
  - a metabolite whose AUC_p at steady-state is <10% that of parent needs no further study
  - if AUC_p is >10% of parent, "coverage" (i.e. exposure margin $\geq$1) needs to be demonstrated in at least one tox species
  - otherwise, human metabolite is "disproportionate" and may require testing

- Types of toxicology studies that may be required:
  - general tox (3 months), genotoxicity, embryo-fetal development tox, carcinogenicity

http://www.fda.gov/cder/guidance/index.htm

ICH Topic M3 (R2)
Non-Clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals

- Key recommendations:

  - 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

  - For drugs dosed at <10mg / day, a larger % of the total drug-related material might be appropriate before safety testing is needed

  - Some metabolites do not warrant testing (eg "most GSH conjugates")

  - "Unique human metabolites" should be considered on a case-by-case basis

http://www.emea.europa.eu

Practical Issues with MIST Guidances

- *FDA Guidance*
  - How to assess those metabolites in human plasma that circulate at ≥10% AUC of parent drug under steady-state dosing conditions?
  - Multiple ascending dose (safety/tolerability) study with "cold" drug and LC-MS/MS analysis? Availability of validated assay for metabolite(s)?
- *ICH Guidance*
  - How to assess "total drug-related exposure" in plasma?
  - Radioactive dose (with required GMP synthesis, dosimetry, etc)?

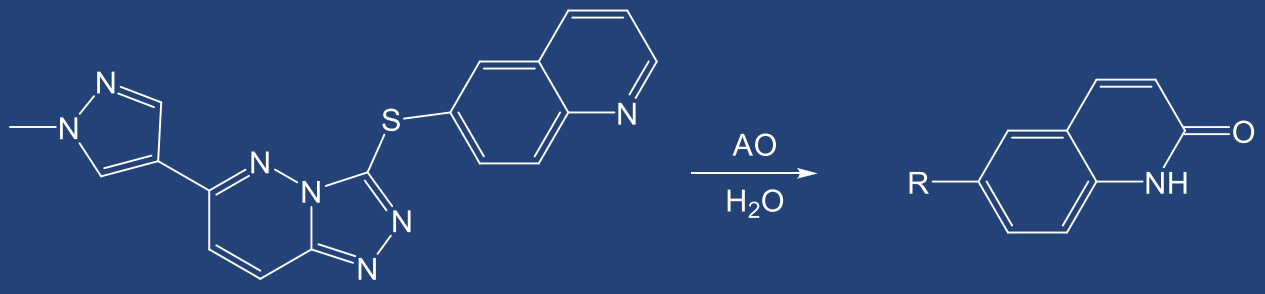
As of January, 2010, where the FDA and ICH guidances differ, the ICH guidance supersedes the FDA guidance

http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/default.htm

T. W. Robinson and A. Jacobs, *Bioanalysis,* **1**: 1193-1200 (2010)

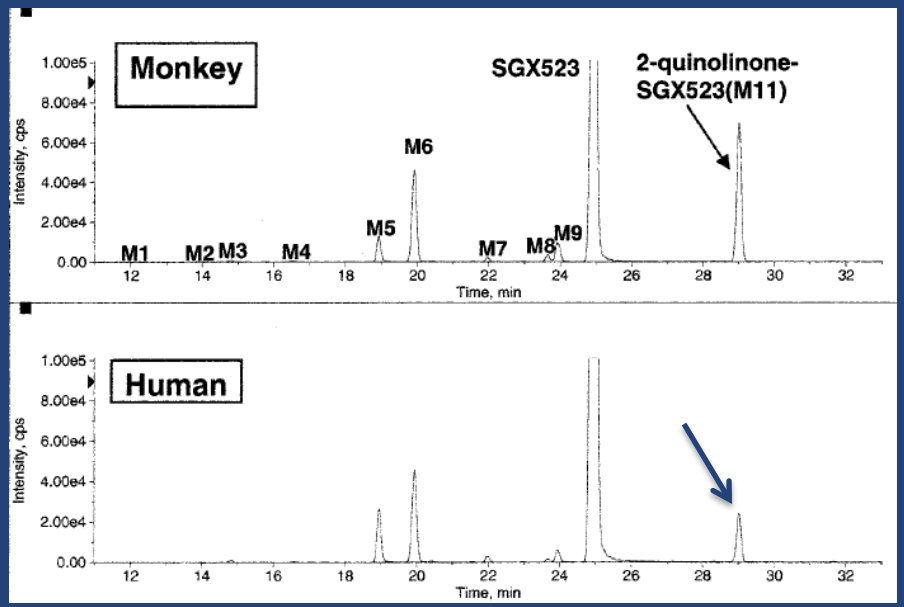
Species Differences in Drug Metabolism

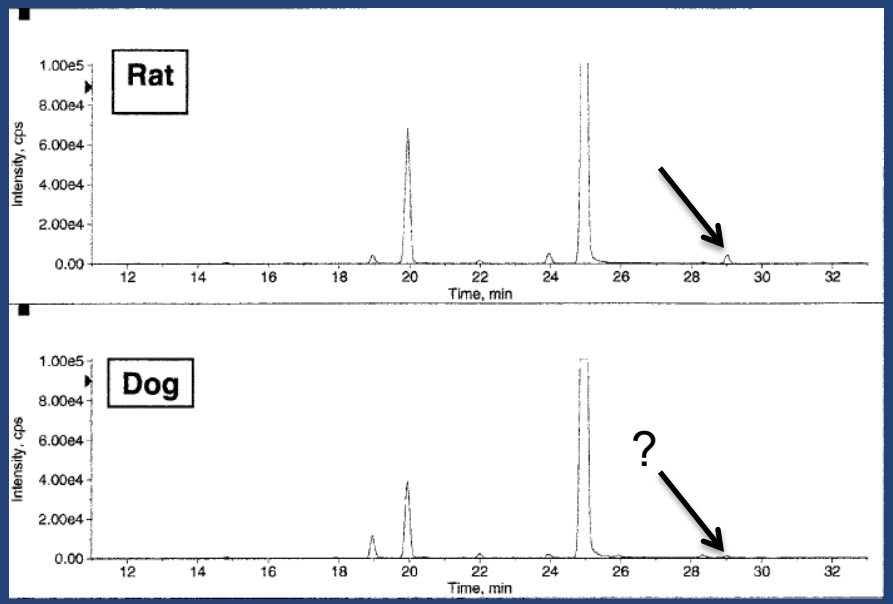
c-MET Inhibitor SGX 523



SGX 523 "M11"

Urinary metabolite profiles





Solubility in monkey urine (pH 8.4): SGX 523 – 13μg/ml M11 – 0.37μg/ml

S. Diamond *et al., Drug Metab. Dispos.*, **38**: 1277-1285 (2010)

Conclusions

- Drug metabolism now plays an integral role in the safety evaluation of new drug candidates and their circulating metabolites
- Both stable and chemically reactive drug metabolites need to be taken into consideration, and the identities of likely human metabolites established, at the lead optimization stage of preclinical development
- 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