Mechanisms of Drug Toxicity & Relevance to Pharmaceutical Development

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Evidentiary Considerations for Integration of Biomarkers in Drug Development
FDA/M-CERSI

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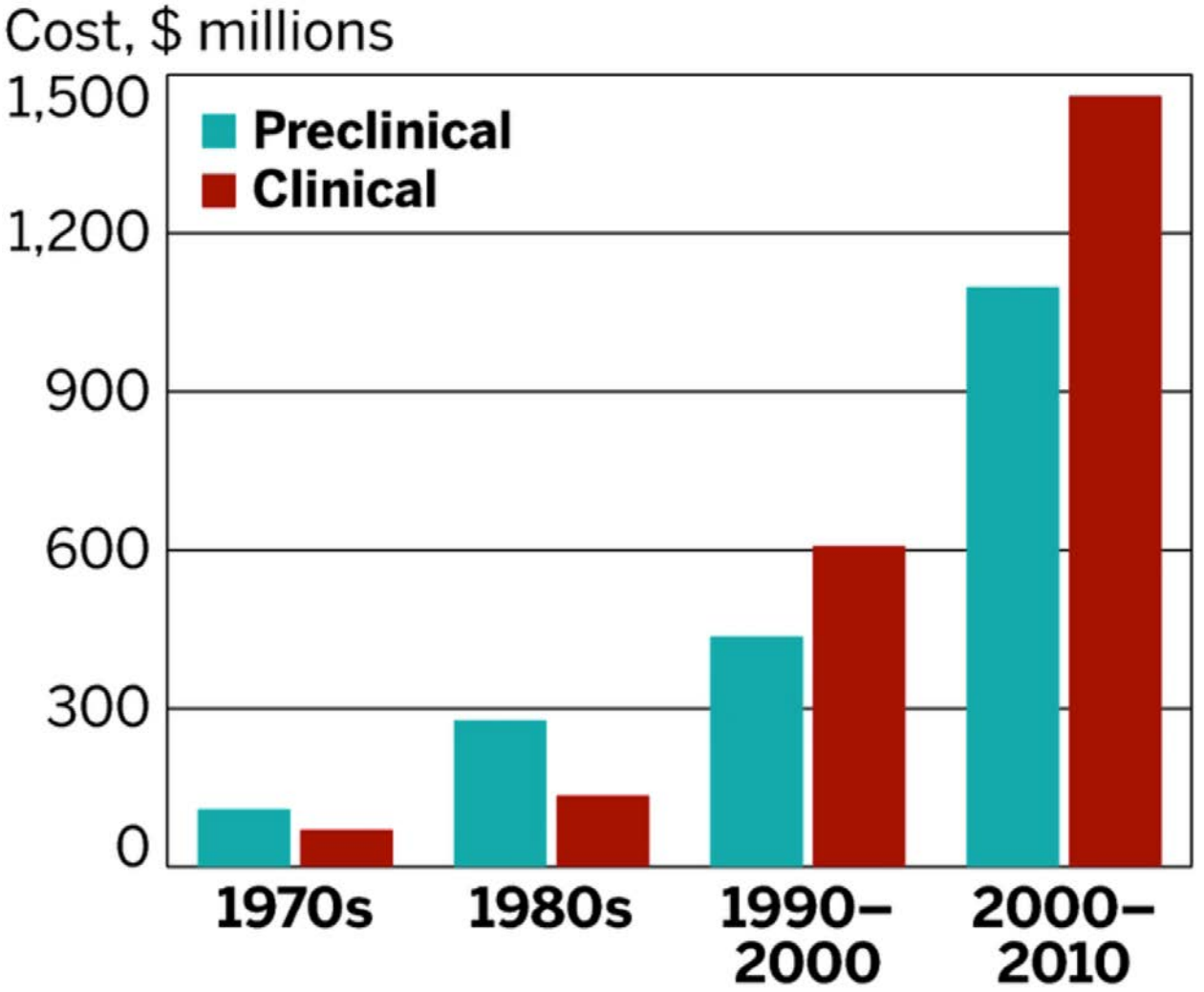
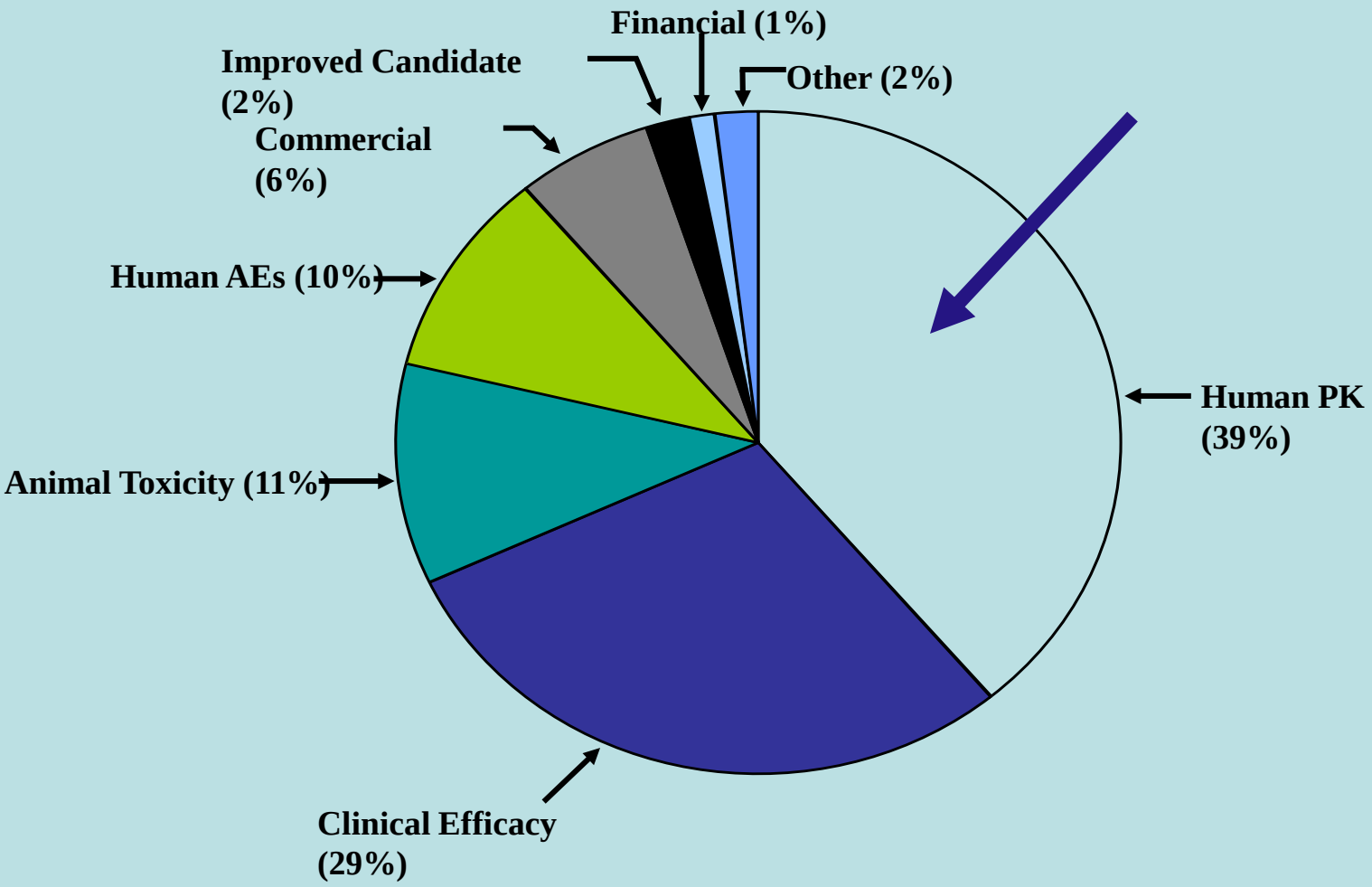


The cost of developing a new drug has skyrocketed since the 1970s. *Source: Tufts Center for the Study of Drug Development.*

Total cost >$2.5 billion/new drug
—and some estimates are even higher!

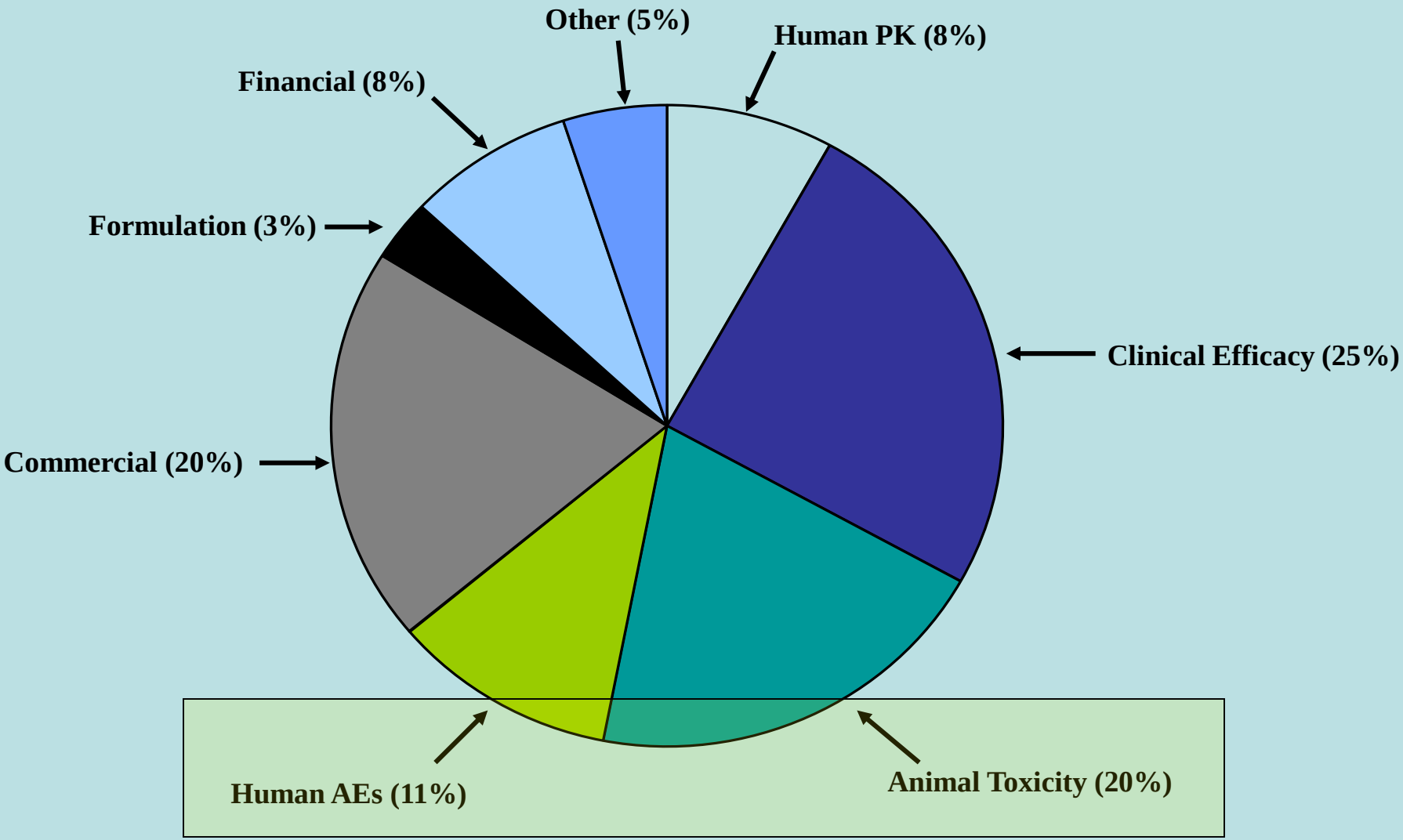
(Tufts Center, November 2014)

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



R.A. Prentis et al. (1988) *Br. J. Clin. Pharmacol.* **25**, 387

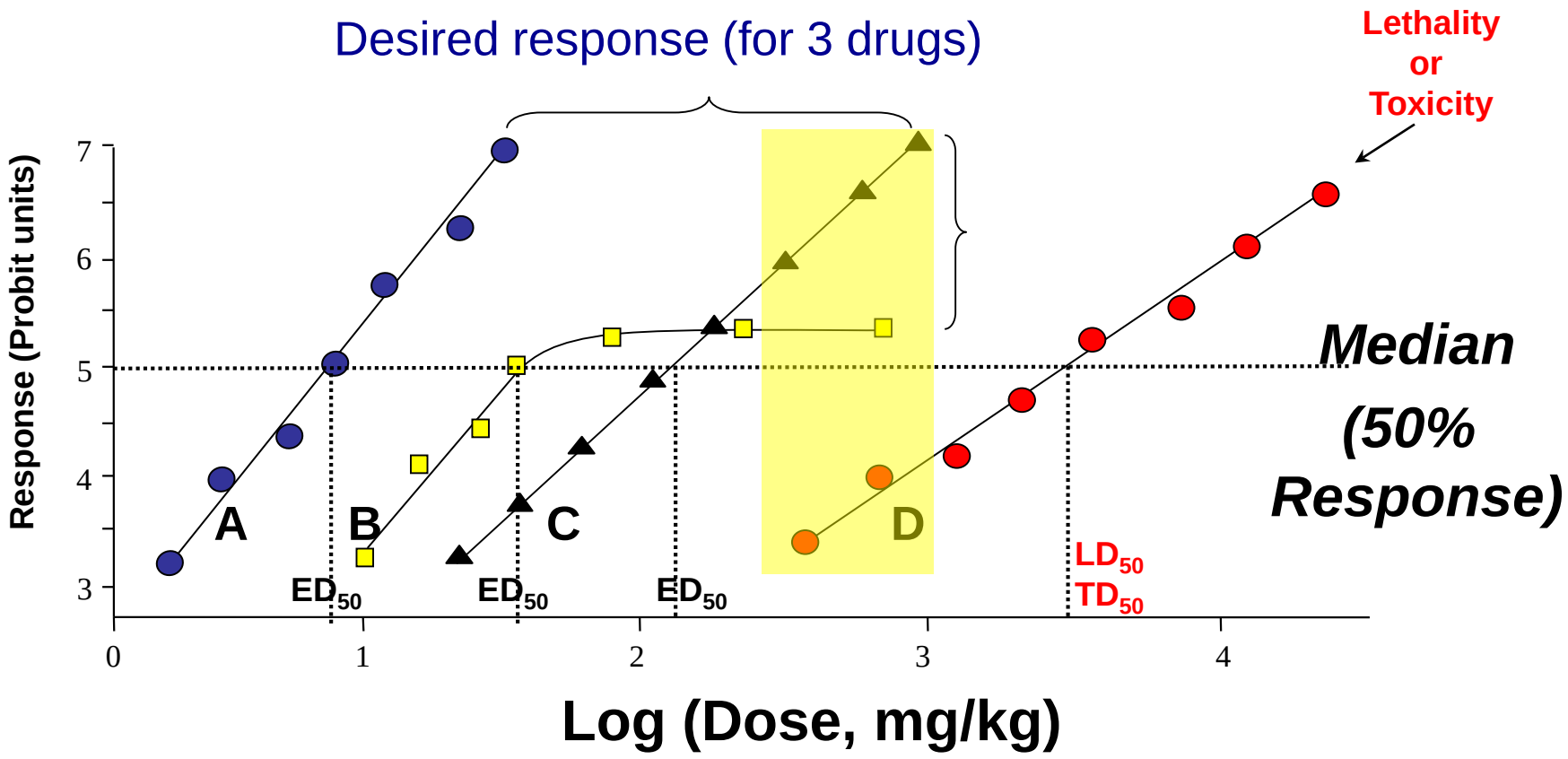
Reasons for Termination of Drug Candidates in Development (2000)



I. Kola and J. Landis (2004) *Nature Rev. Drug Discov.* **3**, 711-715

Dose-response Concepts (Paracelsus)

- **Definitions -** Effective dose = **ED**; Toxic dose = **TD**; Lethal dose = **LD**
- **Potency -** Range of doses over which a drug produces increasing responses
- **Efficacy -** Maximal response; plateau of the dose-response curve

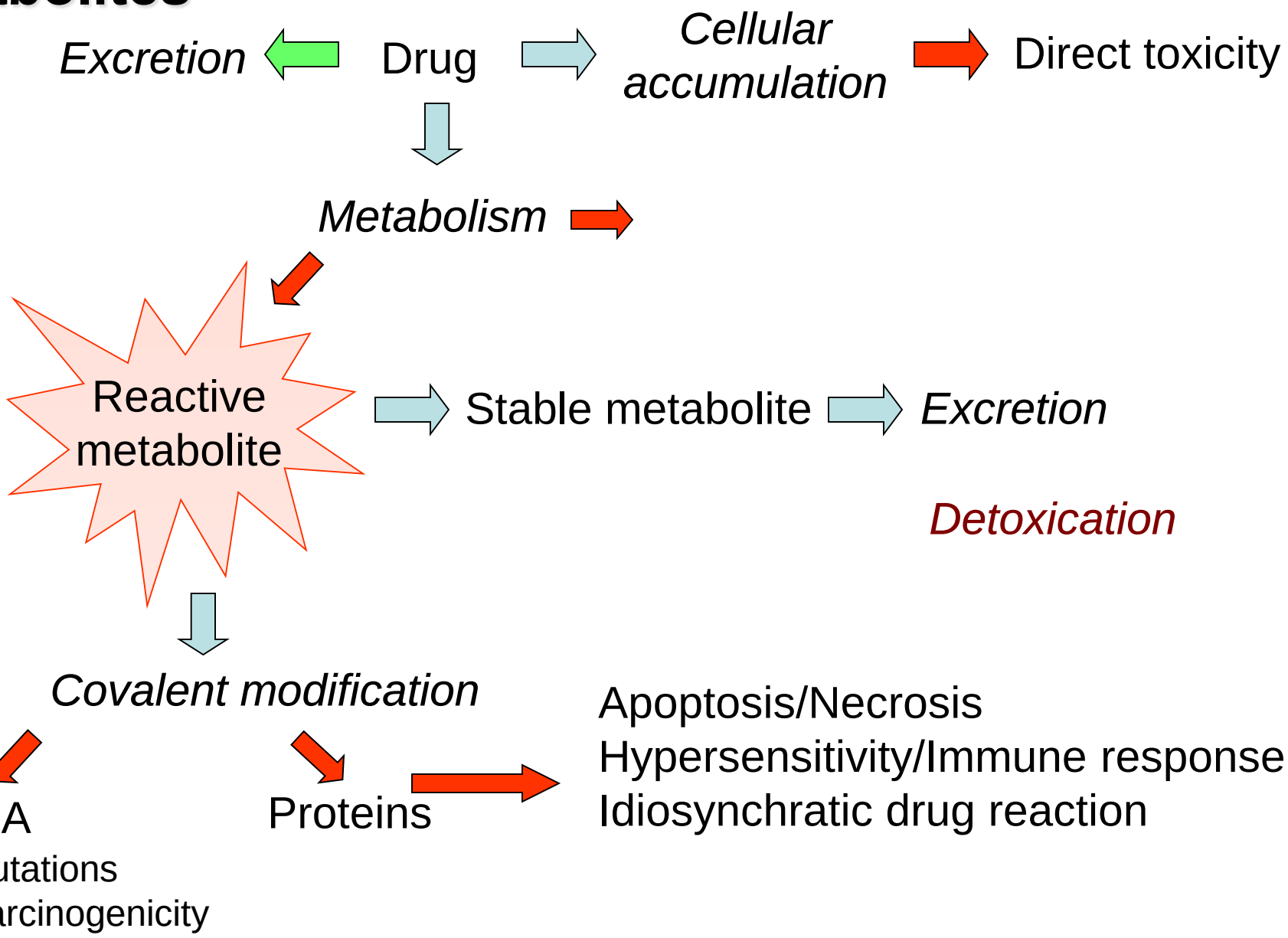


Contexts of Drug Toxicity

- **On-target toxicity** (mechanisms-based): same receptor, wrong tissue (e.g. statins)
- **Hypersensitivity & immunological reactions** (e.g., penicillins)
- **Off-target pharmacology** (e.g., terfenadine & hERG channel effects)
- **Bioactivation to reactive intermediates** (e.g., acetaminophen)
- **Idiosyncratic** toxicities

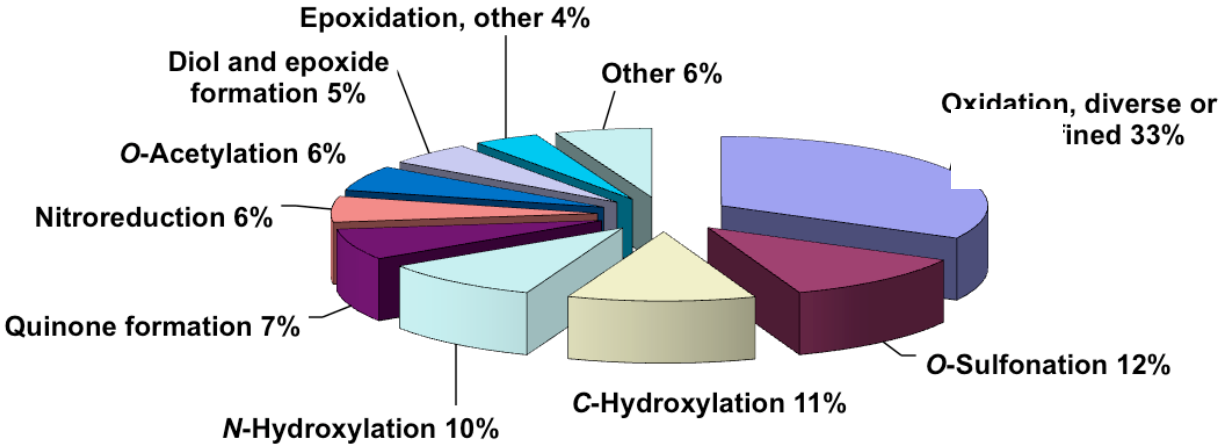
Liebler & Guengerich (2005) *Nature Rev. Drug Discovry* **4**, 410-420

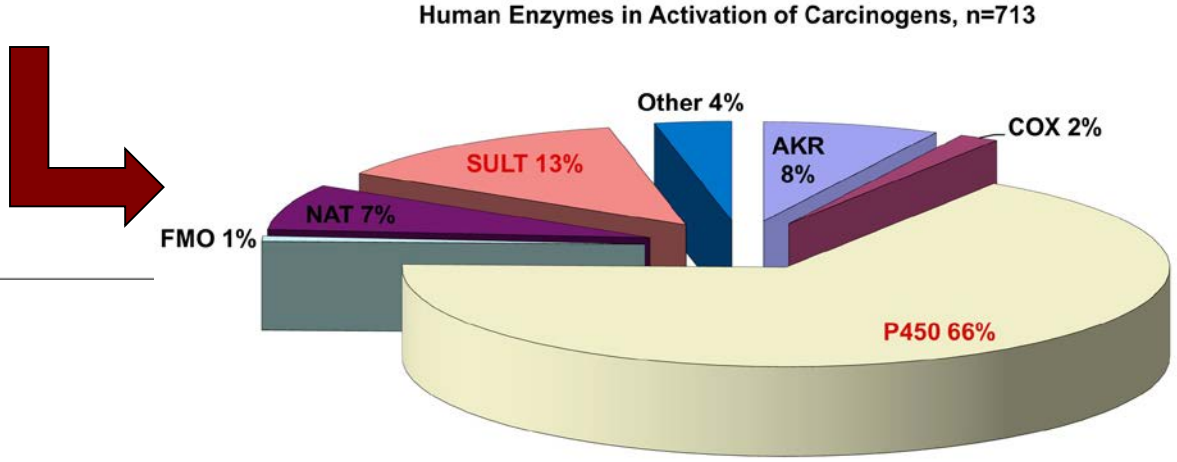
Metabolic Activation of Drugs—"Reactive Metabolites"



Reaction types involved in bioactivation of carcinogens (n = 799 reactions)

Fig. 6





S. Rendic and F. P. Guengerich (2012) *Chem. Res. Toxicol.* **25**, 1316-1383

Structural Alerts for Bioactivation

Hydrazines and hydrazides

Arylacetic or aryl propionic acids

Thiophenes, furans, pyrroles

Anilines and anilides

Quinones and quinoneimines

Medium chain fatty acids

Halogenated hydrocarbons and some halogenated aromatics (Br > Cl > F)

Nitroaromatics

Moities that form $\alpha\beta$-unsaturated enol-like structures

Thiols, thiono compounds, thiazolidinedione

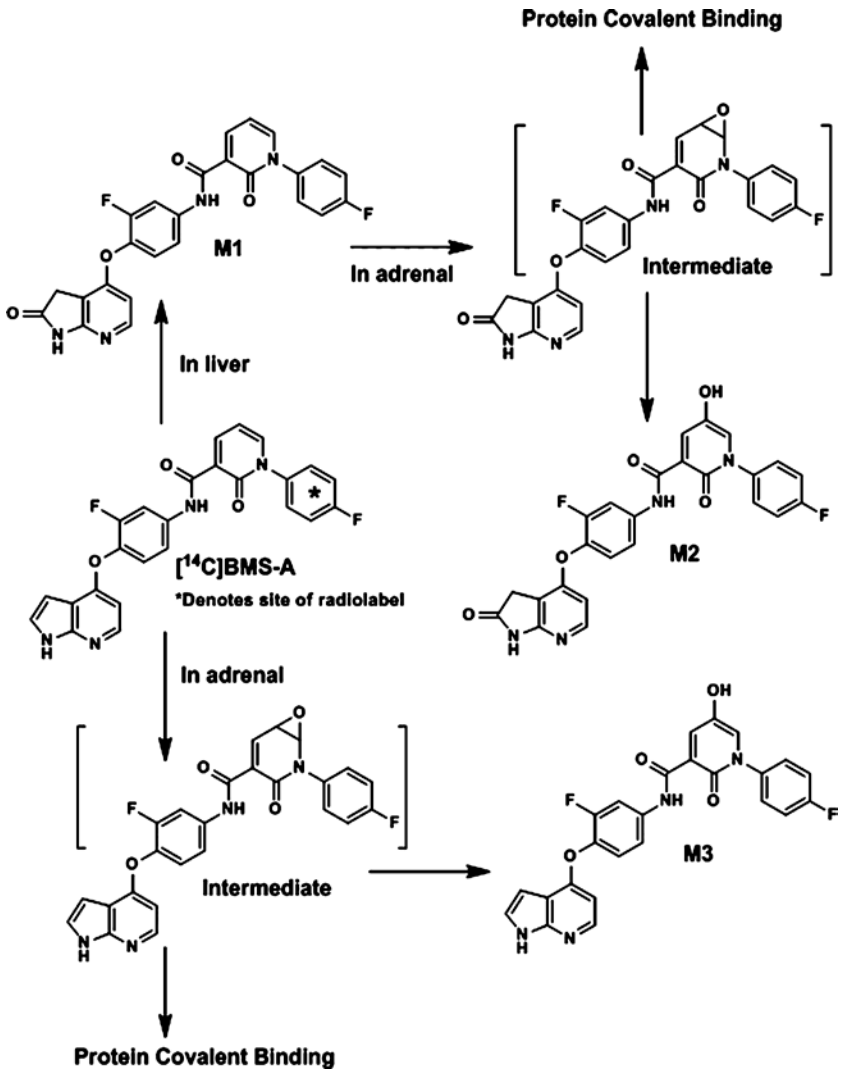
So: What's left to work with?

Also, remember that any phenyl ring is only

1-3 steps away from a reactive intermediate.

Thanks for list to Sid Nelson, U. Wash.

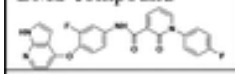
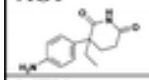
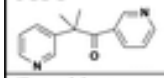
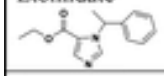
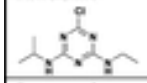
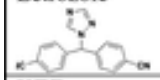
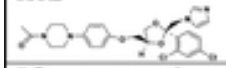
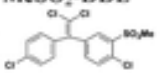
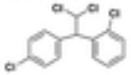
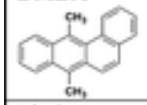
BMS-A
& P450 11A1



Proposed bioactivation pathway of [^{14}C]BMS-A in rat adrenal gland.

Published in: Donglu Zhang; Oliver Flint; Lifei Wang; Ashok Gupta; Richard A. Westhouse; Weiping Zhao; Nirmala Raghavan; Janet Caceres-Cortes; Punit Marathe; Guoxiang Shen; Yueping Zhang; Alban Allentoff; Jonathan Josephs; Jinping Gan; Robert Borzilleri; W. Griffith Humphreys; *Chem. Res. Toxicol.* **2012,** 25, 556-571.
DOI: 10.1021/tx200524d

Comparison of Selected Adrenal Toxicants That Affect Steroidogenic Enzymes

Compounds	Adrenal Toxicity	Toxicity Mechanism	Reference
BMS compound 	Adrenal vacuolar degeneration and necrosis	Mitochondrial bioactivation by CYP11A1	This study
AGT 	Inhibition of cortisol secretion	Inhibition of CYP11A1	(34)
MTY 	Stimulation of ACTH release	Inhibition of CYP11B1	(16)
Etomidate 	Adrenal insufficiency	Inhibition of CYP11B2/1	(35)
Atrazine 	Adrenal weight increase	Induction of CYP19	(36)
Letrozole 	Mild adrenal suppression	Inhibition of CYP19	(25)
KTZ 	Reversible adrenal insufficiency	Inhibition of CYP17 and 11 beta hydroxylase	(37)
Pfizer compound	Formation of vacuoles in adrenal	Inhibition of CYP21	(38)
$MeSO_2$-DDE 	Adrenal disorganization loss of central cristae of mice	Cytotoxic to parenchymal cells, bioactivation by and inhibition of CYP11B1	(39, 40)
Mitotane 	Membrane disruption an dissolution of adrenal	Bioactivation by CYP11B1 and other enzymes	(32, 41)
DMBA 	Adrenal capiliary endothelial lesion to bleeding	Oxidation of mitochondrial GSH, involvement of CYP11B1	(42, 43)
Lindane Hexachloro-cyclohexane	Adrenal weight increase	Inhibition of stAR	(44)

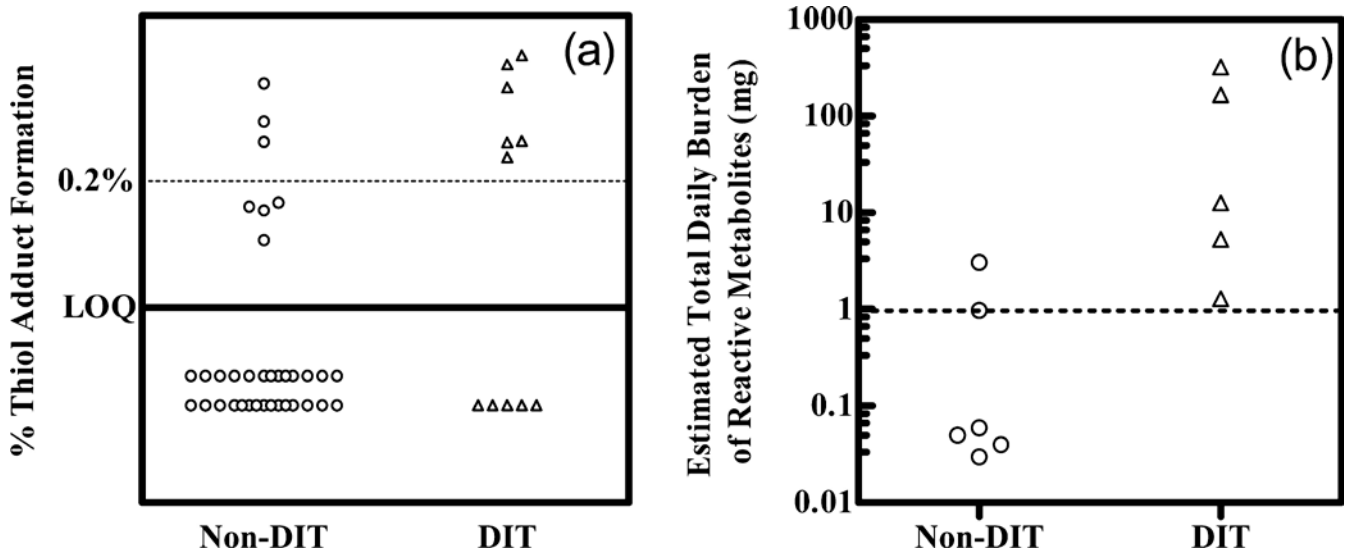
<u>P450s</u>:
11A1
11B1
11B2
19A1
17A1
21A2

*Zhang et al. (*2012) *Chem. Res. Toxicol.* **25**, 556-571

Covalent binding of chemicals to proteins: Issue or not?

- Correlates with *in vivo* toxicity
- Treatment of a purified enzyme with a chemical modifier can destroy activity
- Block covalent binding (e.g. N-Ac Cys), prevent toxicity
- Knock out P450s —> prevent acetaminophen toxicity
- Idiosyncratic toxicity:
  - Majority of culprits show covalent binding
  - Only seen with higher dose drugs (>10 mg/day), consistent with binding overload

- No direct proof of involvement in toxicity
- Alternative mechanisms, e.g. ox stress, would show similar profiles re *N*-AcCys
- Some drugs have high covalent binding but no apparent toxicity
- Delete other genes (non-P450) & see effects on toxicity, implying downstream issues

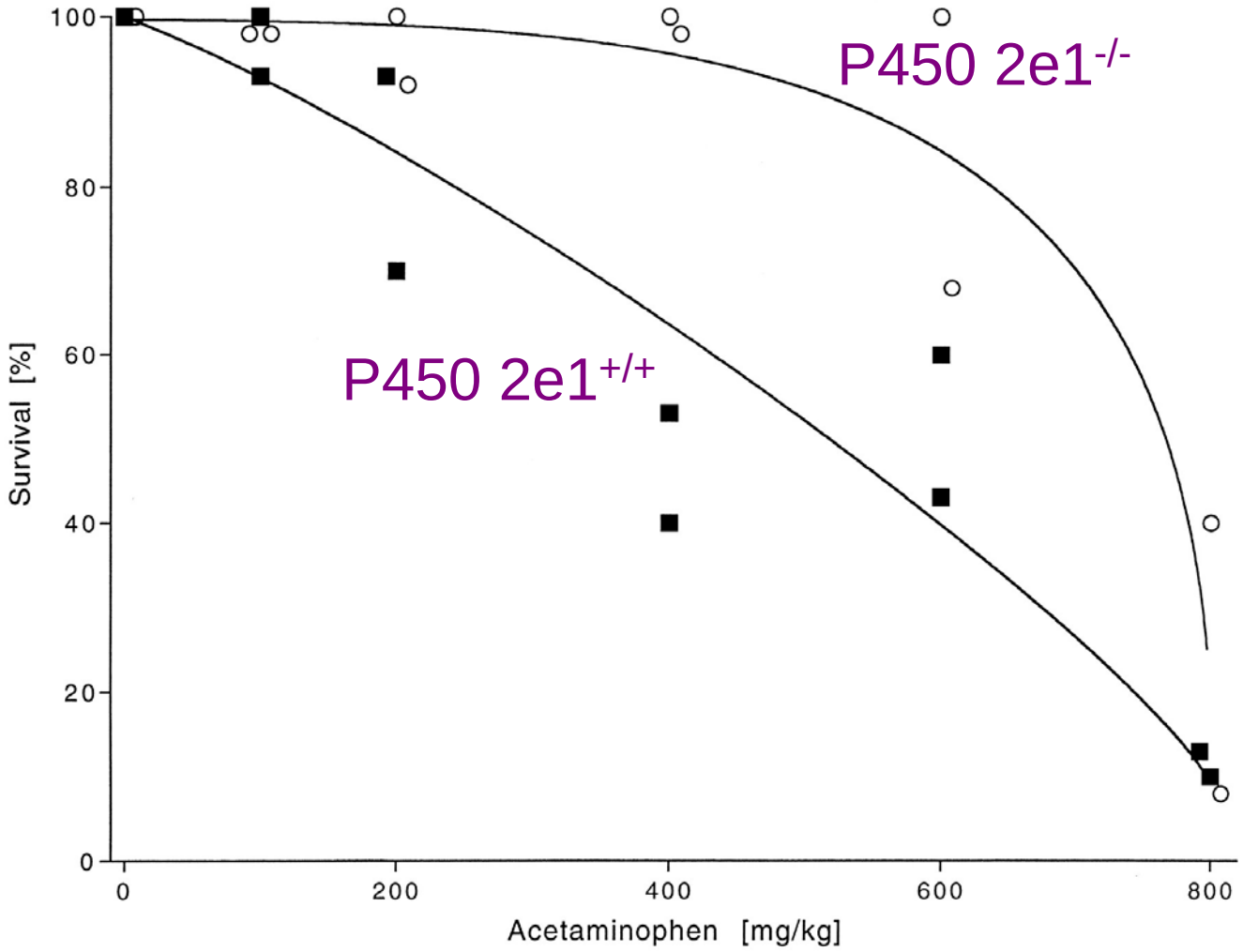


(DIT: drug-induced toxicity)

Scatter plot of % dGSH adduct formation (a) and estimated total daily burden (b) in the DIT and non-DIT groups. The open circles and triangles represent drugs not associated and associated with DIT, respectively. For illustrational purposes, a horizontal dotted line is plotted at 0.2% adduct level in panel a, and another is plotted at the 1 mg level in panel b. Adduct levels of omeprazole, lansoprazole, and montelukast are not shown in this figure.

Published in: Jinping Gan; Qian Ruan; Bing He; Mingshe Zhu; Wen C. Shyu; W. Griffith Humphreys; *Chem. Res. Toxicol.* **2009,** 22, 690-698.
DOI: 10.1021/tx800368n

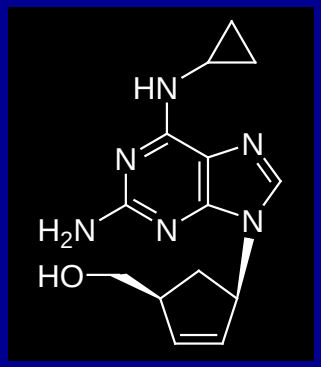
Significance of acetaminophen metabolism in toxicity in mice

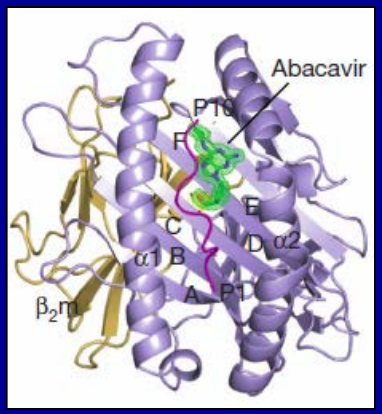


Lee, S. S. T. et al. (1996) *J. Biol. Chem.* **271**, 12063-12067
See also Zaher et al. (1998) *Toxicol. Sci.* **152**, 193-199 regarding deletion of both 2e1 and 1a2

Molecular Basis of Immune Hypersensitivity

- Abacavir use associated with immune hypersensitivity syndrome
  - occurs in individuals with *HLA-B*57:01* allele
- X-ray cocrystal of abacavir bound to *HLA-B*57:01*
  - binds to bottom of antigen binding cleft of the F pocket
- Abacavir binds to 2 amino acids unique to *HLA-B*57:01*
- Cyclopropyl moiety projects into F pocket
  - reduces pocket size; alters peptide binding preference
  - smaller Leu and Ile side chains preferred over Trp and Tyr
- Co-crystal structure of carbamazepine with *HLA-B*15:02*
  - indicates similar mechanism of hypersensitivity





P.T. Illing *et al*., *Nature,* 2012, 486, 554-560
E.L. Reinherz, *Nature,* 2012, 486, 479-481

Mechanistic Causes of Toxicology Attrition

	percent of all advanced molecules[b]
biotransformation-related	27
target-based	28
single or multiple ion channel inhibition	18
immune-mediated	7
all other mechanisms	36

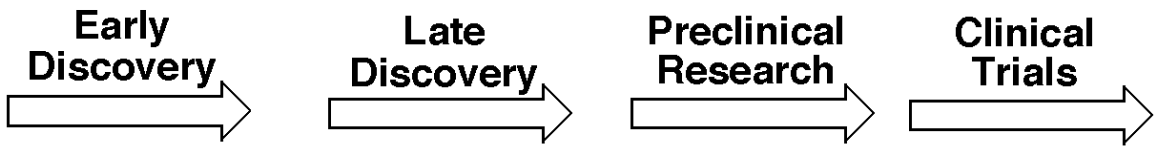
[a]Based on experience form DuPont-Merck and Bristol-Myers Squibb, 1993-2006. Information kindly provided by B. Car.

[b]n=88, note as categories are partially overlapping, the total is > 100%.

Thanks to Bruce Car, BMS

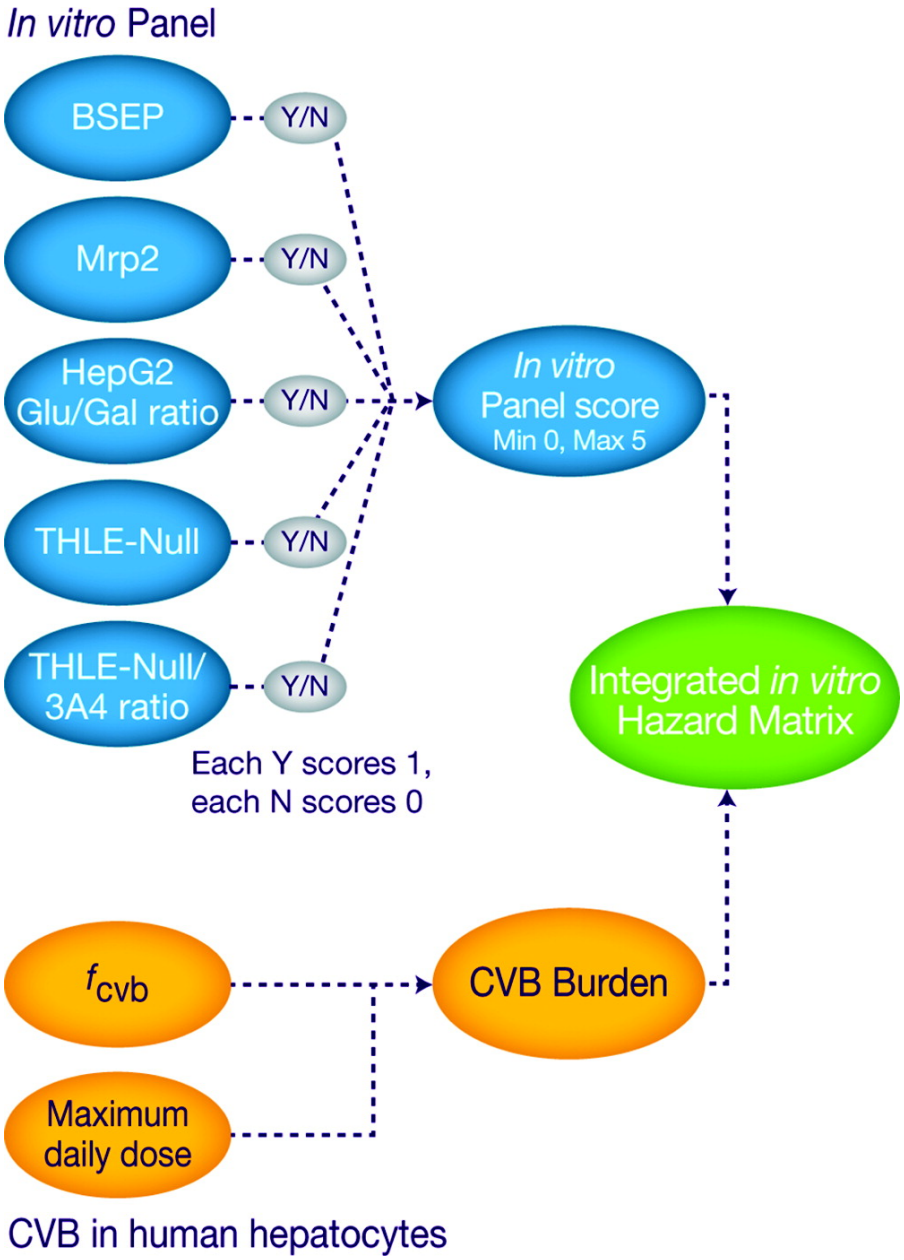
Trends in safety assessment

Assessing toxicity earlier



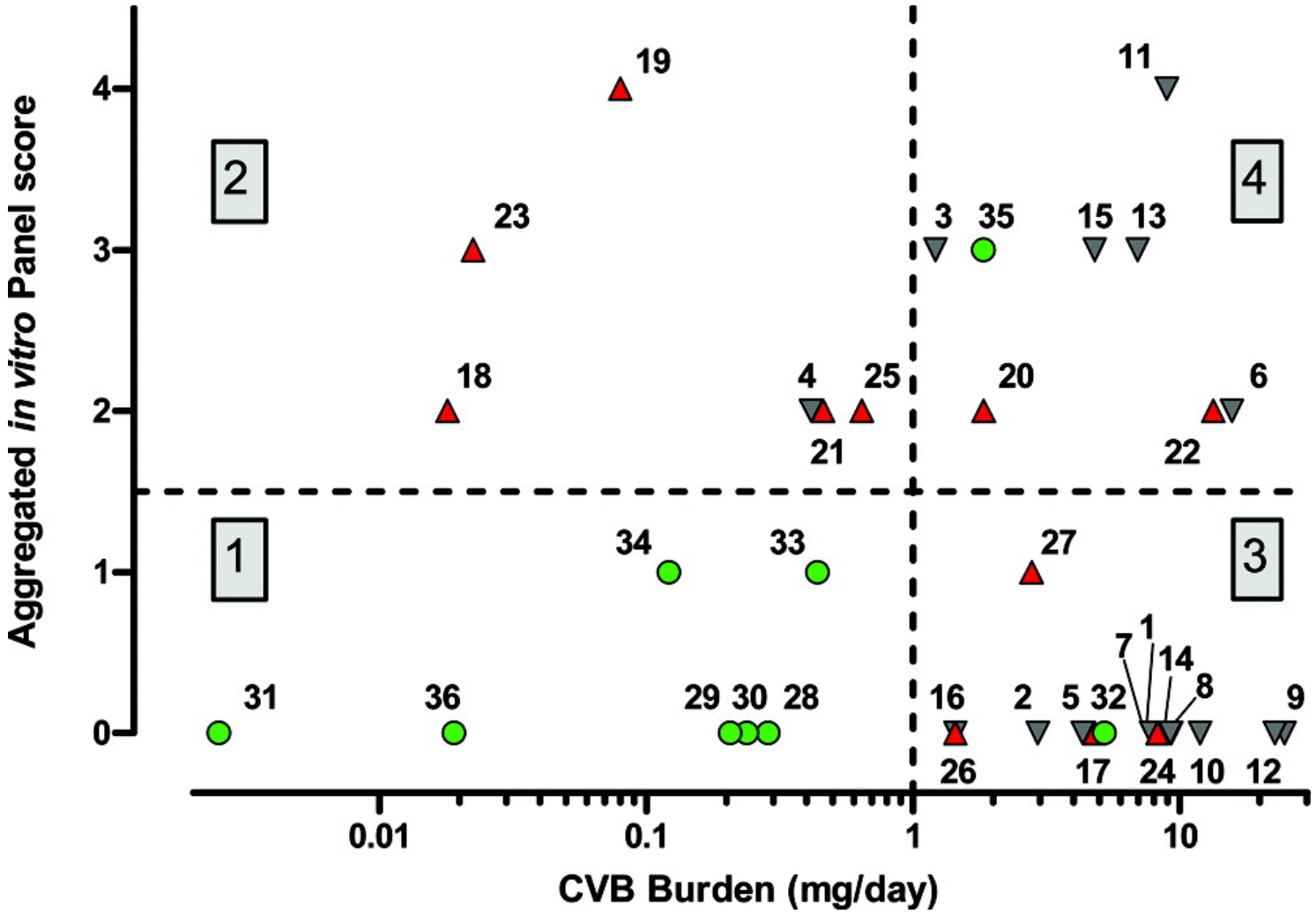
Early Discovery	Late Discovery	Preclinical Research	Clinical Trials
In vitro exprssion profiling	Expression profiling	Animal studies	Clinical trials
hours-days	1-3 days dosing	Weeks of dosing	Days-weeks of dosing
1-5 mg drug	1-10 g drug	100s-1000s g drug	kgs of drug
$1K	$1K-$10K	$100K-$1M	$1M-$100M

Thanks to Eric Blomme, Abbott



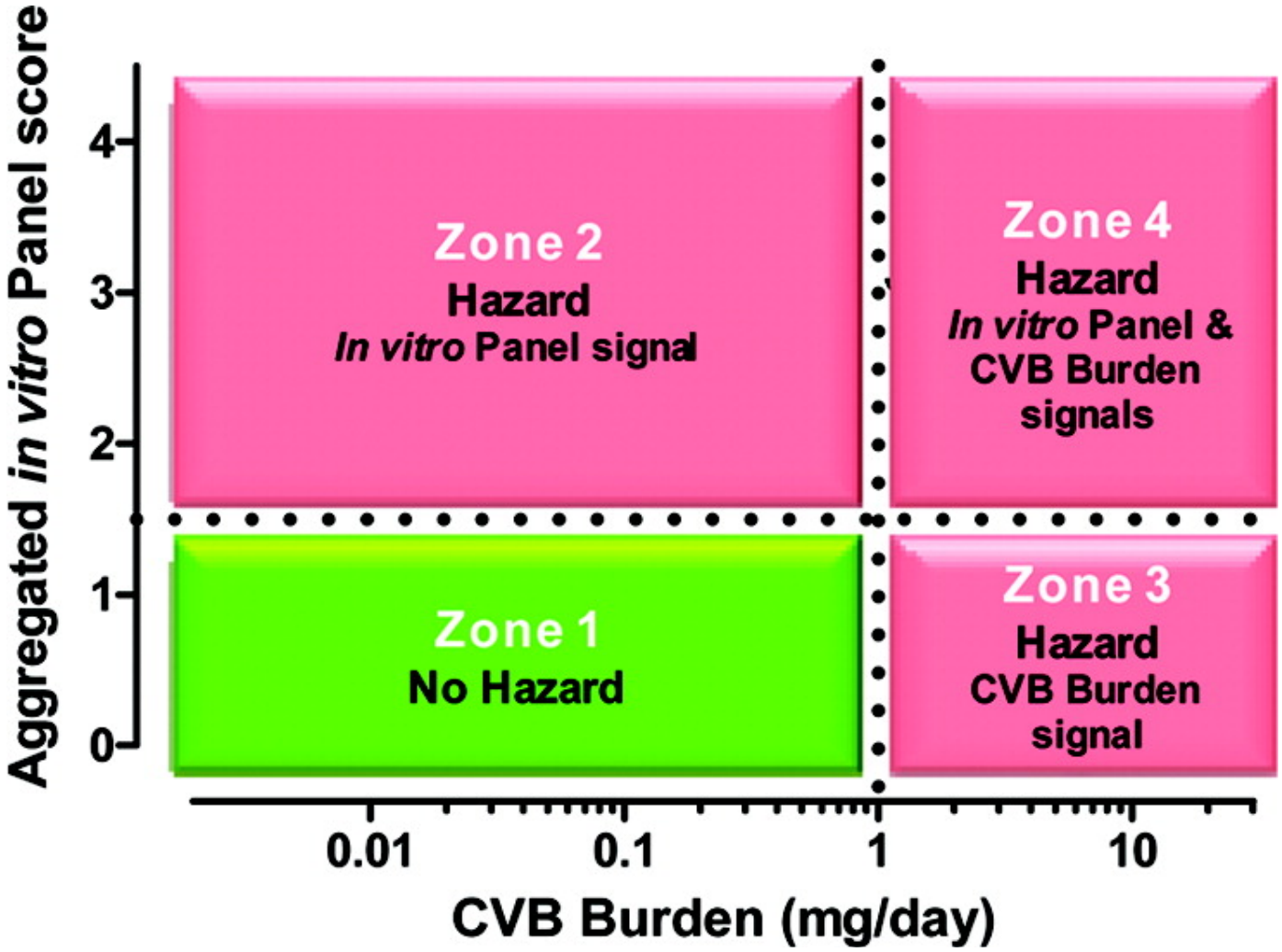
Overview of assays and their interrelationship.

Published in: Richard A. Thompson; Emre M. Isin; Yan Li; Lars Weidolf; Ken Page; Ian Wilson; Steve Swallow; Brian Middleton; Simone Stahl; Alison J. Foster; Hugues Dolgos; Richard Weaver; J. Gerry Kenna; *Chem. Res. Toxicol.* Article ASAP
DOI: 10.1021/tx300091x



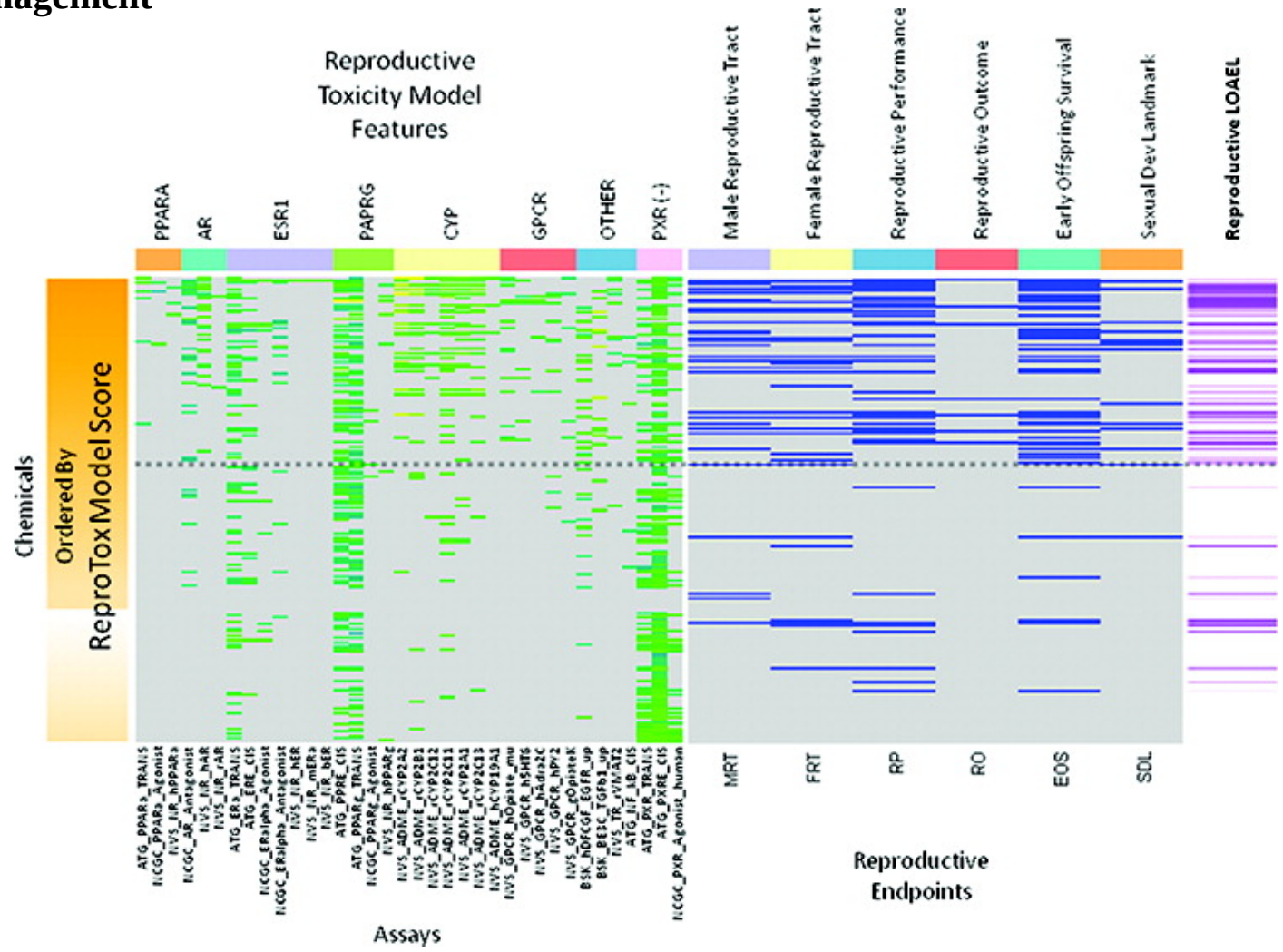
Integrated *in vitro* Hazard Matrix. IADR categories are Severe concern (black inverted triangles), Marked concern (red triangles), and Low concern (green circles).

Published in: Richard A. Thompson; Emre M. Isin; Yan Li; Lars Weidolf; Ken Page; Ian Wilson; Steve Swallow; Brian Middleton; Simone Stahl; Alison J. Foster; Hugues Dolgos; Richard Weaver; J. Gerry Kenna; *Chem. Res. Toxicol.* Article ASAP
DOI: 10.1021/tx300091x



Published in: Richard A. Thompson; Emre M. Isin; Yan Li; Lars Weidolf; Ken Page; Ian Wilson; Steve Swallow; Brian Middleton; Simone Stahl; Alison J. Foster; Hugues Dolgos; Richard Weaver; J. Gerry Kenna; *Chem. Res. Toxicol.* Article ASAP
DOI: 10.1021/tx300091x

Update on U. S. EPA's ToxCast Program: Providing High Throughput Decision Support Tools for Chemical Risk Management



No provision for bioactivation!

Published in: Robert Kavlock; Kelly Chandler; Keith Houck; Sid Hunter; Richard Judson; Nicole Kleinstreuer; Thomas Knudsen; Matt Martin; Stephanie Padilla; David Reif; Ann Richard; Daniel Rotroff; Nisha Sipes; David Dix; *Chem. Res. Toxicol.* **2012,** 25, 1287-1302.
DOI: 10.1021/tx3000939

Special Issue OUTLINE (early 2016)
Chemical Research in Toxicology
Toxicology Strategies for Drug Discovery—Present and Future

Introduction: *W. G. Humphreys, BMS; Y. Will, Pfizer; F. Guengerich, Vanderbilt*

Physicochemical properties of molecules: *N. Meanwell, BMS*
In silico stratification/computational models: *Grace Patlewicz, EPA*

Transporters: *Yurong Lai, BMS*
Reactive metabolites: *Richard Thompson, AZ*

Hepatic issues: *Gerry Kenna, FRAME*
Cardiovascular issues: *Paul Levesque, BMS*
New methods in reproductive toxicology: *Karen Augustine, MBS*

New technologies: *Donna Dambach, Genentech*
Overview—pulling it all together: *Eric Blomme, Abbott; Y. Will, Pfizer*

Summary

- General issues in the pharmaceutical industry
  -Toxicity/safety is a big issue
- Bioactivation is an important issue but not the only one
- Issues with “endogenous substrate” P450s
- Covalent binding: general, issues-bad, good
- *In vitro* strategies in discovery toxicology