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# Translational Preclinical Evaluations (DMPK, Toxicology, Risk Assessment)

**Dolo Diaz, PhD, DABT**

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Altos Labs

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Senior Director, Toxicology  
DICE Therapeutics (a wholly owned subsidiary of Eli Lilly)

# SESSION OUTLINE (180 minutes)

- Intro from Dolo Diaz
- Basic Toxicology and DMPK concepts
- Toxicology in the Discovery Space
- Q&A
- Mini break ----- 11:15 pm PT
  
- Toxicology in the Development Space
- Q&A
- Mini break ----- 12:30 pm PT
- Case studies (time allowing)
  
- Office hours





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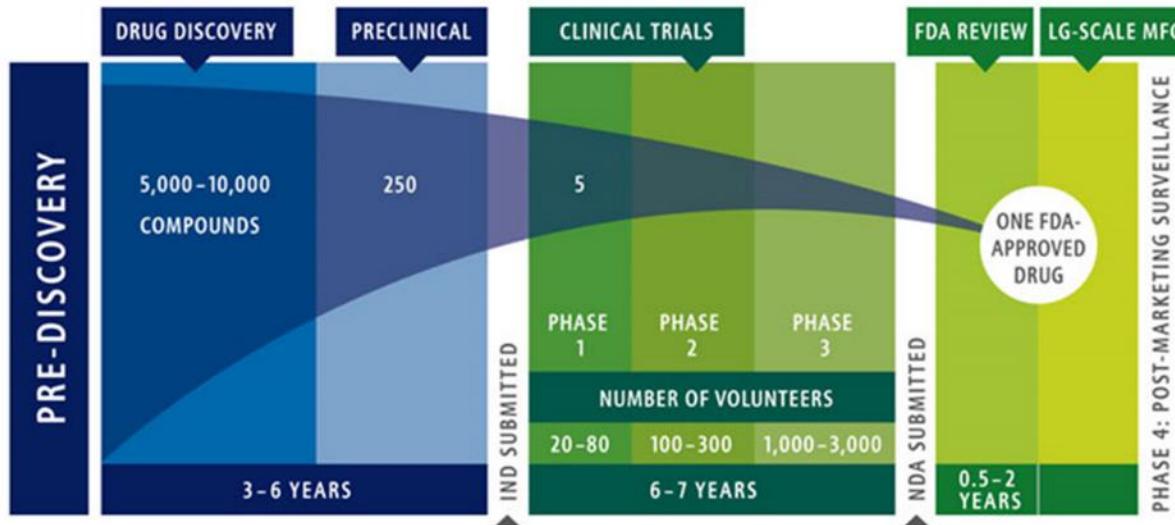
# Toxicology in the Discovery Space

**Rebecca Erickson, PhD, DABT**

Senior Director, Toxicology  
DICE Therapeutics (a wholly owned subsidiary of Eli Lilly)

# The Drug Development Process is Long and Challenging

## Drug Discovery and Development: A LONG, RISKY ROAD



Source: Pharmaceutical Research and Manufacturers of America

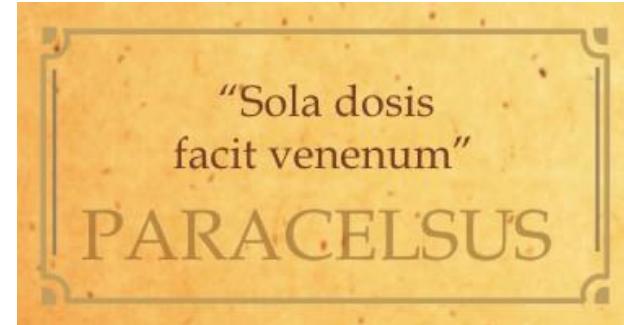
- ⑩ Only 1 in every 250 compounds entering preclinical development is ultimately approved
- ⑩ Average cost ~ \$1B per drug
- ⑩ Profits made from one drug need to cover the costs of previous "failed drugs"
- ⑩ Translational phase between preclinical and clinical development accounts for a large % of the attrition
- ⑩ **Toxicities associated with a narrow Therapeutic Index (TI) lead to attrition**

# What Is Toxicology?

The study of the adverse effects of chemicals (including drugs) on living systems and the means to prevent or mitigate such effects

Toxicology is a multidisciplinary field, it overlaps with

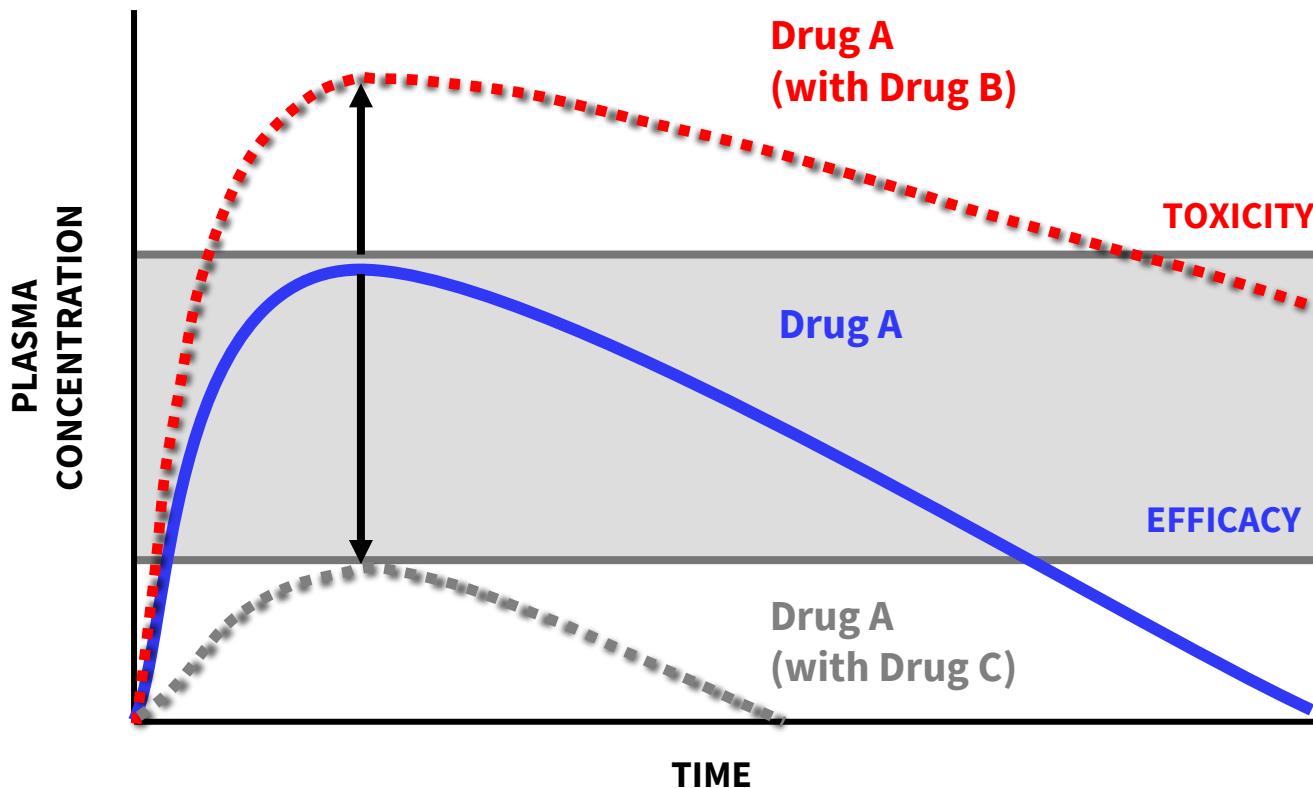
- Biology
- Chemistry
- Pharmacology
- Medicine
- Regulatory
- CMC



LD <sub>50</sub> translation	Mg/kg – bw (body weight) Toxicities LD <sub>50</sub> rating	Active Constituent
<b>Toxic in very small doses</b>	0.000001	Botulinum Toxin
	0.02	Dioxin
	<1	Brodifacoum, aldicarb
	2	Strychnine, parathion, 1080
	4	Cyanide
	10	Nicotine, abamectin, Vitamin D
	50	Omethoate
	150	Petrol, Pirimicarb
	180	Fluorine
	250	Caffeine
	280	Paraquat dichloride
	408	Diquat dibromide
	639	2,4-D
	3 320	Table salt
	5 600	Glyphosphate, Simazine
	11 900	Vitamin C
<b>Toxic in very large doses</b>	90 000	Water

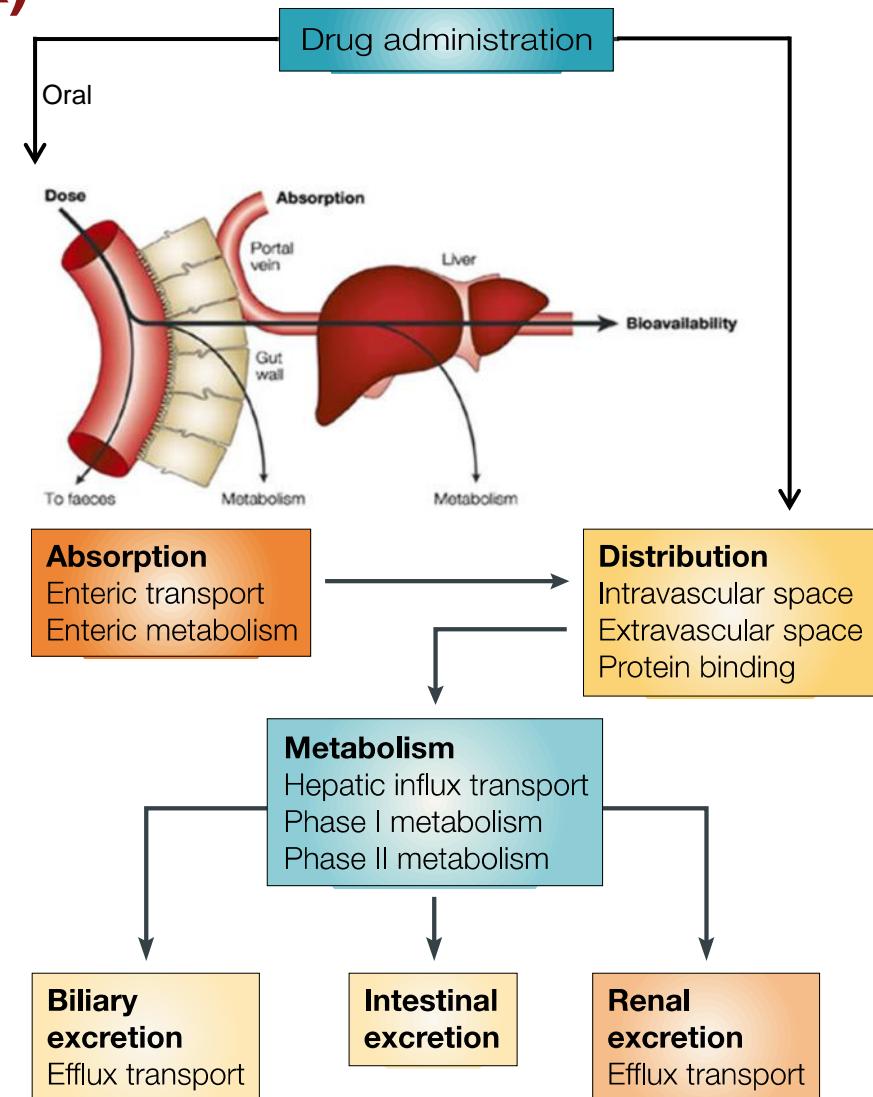
# What is the most important determinant of Toxicity?

Pharmacokinetics...  
How the body “sees” the DOSE



# Drug Metabolism and Pharmacokinetics (DMPK)

- Elucidates biological and biochemical basis of what the **body does to the drug**: how it is
    - **Absorbed,**
    - **Distributed,**
    - **Metabolized (biotransformed)**
    - **Excreted**
    - **ADME**
  - Goal in drug discovery/development:  
Select drug candidates with optimal ADME properties to
    - **Maximize therapeutic benefit**
    - **Minimize safety risks**



S Undevia. 2005. Nature Reviews Oncology.

# Drug Clearance is a Primary Determinant of Exposure

- **Clearance (CL):** The efficiency of irreversible elimination of a drug from systemic circulation
  - Expressed as volume of blood cleared of drug per unit time (e.g. mL/h)
  - Occurs in many organs or cell types, and is additive across mechanisms

$$CL = \frac{Dose}{AUC_0^\infty} = CL_{hepatic} + CL_{renal} + CL_{other}$$



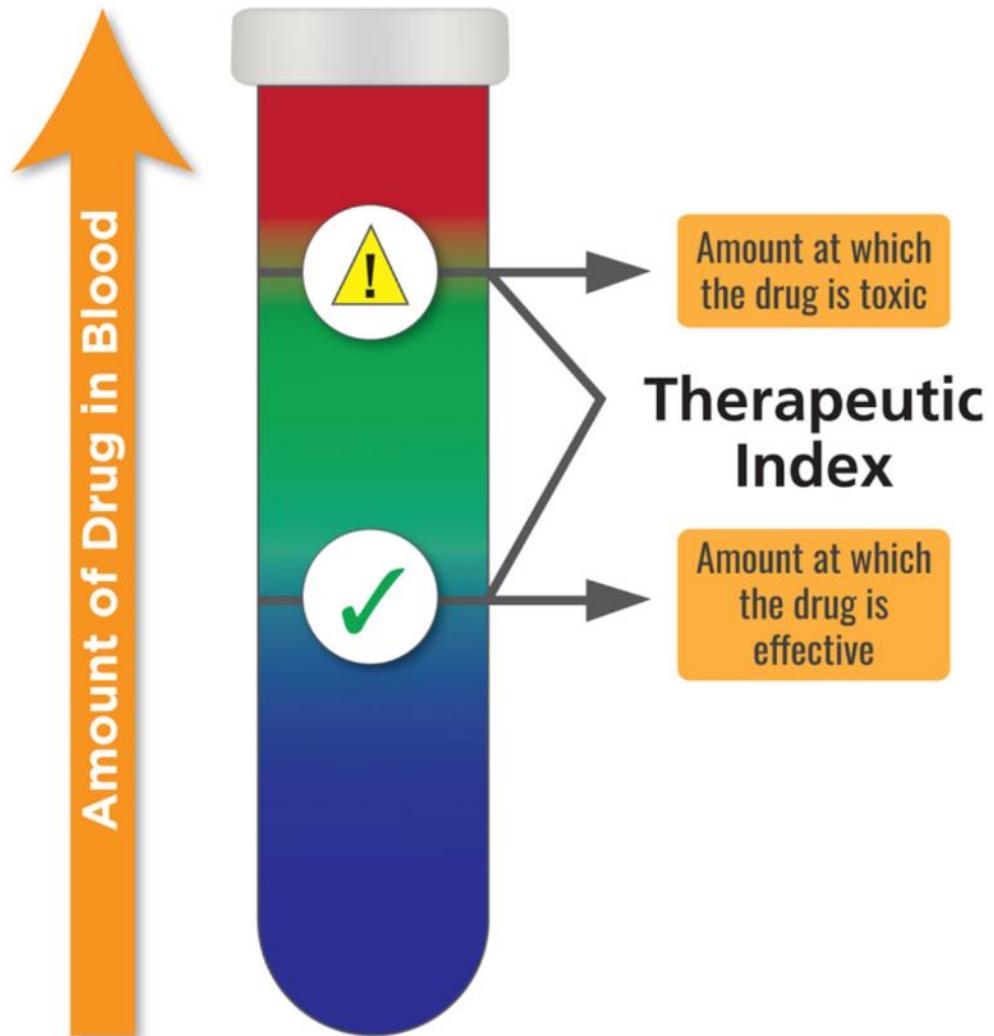
## Goal during Drug Discovery:

- Reduce CL to ensure sufficient drug concentration to elicit pharmacological response with reasonable dosing frequency
  - › Drug half-life is dependent on CL
- Elucidate CL mechanisms to understand factors that may lead to CL changes in humans

## Why understand Clearance?

- Drug efficacy and safety may be impacted*
- Unexpected increases or decreases in CL may result in changes in benefit:risk

# The Therapeutic Index (TI): Wide or Narrow?



- A **ratio** that compares the blood concentration at which a drug becomes toxic and the concentration at which the drug is effective
- The **larger** the TI, the **safer** the drug
- If the **TI is small**, the drug must be dosed carefully and the subject/patient should be **monitored closely** for any signs of drug toxicity
- **How much TI is needed depends on benefit:risk:** If the therapeutic benefit is large, there is more acceptability for safety risks (ie, healthy volunteers vs life saving drug in patients)

EXAMPLES	
Wide TI Drugs	Narrow TI Drugs
Ibuprofen Acetaminophen Antihistamines Most antibiotics	Digoxin Warfarin Cyclosporine Gentamicin

<https://clinicalinfo.hiv.gov/en/glossary/therapeutic-index-ti>

# POLL QUESTION 1

**Which is an incorrect statement about the “Therapeutic Index”**

- A. The therapeutic index is a ratio that compares the blood concentration at which a drug becomes toxic and the concentration at which the drug is effective
- B. If the concentration at which the drug is effective is similar or higher than the concentration at which the drug is toxic, the therapeutic index is wide
- C. Narrow therapeutic index drugs are used in clinical practice
- D. Over-the-counter drugs have a wide therapeutic index



# Toxicity: One of the Most Important Aspects to Distinguish is

## On-Target



- Pharmacological engagement of intended molecular target
- Typically unavoidable
- Therapeutic Index (TI) typically narrow
- All drug modalities

## Off-Target



- Pharmacological engagement of unintended molecular targets
- Physio-chemical property driven
- TI may be narrow or wide
- Small Molecules, gene editing, protein degraders, etc. (Biologics are less likely)

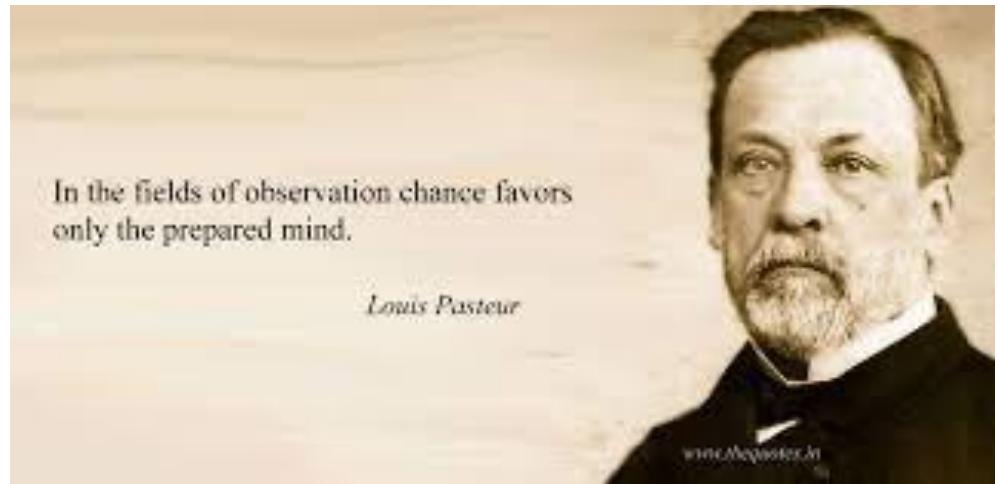
### STRATEGY

- Determine if a **path forward** exists:
  - Toxicity acceptable for indication?
  - Consider targeted drug delivery?

### STRATEGY

- Optimize **selectivity** during lead optimization
- Minimize toxicity rationally or empirically during drug development

# On-Target Toxicity



In the fields of observation chance favors  
only the prepared mind.

*Louis Pasteur*

# Toxicology in the Drug Development Process



**On-target safety issues:**  
**Understand / Manage**

**Off-target safety issues:**  
**Minimize / Mitigate**



# Criteria Consistent with On-Target Toxicity: What to Think About

## BIOLOGY

- Toxicity consistent with biology of the target?
- Toxicity consistent with other pathway modulators?
- Expression of target in tissue that has toxicity?

## KO MOUSE TOOLS

- Toxicity consistent with phenotype of KO animal?
- If no phenotype, toxicity *not* present when inhibitor drug is dosed to KO animal?

## CHEMICAL TOOLS

- Toxicity is present with chemically-diverse modulators?
- Toxicity *not* present with an inactive analog?

## OTHER

- Relationship between toxicity and target potency?
- Clean in vitro off-target profile?
- Toxicity consistent across species?

The more “Yes” to the above questions, the more likely on-target



# Criteria Consistent with On-Target Toxicity: More Granularity

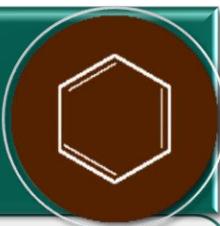
Criteria	Considerations Supporting ON Target
Consistent with the biology of the target	<ul style="list-style-type: none"> <li>- Caution with potential lack of relevance of biological link</li> <li>- Ruling out biological plausibility can be difficult</li> </ul>
Expression of target in the tissue that manifests toxicity	<ul style="list-style-type: none"> <li>- Tissue expression can be inconclusive or misleading and different expression sources might be conflicting</li> <li>- RNA expression does not imply protein expression</li> <li>- Species differences in tissue expression are possible</li> <li>- Target tissue might differ from tissue that manifests the toxicity</li> </ul>
Toxic effects consistent with other pathway inhibitors/effectors	<ul style="list-style-type: none"> <li>- More informative for linear, as opposed non-linear, complex pathway</li> </ul>
Toxic effects mitigated with pathway modulation	<ul style="list-style-type: none"> <li>- Pathway modulation to explore mitigation is not always possible</li> <li>- Difficult to achieve for inhibitor molecules (need to activate pathway to bypass toxicity)</li> </ul>
Consistent w phenotype of KO mice	<ul style="list-style-type: none"> <li>- Embryonic KOs can overestimate toxicity risk if target has function in development, or underestimate toxicity if compensatory mechanisms are triggered during development.</li> <li>- Full KOs can overestimate toxicity if protein has scaffolding functions that is affected by pharmacological intervention</li> <li>- KO phenotypes can vary across species, and they can over- or under-predict human risk</li> </ul>
Toxicity not present in KO dosed with inhibitor	<ul style="list-style-type: none"> <li>- Positive control with toxicity should be wild-type littermates to avoid confounding effects due to strain differences</li> <li>- Ensure adequate exposure to avoid a false negative outcome</li> </ul>
Toxicity present with chemically-diverse inhibitors	<ul style="list-style-type: none"> <li>- Chemical diversity should be significant for this diagnostic to be relevant</li> <li>- Chemically diverse molecules can share common off-targets</li> </ul>
Toxicity not present with an inactive analog	<ul style="list-style-type: none"> <li>- Analogs should be very structurally similar to reduce the chance of confounding off-target toxicities</li> </ul>
Strong relationship between toxicity and target potency	<ul style="list-style-type: none"> <li>- Easier to demonstrate using in vitro models</li> <li>- Wide range of target potencies needed (10-100 fold) to establish a clear relationship</li> </ul>
Clean in vitro off-target profile	<ul style="list-style-type: none"> <li>- Screens cover limited pharmacological space, cannot rule out possibility of off-target hits</li> </ul>
Toxicity consistent across multiple species	<ul style="list-style-type: none"> <li>- Toxicity could also be driven by a conserved off-target hit</li> </ul>

# Target Safety Assessment (TSA)



- Is the target tractable from a safety perspective?
- Is the Benefit:Risk appropriate for our patient population?
- Can we take advantage of early opportunities to test hypotheses and inform timely attrition, if necessary?

## Target Pharmacology across species



- Any species sensitivity differences?
- Probability of translation to human?

## Target Population



- Impact, if translation to human?
- Comeds, combination therapies with overlapping toxicities?

## Safety Screening Strategy during Lead Optimization



- Add biomarker endpoints to toxicology studies?
- Develop animal models (eg, KOs)?
- Enhance clinical monitorability with ID of new safety biomarkers?

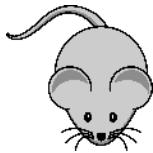
# TSA: Risk Table Example for “Target X”

- Target X inhibitors are first in class, very limited knowledge about safety of the target
- Target X KO mice are a great start: viable, fertile, normal lifespan, but pathology evaluations reveal phenotype affecting lung, kidney and heme system

Potential Target Tissues	Rationale	Probability of Occurrence (Translation to Humans)	Anticipated Impact if Present in Humans	Proposed De-risking Strategy	References
Lung	Highly expressed, non adverse lung findings in KO mice	High	Low (non adverse)	<ul style="list-style-type: none"><li>• Safety characterization of mouse KO</li><li>• Standard toxicology studies with drug</li><li>• Clinical monitoring: add <u>lung function</u></li></ul>	x, y, z
Kidney	Highly expressed, non adverse kidney findings in KO mice	Medium	Low (non adverse)	<ul style="list-style-type: none"><li>• Safety characterization of mouse KO</li><li>• Standard toxicology studies with drug + add <u>renal biomarkers</u></li><li>• Clinical monitoring: add <u>renal biomarkers</u></li></ul>	x, y, z
Hematological	Highly expressed, KO mice have reduced RBC	High	Medium (potentially adverse)	<ul style="list-style-type: none"><li>• Standard toxicology studies with drug</li><li>• Clinical monitoring (standard)</li></ul>	X, y, z



# TSA: Different KO Models: Advantages And Limitations



**TABLE 19.1** Genetic Models in Discovery Toxicology

	Advantages	Limitations
Germ line KOs	<ul style="list-style-type: none"> <li>• Rapid model generation</li> <li>• Complete KO in all tissues</li> <li>• Can be leveraged for developmental assessment</li> </ul>	<ul style="list-style-type: none"> <li>• Can be embryonic lethal</li> <li>• Potential confounding developmental effects, which can over or underestimate toxicity</li> <li>• Potential scaffolding effects can result in overestimation of toxicities</li> </ul>
Germ line—tissue-specific KO	<ul style="list-style-type: none"> <li>• Can bypass embryonic lethality if the KO gene is not critical for development in the particular tissue</li> <li>• Useful when interrogating the role of a gene in a particular tissue, including its role in development</li> </ul>	<ul style="list-style-type: none"> <li>• Can be embryonic lethal if KO tissue has a critical role in development</li> <li>• Potential confounding developmental effects</li> <li>• Potential scaffolding effects can result in overestimation of toxicities</li> <li>• Assessment limited to tissue with KO gene</li> </ul>
Conditional KO in adulthood	<ul style="list-style-type: none"> <li>• More relevant to pharmacological inhibition in adults</li> <li>• Flexibility in timing of the KO</li> </ul>	<ul style="list-style-type: none"> <li>• Potential scaffolding effects</li> <li>• Potential incomplete KO</li> <li>• Limitations in obtaining KO in brain</li> <li>• Potential confounding effects of tamoxifen if used</li> <li>• Potential scaffolding effects</li> </ul>
Conditional KO in adulthood—tissue specific	<ul style="list-style-type: none"> <li>• More relevant to pharmacological inhibition in adults</li> <li>• Useful when interrogating the role of a gene in a particular tissue in isolation</li> </ul>	<ul style="list-style-type: none"> <li>• Assessment limited to tissue with KO gene</li> <li>• Potential incomplete KO</li> <li>• Limitations in obtaining KO in brain</li> <li>• Potential confounding effects of tamoxifen if used</li> <li>• Potential scaffolding effects</li> </ul>
Germ line KI (inactive drug-binding site)	<ul style="list-style-type: none"> <li>• Complete KO in all tissues</li> <li>• Preserves scaffolding function and more relevant to pharmacological inhibition.</li> </ul>	<ul style="list-style-type: none"> <li>• Can be embryonic lethal</li> <li>• Potential confounding developmental effects</li> </ul>
Conditional KI on adulthood (inactive drug-binding site)	<ul style="list-style-type: none"> <li>• Complete KO in all tissues</li> <li>• Most relevant model to mimic pharmacological effects in adults</li> </ul>	<ul style="list-style-type: none"> <li>• Potential incomplete KO</li> <li>• Limitation in obtaining KO in brain</li> <li>• Potential confounding effects of tamoxifen</li> </ul>

- **Many models may be useful, but be mindful of limitations when interpreting findings**
- **Consider utility of rat KO models**

**Most relevant KO model to inform safety**

(Diaz and Maher, 2016)

# Examples of On-target Toxicity and Decision Making

## NAMPT Inhibitors for Oncology

- ⑩ Inhibitors caused on-target toxicities in rats and dogs  
❖ Toxicities are likely relevant to humans

Risks	Therapeutic Index	Monitorability	Level of Concern (for program progression)
Thrombocytopenia	Narrow	Yes	Low
Acute Cardiovascular	Narrow	No	High
Retinal Toxicity	Narrow	No	High

Sampath et al, 2015. Pharmacol Ther.

- ⑩ **Decision to not progress program**

## BTK Inhibitors for Autoimmune Disease

- ⑩ Inhibitors caused a pancreatic toxicity in rats, not dogs  
❖ Toxicity is not likely relevant to humans

Risks	Therapeutic Index	Monitorability	Level of Concern (for program progression)
Pancreas	Narrow	Limited	High (initially)

A Early-stage Peri-islet hemorrhage

B Late-stage Exocrine atrophy Hemorrhage with mixed cell infiltrates

Erickson, Schutt et al, 2017.  
J. Pharmacol. Exp Ther.

- ⑩ Investigation, learnings:  
❖ **Literature review:** Certain rat strains are susceptible to a background, age/obesity related pancreatic pathology that strongly resembles the SM BTK inhibitor lesions  
❖ **Btk KO rats** were generated: have the lesion (but Btk KO mice do not); thus, the toxicity is **on-target in rats**  
❖ **Humans** lacking functional BTK enzyme have **no reports** of pancreatic or metabolic-related diseases
- ⑩ **Decision to progress program**

# POLL QUESTION 2

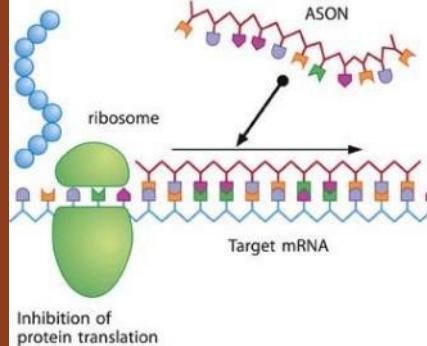
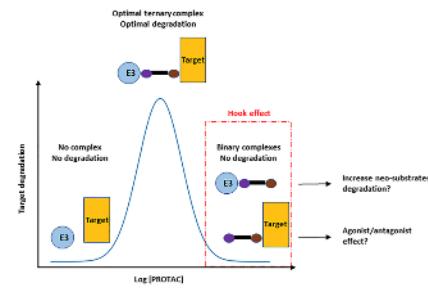
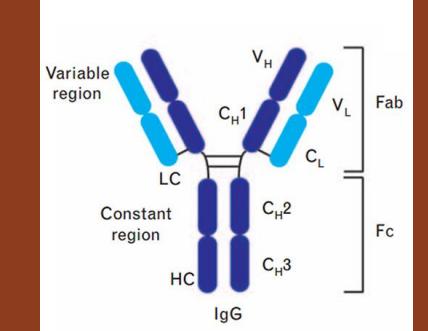
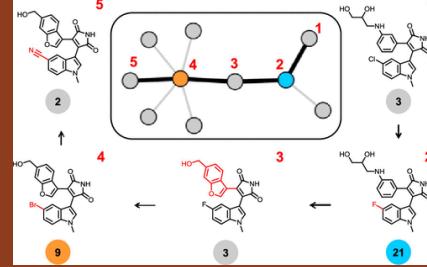
**Which is not a criteria supportive of on-target toxicity:**

- A. Toxicity present in an organ where the target has high expression levels.
- B. Toxicity present with two chemically distinct target modulators.
- C. Toxicity present when drug is administered to a knockout mouse model.
- D. Toxicity absent with analog that has weaker target potency.



# Off-Target Toxicity

# Off-target “Class effects” of different therapeutic modalities: a short introduction

Gene therapy (eg, AAV)	Nucleic acid based drugs (eg, antisense)	Protein degraders (eg, PROTAC)	Large Molecules (eg, monoclonal antibody)	Small Molecules (eg, most kinase inhibitors)
 <p>Hutt et al. 2022, Tox Path</p>	 <p>Inhibition of protein translation</p> <p>Goyenvalle et al. 2023, Nucleic Acid Therapeutics.</p>	 <p>Moreau et al. 2019, British J. Pharmacology</p>	 <p>acrobiosystems.com</p>	 <p>References on slide 27</p>
<p>Biodistribution outside of target tissue; <b>immune response</b> against transgene/product and <b>cytotoxicity</b></p> <ul style="list-style-type: none"> <li><b>Liver</b>, major site of transduction</li> <li><b>Dorsal root ganglia (DRG)</b></li> </ul>	<ul style="list-style-type: none"> <li><b>Immune stimulation</b> (Toll receptors, complement)</li> <li><b>Platelets</b>, including thrombocytopenia and coagulation effects</li> <li>Biodistribution and accumulation in proximal tubules of the <b>kidney</b>, and macrophages of the <b>liver</b></li> </ul>	<ul style="list-style-type: none"> <li><b>Off-target protein degradation</b></li> <li><b>Proteasome saturation</b> by ubiquitinated proteins</li> <li><b>“Hook effect”</b>: at high concentrations, saturated binding leads to formation of binary complexes (off-target degradation, agonist effects)</li> </ul>	<ul style="list-style-type: none"> <li><b>Infusion reactions</b>, cytokine release syndrome (innate immunity)</li> <li><b>Anti-drug antibodies</b> (adaptive immunity)</li> <li>Antibody-dependent cell-mediated cytotoxicity (ADCC) and Fc effector function</li> </ul>	<ul style="list-style-type: none"> <li><b>Promiscuity</b> against kinases, receptors, ion channels; may be due to physio-chemical properties that are modifiable           <ul style="list-style-type: none"> <li><b>Genotoxicity</b></li> <li><b>Hepatotoxicity</b></li> <li><b>Cardiovascular</b></li> <li><b>CNS</b></li> </ul> </li> </ul>

- An opportunity to optimize molecules, to mitigate these intrinsic properties that cause toxicities
- Often present in therapeutic ranges, but because of OFF-target mechanism, can be “dialed out” to ↑TI
- Toxicologist may partner with protein engineering, discovery biology and medicinal chemistry

# Toxicology in the Drug Development Process



On-target safety issues:  
Understand / Manage

Off-target safety issues:  
Minimize / Mitigate

# Safety is an Important Component of Small Molecule Lead Optimization

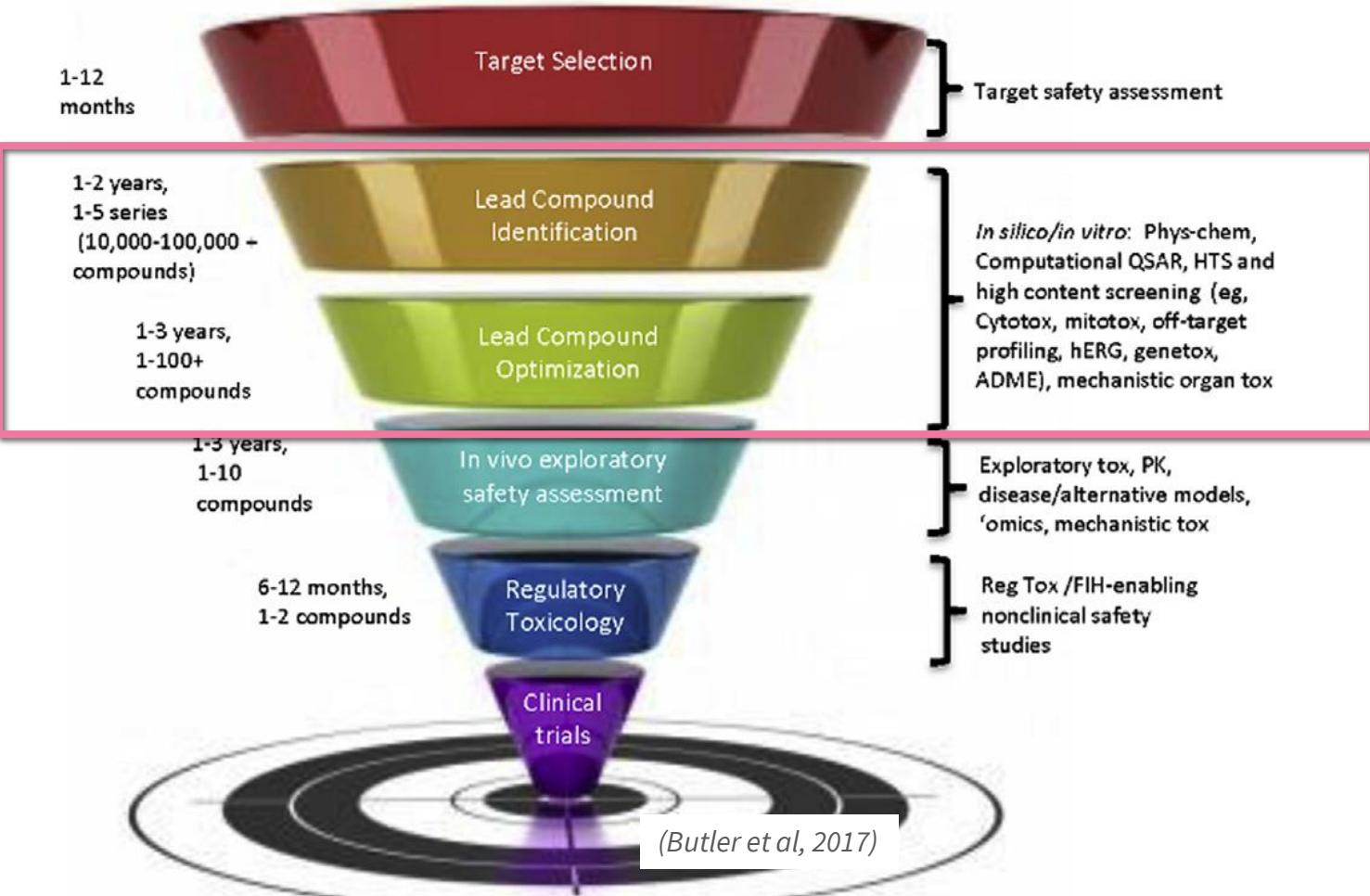


Fig. 1. Tiered Screening Approach and Associated Attrition (all causes) from Target Selection to First Phase Clinical Trials.

## Safety Lead Optimization

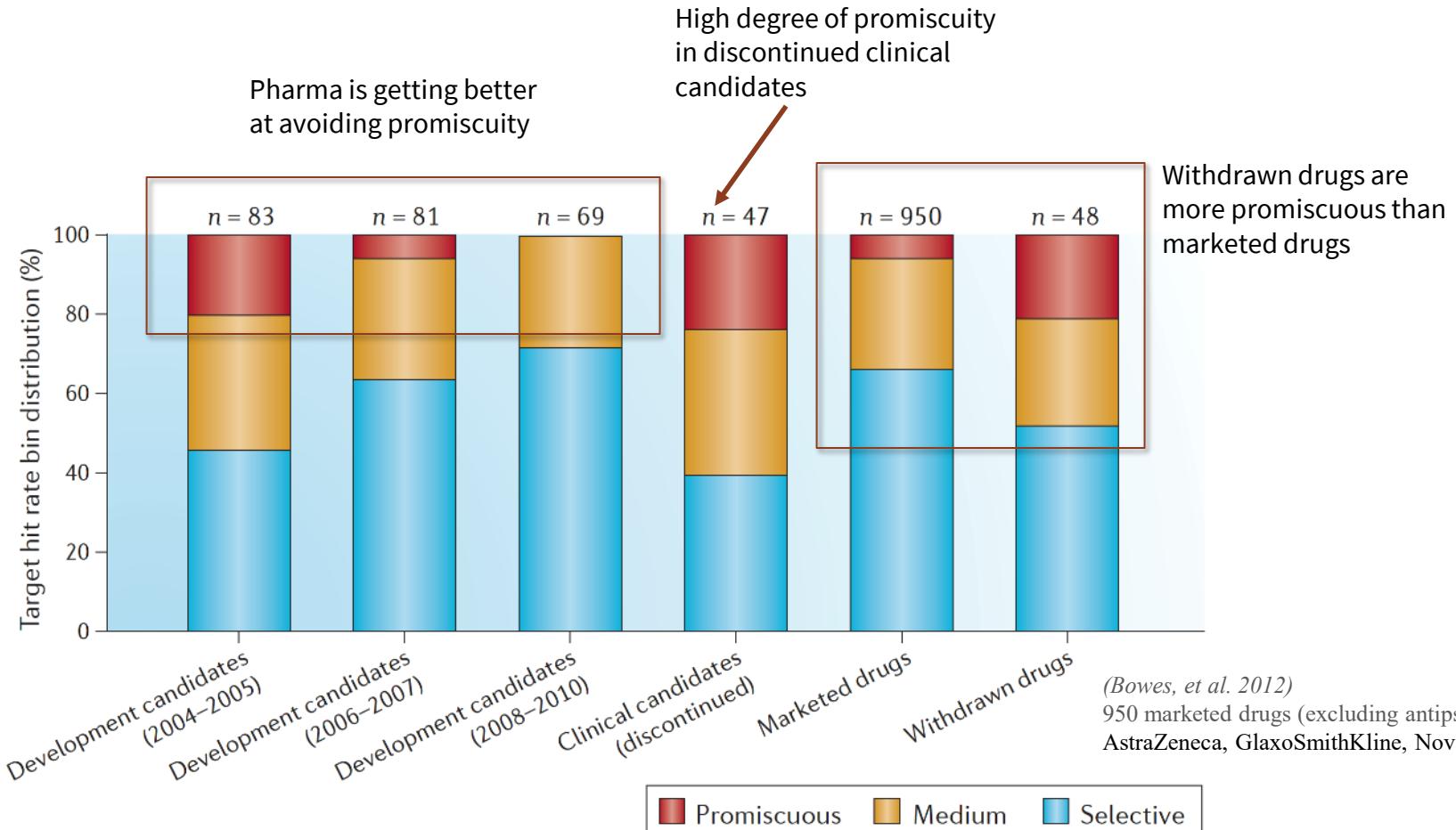
- Optimize progression of safe molecules
- Minimize discarding good molecules
  - Avoid Promiscuity
  - Optimize Selectivity

### Focus on

- Cardiovascular
- Genotoxicity
- Liver toxicity

# Promiscuity for Off-Targets Is Associated With Drug Attrition

- Property of a drug to interact with multiple molecular targets and exhibit distinct pharmacological effects





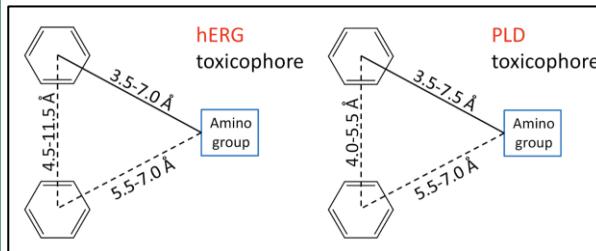
# Physio Chemical Properties Can Be Modified to Mitigate Promiscuity and Non-Specific Toxicity

## Promiscuity (hitting other molecular targets)

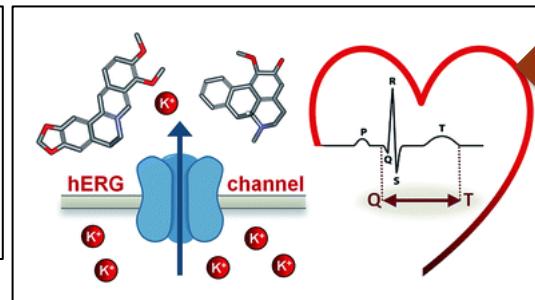
- Lipophilicity or ↑ cLogP
- Basicity or ↑ pKa
- 2<sup>o</sup> or 3<sup>o</sup> amines
- Polarity or ↓ TPSA

Gould and Templin 2023. Toxicology Letters.  
 Brown et al. 2020. J. Med Chem.  
 Rao et al. 2019 Front Big Data.  
 Lee et al. 2017. Bio and Med Chem Letters.  
 Peters et al. 2009 ChemMedChem.  
 Sun et al. 2013. Bioorg Med Chem Lett.  
 Azzaoui et al., 2007. ChemMedChem.

"two aromatic rings and an amino group"



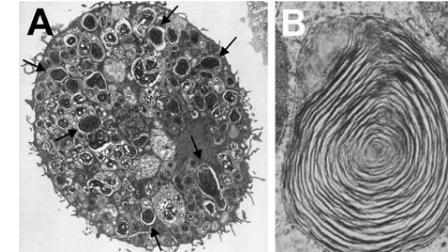
Slavov et al. 2017. Arch Toxicol.



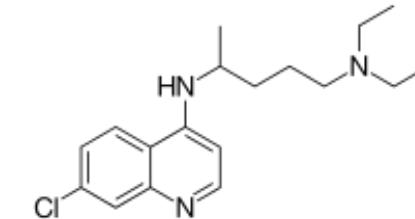
Some do both!

## Non-Specific Toxicity

- Cationic Amphiphilic Drugs (CAD's): cause drug-induced phospholipidosis (DIPL) in kidney, liver, lung, brain, cornea...resulting in lamellar body formation



Alveolar macrophage from a rat treated with Chlorphentermine shows numerous lamellar bodies. Breiden and Sandhoff 2020. Biol. Chem.

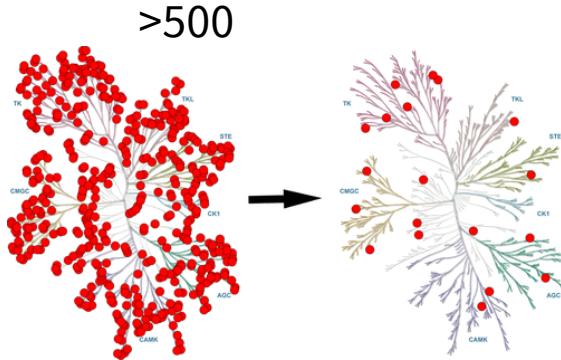


hERG IC<sub>50</sub> = 3uM

- ~80% of DIPL also bind hERG; this is associated with certain toxicophores: "two aromatic rings and an amino group"

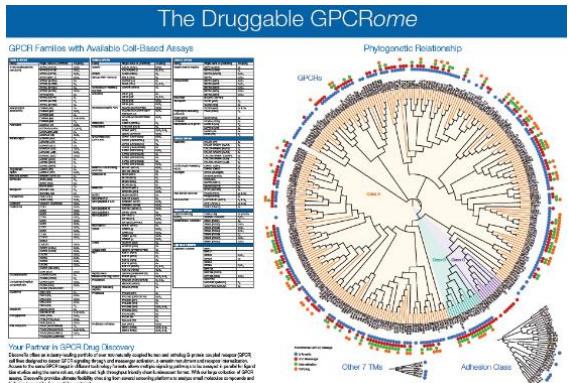
# Small Molecules are Screened in Extensive Assay Panels

## Kinase Panels



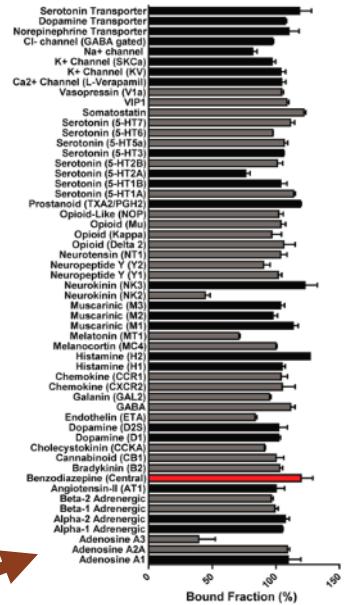
Bembenek et al. 2018. J. Chem. Inf. Model

## GPCR Panels

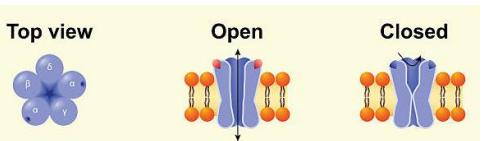


Can customize  
with your project safety in mind

## Receptor Panels



## Ion Channel Panels



- hERG
  - CaV
  - NaV
  - K<sub>ATP</sub>
  - GABA
  - Glycine
  - Glutamate
- Ligand binding assays**
- Flux-based assays**
- Automated or manual patch clamp**

Yu et al. 2016. Acta Pharmacologica Sinica

- *In silico* QSAR methods can be an alternative (or supplemental) approach to empirical testing

(eg, Zhao and Bourne, 2021 book chapter on structural kinome)

# Understanding Selectivity: Good Resources

## Reducing safety-related drug attrition: the use of *in vitro* pharmacological profiling

Joanne Bowes, Andrew J. Brown, Jacques Hamon, Wolfgang Jarolimek,

Arun Sridhar, Gareth Waldron and Steven Whitebread

(AstraZeneca, GSK, Novartis and Pfizer)

Table 1 | Recommended targets to provide an early assessment of the potential hazard of a compound or chemical series

Targets (gene)	Hit rate*		Main organ class or system	Effects	Refs <sup>§</sup>	
	Binding	Functional or enzymatic		Agonism or activation		
<b>G protein-coupled receptors</b>						
Adenosine receptor A <sub>2A</sub> (ADORA2A)	High	Low(agonist)	CVS, CNS	Coronary vasodilation; ↓ in BP and reflex; ↑ in HR; ↓ in platelet aggregation and leukocyte activation; ↓ in locomotor activity; sleep induction	Potential for stimulation of platelet aggregation; ↑ in BP; nervousness (tremors, agitation); arousal; insomnia	57
α <sub>1A</sub> -adrenergic receptor (ADRA1A)	High	Low(agonist); high (antagonist)	CVS, GI, CNS	Smooth muscle contraction; ↑ in BP; cardiac positive ionotropy; potential for arrhythmia; mydriasis; ↓ in insulin release	↓ in smooth muscle tone; orthostatic hypotension and ↑ in HR; dizziness; impact on various aspects of sexual function	58

Potential functional and pathological side effects related to off-target pharmacological activity

James J. Lynch III \*<sup>†</sup>, Terry R. Van Vleet, Scott W. Mittelstadt, Eric A.G. Blomme

AbbVie Inc., 1 North Waukegan Road, North Chicago, IL 60064, USA

Table 1  
The most commonly observed effects due to pharmacological activity.

Pharmacological target	Main organs & systems affected	Agonism/activation effects	Antagonism/inhibition effects	References
Acetylcholinesterase	NS, respiratory, GI, CV	Insufficient data	Apnea; bronchoconstriction; ↑ bronchial secretions; muscle relaxation; ataxia; ↑ salivation; lacrimation; ↑ pupil diameter; blurred vision; diarrhea; vomiting; ↑ → ↓ BP; ↑ ↓ HR; convulsions; coma; death	Carey et al. (2013); De Araujo Furtado et al. (2012); Gordon and Padnos (2000); Jokanović (2009); Mimica and Presecki (2009); Namba (1971)
Adenosine A <sub>1</sub> receptor	NS, renal, CV	↓ Pain; ↓ HR; ↓ BP; AV block; cardiac arrhythmia; heart failure; death	Convulsions; ↑ urine excretion; ↑ urinary sodium excretion	Bonizzoni, Milani, Ongini, Casati, and Monopoli (1995); Elzein and Zabolcky (2008); Fishberger et al. (1998); Rajaram and Joseph (2007)

## Safety screening in early drug discovery: An optimized assay panel

Stefanie Bendels<sup>a</sup>, Caterina Bissantz<sup>a</sup>, Bernhard Fasching<sup>a</sup>, Grégoire Gerebtzoff<sup>a</sup>, Wolfgang Guba<sup>a</sup>, Manfred Kansy<sup>a</sup>, Jacques Migeon<sup>b</sup>, Susanne Mohr<sup>a</sup>, Jens-Uwe Peters<sup>a</sup>, Fabien Tillier<sup>b</sup>, René Wyler<sup>a</sup>, Christian Lerner<sup>a</sup>, Christian Kramer<sup>a</sup>, Hans Richter<sup>a</sup>, Sonia Roberts<sup>a,\*</sup>

<sup>a</sup> Roche Pharma Research and Early Development, Pharmaceutical Sciences, Roche Innovation Center Basel, Switzerland

<sup>b</sup> Eurofins Cerep Panlabs, Le bâti l'Évêque, 86600 Celle l'Evescault, France

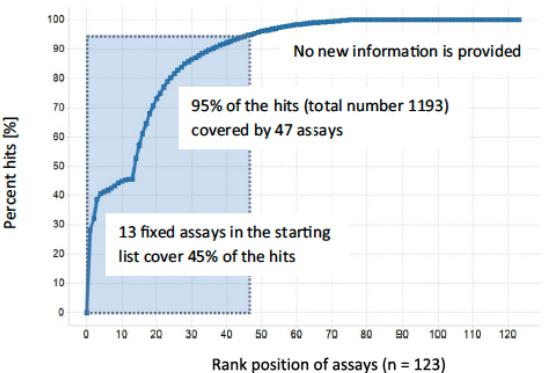
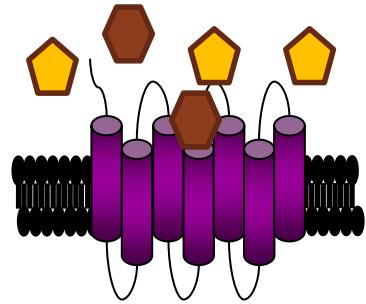


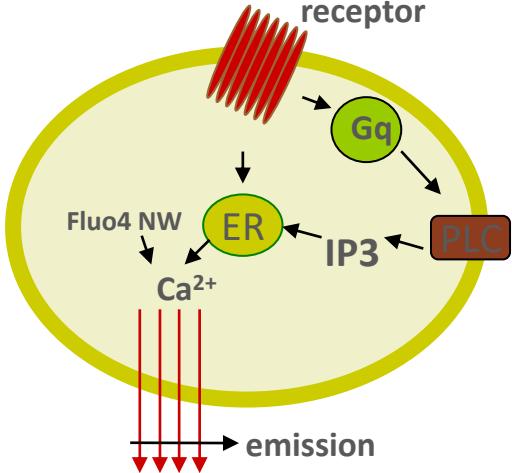
Fig. 4. Percentage of hits (compounds that are active in ≥1 assay) covered by the 123 assays after the ranking procedure.

# Selectivity: Important to Understand Functional Translation

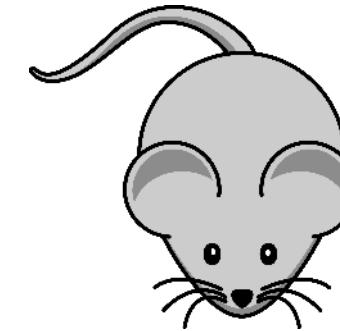
**Tier 1**  
e.g. Binding Assay



**Tier 2**  
e.g. Functional Assay



**Tier 3**  
e.g. *In vivo*



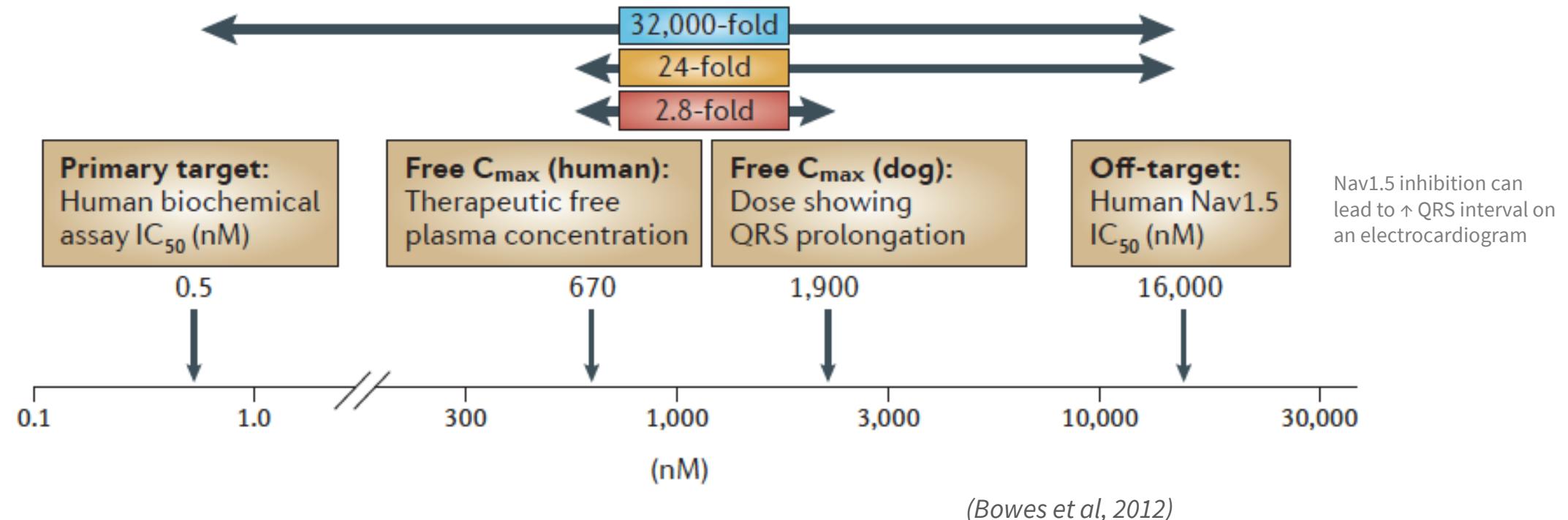
- Direct measure of affinity
- Single, defined binding site
- No differentiation of modes of action (agonist vs antagonist)

- Functional Translation – signal transduction or phenotypic screen
- Can differentiate agonist vs antagonist

- Assumptions:**
- Free Drug Hypothesis: only unbound drug can interact with the target
  - Free  $C_{max}$  frequently used as a relevant measure of drug exposure

# Selectivity: Off-Target Hits In The Context Of Exposure

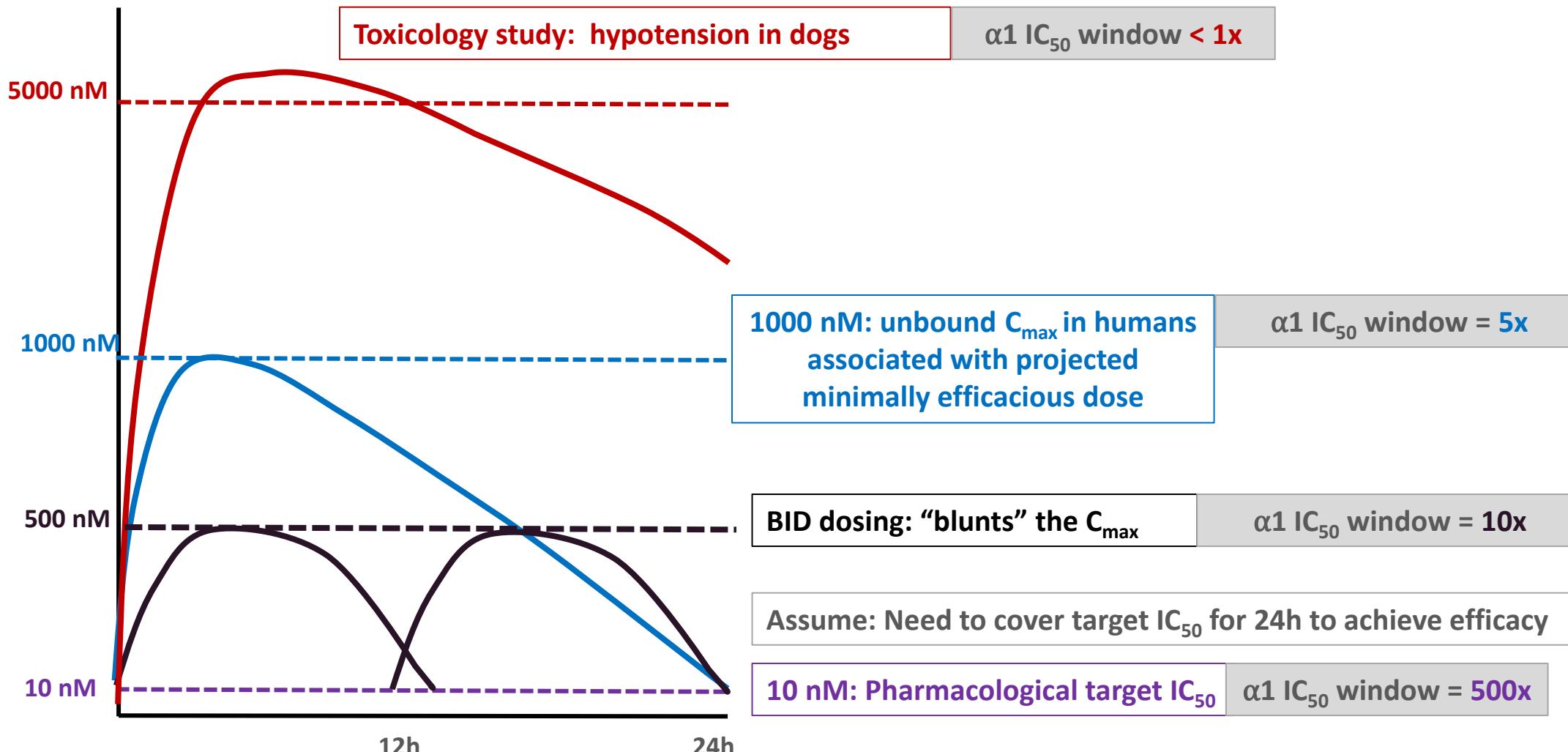
- Toxicities from off-target hits (eg ion channels) are typically assumed to be driven by unbound (free) drug
- In vitro and in vivo concentrations should consider protein binding



# Small molecule Selectivity Exercise:

Drug X hits alpha1 adrenergic receptor ( $IC_{50} = 5000\text{nM}$ )

Off-target effects manifested as hypotension



# Example of Mitigating Off-Target Toxicity by Improving Selectivity

## Mitigation of opioid off-target effects and identification of structural drivers of opioid receptor engagement for BACE-1 small molecule inhibitors

Dolores Diaz<sup>1</sup>, Donna Dambach<sup>1</sup>, Michael Siu<sup>2</sup>, Kevin Hunt<sup>3</sup>, Allen Thomas<sup>3</sup>, Joseph Lyssikatos<sup>2</sup>, Xingrong Liu<sup>4</sup>, Sock Lewin-Koh<sup>5</sup>, Bobby McCray<sup>1</sup>, and Kevin Ford<sup>1</sup>

<sup>1</sup>Safety Assessment, Genentech Inc., South San Francisco, CA, USA, <sup>2</sup>Medicinal Chemistry, Genentech Inc., South San Francisco, CA, USA,

<sup>3</sup>Array Biopharma, Boulder, CO, USA, <sup>4</sup>Drug Metabolism and Pharmacokinetics, Genentech Inc., South San Francisco, CA, USA, and

<sup>5</sup>Biostatistics, Genentech Inc., South San Francisco, CA, USA

### Tier 2

Table 1. EC<sub>50</sub> values in μM for MOP, KOP, and DOP agonistic activity in human recombinant CHO-K1 cells for GNE-962, GNE-892, and GNE-629.

	MOP	KOP	DOP
GNE-962	0.6	7.2	>30
GNE-892	0.5	8.9	>30
GNE-629	15.0	>30	>30

GNE-892  
BACE-1 SM inhibitor:  
off-target agonistic  
activity on opioid  
receptors (MOP)

### MOP off-target effects translate in vivo

### Tier 3

Table 3. Clinical observation of mild or moderate hypoactivity in rats dosed with GNE-892 at 100, 300, and 600 mg/kg for 6 consecutive days; n = 5 rats/group.

	Number of rats with hypoactivity (7 h post-dose)			
	d1	d2	d3	d6
Vehicle control	0/5	0/5	0/5	0/5
GNE-892, 100 mg/kg	0/5	0/5	0/5	0/5
GNE-892, 300 mg/kg	5/5	5/5	5/5	0/5
GNE-892, 600 mg/kg	5/5	5/5	5/5	0/4

### Risk assessment using unbound drug exposures

Table 4. Relationship between the absence and the presence of clinical observation of hypoactivity in mice (GNE-962) and rats (GNE-892 and GNE-629), and free brain levels in μM for GNE-962, GNE-892, and GNE-629.

	Hypoactivity (lowest brain levels, μM fu)	No hypoactivity (highest brain levels, μM fu)	Minimally efficacious brain levels (μM fu)	Brain Aβ reduction (%)	Brain (fu)	Safety margin
GNE-962	0.14	0.07	0.03	51	0.03	2.3x
GNE-892	0.44	0.32	0.2	55	0.20	1.6x
GNE-629	NA	0.70	0.03	58	0.03	23x

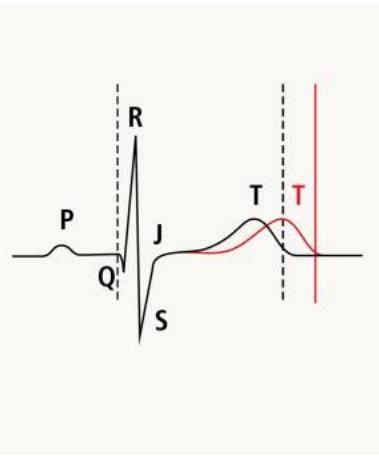
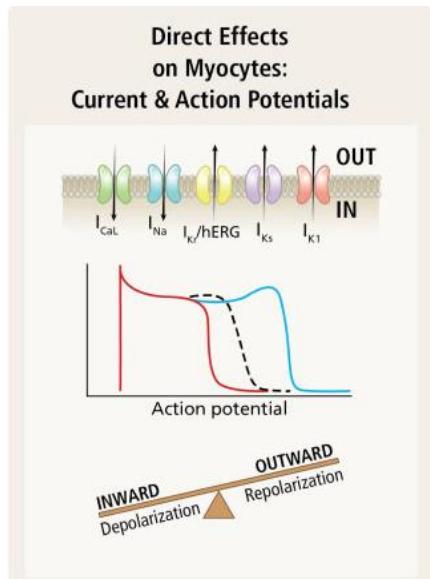
**Safety margin (~ Therapeutic Index): Ratio of safe concentration to efficacious concentration Expanded with backup molecule (GNE-629)**

# Cardiovascular Toxicity: Minimizing Risk in Lead Optimization



## Screening assays (tiered)

1. Ion channel patch clamp: **hERG**, NaV1.5, CaV1.2 (2-pt screen, IC<sub>50</sub>)
2. Human cardiomyocytes
3. Rabbit or guinea pig Langendorff, isolated heart assay
4. In vivo rodent CV (does not derisk hERG hit)
5. In vivo non-rodent CV (may be anesthetized)



Valentin et al. 2022. Toxicol Sci.

## GLP studies to support clinical trials follow the ICH S7 guidance

### 2.7.2 Cardiovascular System



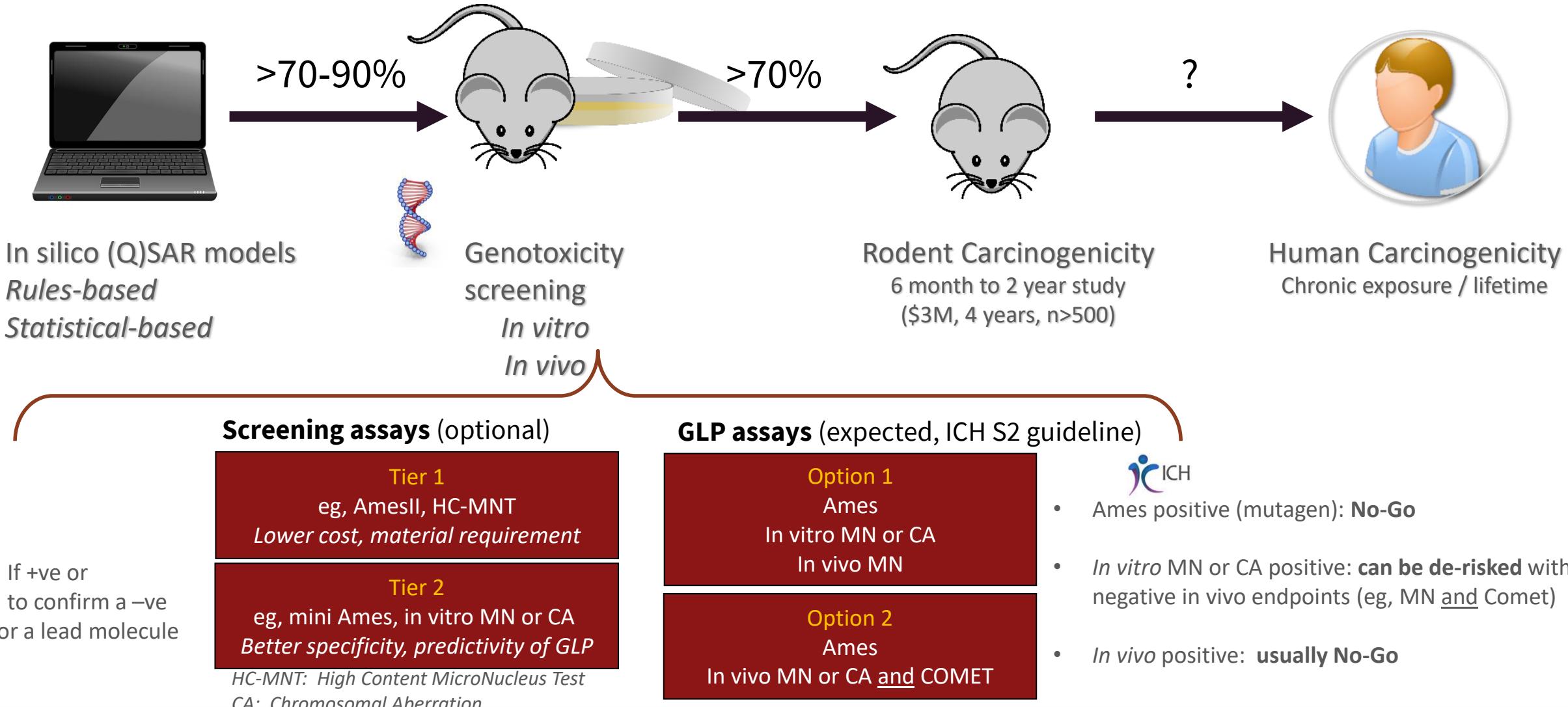
Effects of the test substance on the cardiovascular system should be assessed appropriately. Blood pressure, heart rate, and the electrocardiogram should be evaluated. In vivo, in vitro and/or ex vivo evaluations, including methods for repolarization and conductance abnormalities, should also be considered.

Typically...

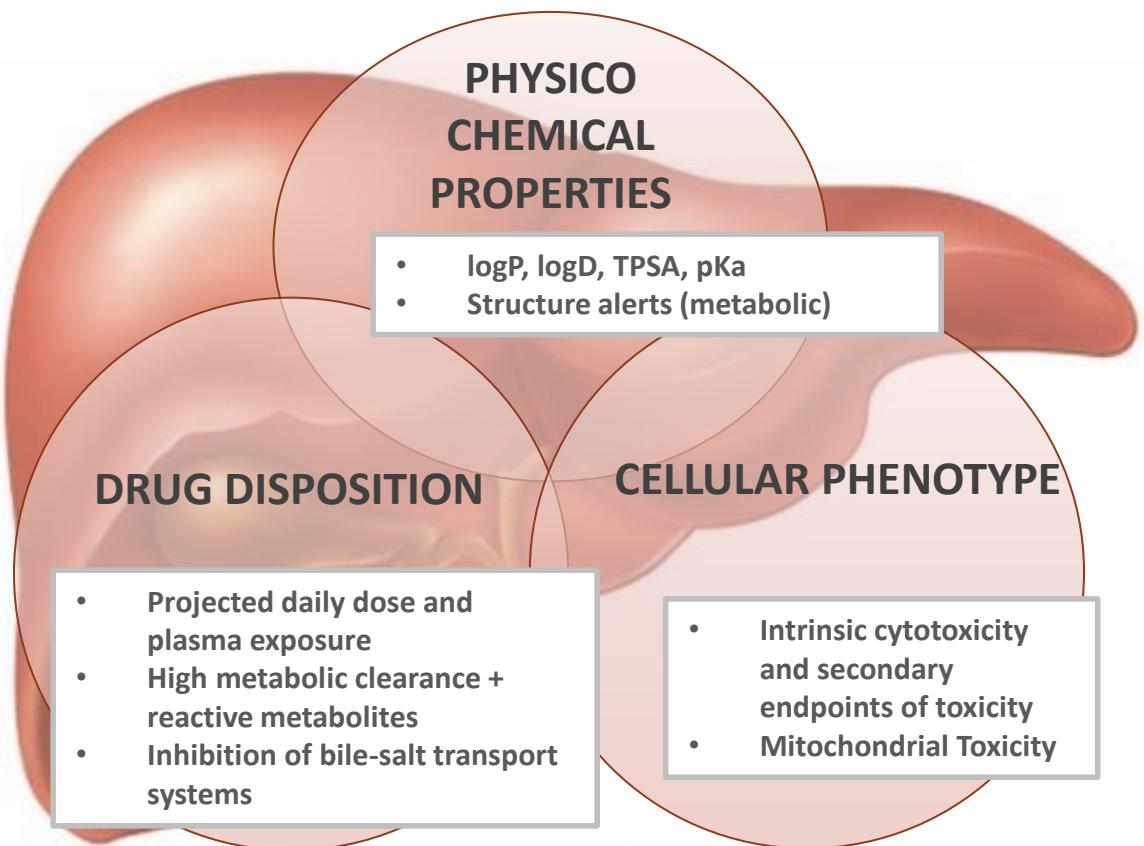
1. Telemetry-instrumented non-rodent single dose
2. Electrocardiograms in repeat-dose non-rodent study

# Genotoxicity: Minimizing Risk in SM Lead Optimization

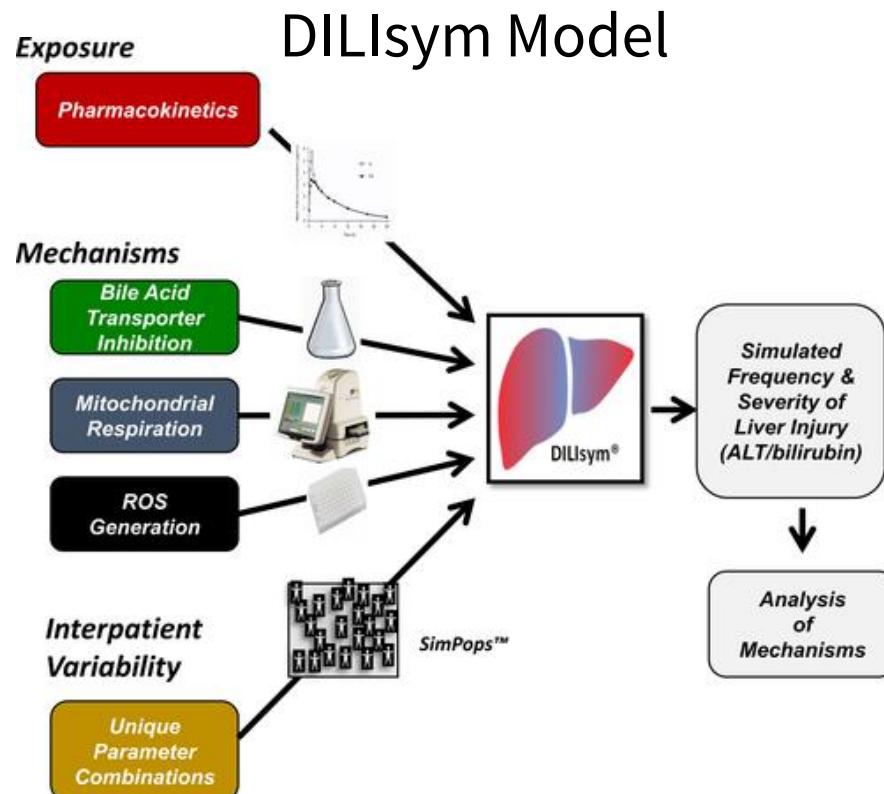
- Non-Oncology > Oncology



# Intrinsic Drug Induced Liver Injury (DILI): Minimizing Risk in Lead Optimization



Modified from Will Proctor. Donna Dambach 2014. Drug-Induced Hepatotoxicity: Advances in Preclinical Predictive Strategies and Tools, in Predictive ADMET

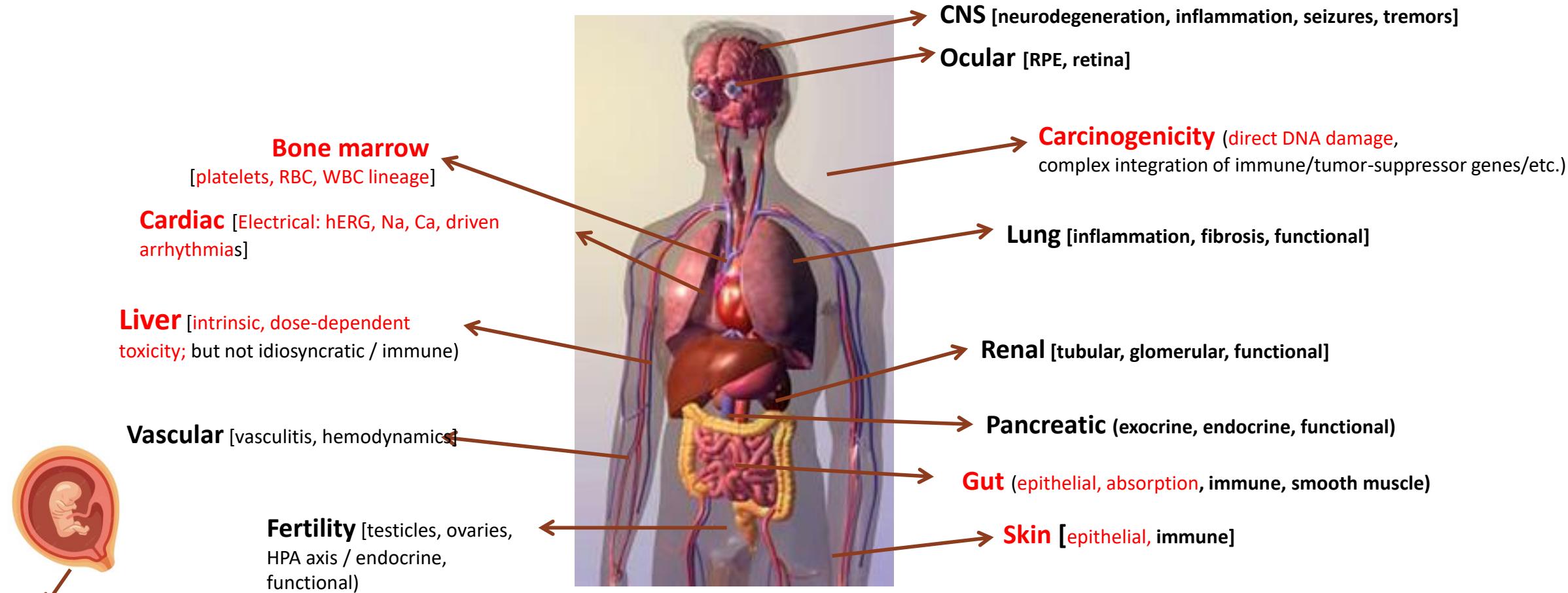


Paul Watkins, Clin Transl Sci 2019. The DILI-sim Initiative: Insights into Hepatotoxicity Mechanisms and Biomarker Interpretation

- “...most **dose-dependent [intrinsic] hepatotoxicity** can be accounted for by combinations of **just three mechanisms** (oxidative stress, interference with mitochondrial respiration, and alterations in bile acid homeostasis)..."

Major Caveat: idiosyncratic or immune-mediated DILI is much harder to predict

# Many Toxicities cannot be Recapitulated *In Vitro*



Embryo-fetal development

- In red: toxicities for which reasonably good *in vitro* models exist
- Animal studies are still necessary for identifying many on- and off-target risks and defining a TI

# POLL QUESTION 3

**Which of the following statements is false:**

- A. Genotoxicity is determined by carcinogenicity studies prior to initiating clinical trials.
- B. Promiscuity is associated with physio-chemical properties like increased lipophilicity, increased numbers of basic moieties, and decreased polarity.
- C. Inhibition of the HERG (Human Ether a-go-go Related Gene) potassium ion channel results in a longer QT interval and cardiac arrhythmias.
- D. Intrinsic drug-induced liver injury (DILI) is easier to predict than immune-mediated or idiosyncratic DILI.



# 10 Minute Q&A, Break



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# Toxicology in the Development Space

**Dolo Diaz, PhD, DABT**

Vice President, Development Sciences,  
Denali Therapeutics

# Toxicology in The Drug Development Process



## Preclinical (IND-Enabling)

**GOAL:** Determine if molecule is safe for humans and guide human dosing

- Design, conduct and interpret toxicology studies in animals
- Determine (with clinical) if the molecule is safe to administer to humans and likelihood of a TI
- Guide first-in-human dose selection and monitoring plan for safe dose escalation in human

# Animal testing is an essential component of drug development

Despite the progress made, in vitro systems do not recapitulate the complexity of the human body to a sufficient degree to enable safe clinical dosing of a drug

Animal studies are conducted with a high degree of ethical and animal welfare considerations; the 3Rs are often applied (Reduce, Refine, Replace)

- Regulatory requirement to test drugs in a rodent and a non-rodent species
- Rationale: increase ability to identify toxicity that may affect humans

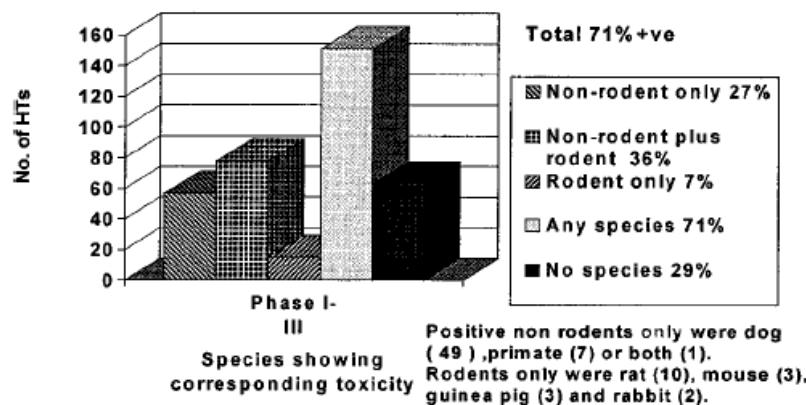
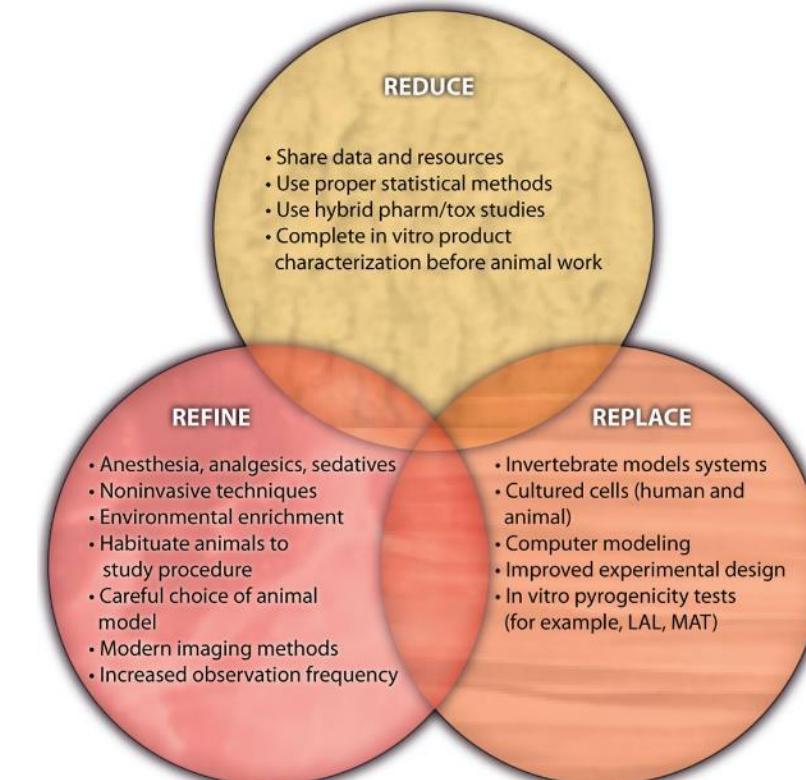


FIG. 3. Concordance of human toxicity from animals.

(Olson et al. 2000)



Science.org

# Animal testing is an essential component of drug development

Animal testing results in a high level of attrition in drug development:  
many molecules that are tested in animals never reach human trials

Toxicology findings drive a large % of drug attrition

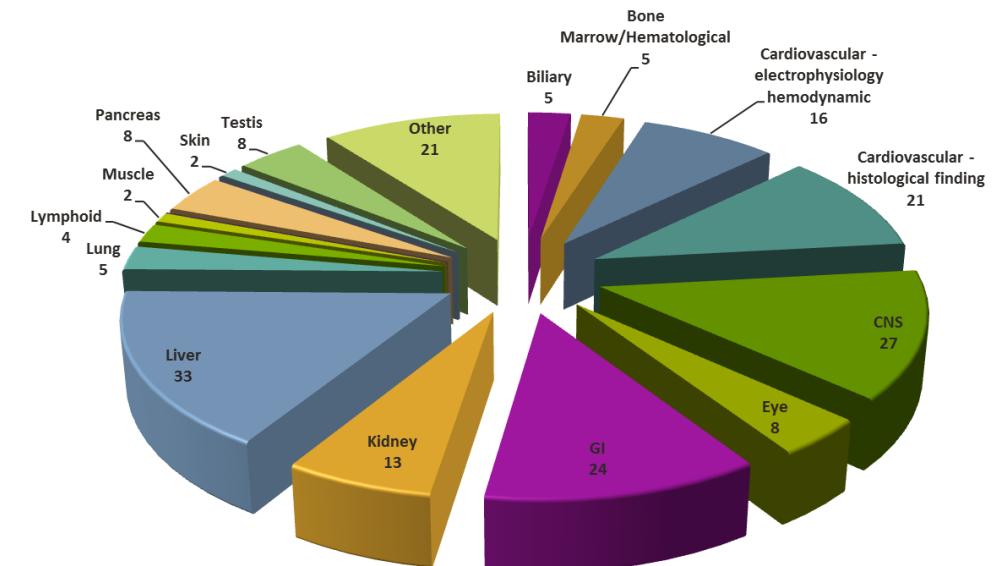
Table 1 | Populations of the primary cause of failure categories for terminated compounds\*

Termination reason	Overall	Period		Phase		
		2000–2005	2006–2010	Candidate nomination	Phase I	Phase II
605 compounds terminated	605	362	243	356	157	89
Clinical safety	68 (11%)	48 (13%)	20 (8%)	5 (1%)	40 (25%)	22 (25%)
Commercial	40 (7%)	23 (6%)	17 (7%)	26 (7%)	10 (6%)	4 (4%)
Efficacy	55 (9%)	45 (11%)	10 (4%)	10 (3%)	14 (9%)	31 (35%)
Formulation	9 (1%)	4 (1%)	5 (2%)	8 (2%)	1 (0.6%)	0
Non-clinical toxicology	240 (40%)	144 (40%)	96 (40%)	211 (59%)	21 (13%)	7 (8%)
Patent issue	1 (0.2%)	0	1 (0.4%)	1 (0.3%)	0	0
Pharmacokinetics or bioavailability	29 (5%)	19 (5%)	10 (4%)	3 (0.8%)	25 (16%)	1 (1%)
Rationalization of company portfolio	124 (21%)	46 (13%)	78 (32%)	75 (21%)	29 (18%)	19 (21%)
Regulatory	2 (0.3%)	2 (0.6%)	0	1 (0.3%)	1 (0.6%)	0
Scientific	33 (5%)	28 (8%)	5 (2%)	13 (4%)	15 (10%)	5 (6%)
Technical	3 (1%)	3 (1%)	0	2 (0.6%)	1 (0.6%)	0
Other	1 (0.2%)	0	1 (0.4%)	1 (0.3%)	0	0
Total	605	362	243	356	157	89

\*Table entries for each column indicate the total number and the percentage in parentheses.

Waring et al, 2015; *Nature Reviews Drug Discovery*; “An analysis of the attrition of drug candidates from four major pharmaceutical companies” (AZ, GSK, Pfizer, Lilly)

Most Common Toxicities Resulting in Attrition



Sherry Ralston. IQ DruSafe Attrition of Pharmaceuticals during Preclinical Development. SOT 2017.

# Selection of Appropriate Animal Species is Key in Toxicology Studies



## RAT

- Most commonly used
- Larger size allows for better sampling
- More historical control databases for findings



## MOUSE

- Less commonly used
- Smaller size is material sparing when needed
- Can use genetically engineered models



## DOG

- Most commonly used for SMs
- Some consider use more ethical than monkey
- Large availability



## MONKEY

- Almost always used for biotherapeutics
- Smaller size can be material sparing
- More reagents available to explore findings

### Criteria For Selection Of Tox Species:

#### Relevant pharmacology

- Is the target expressed?
- Is there evidence that drug has pharmacodynamic effects?
- Need at least one species with relevant pharmacology

#### Exposure (PK)

- Is the exposure sufficient to de-risk? (need multiples of human exposure)

#### Coverage of human metabolites

- Coverage in at least one species?

# POLL QUESTION 4

**Which statement is CORRECT re: species selection in toxicology studies:**

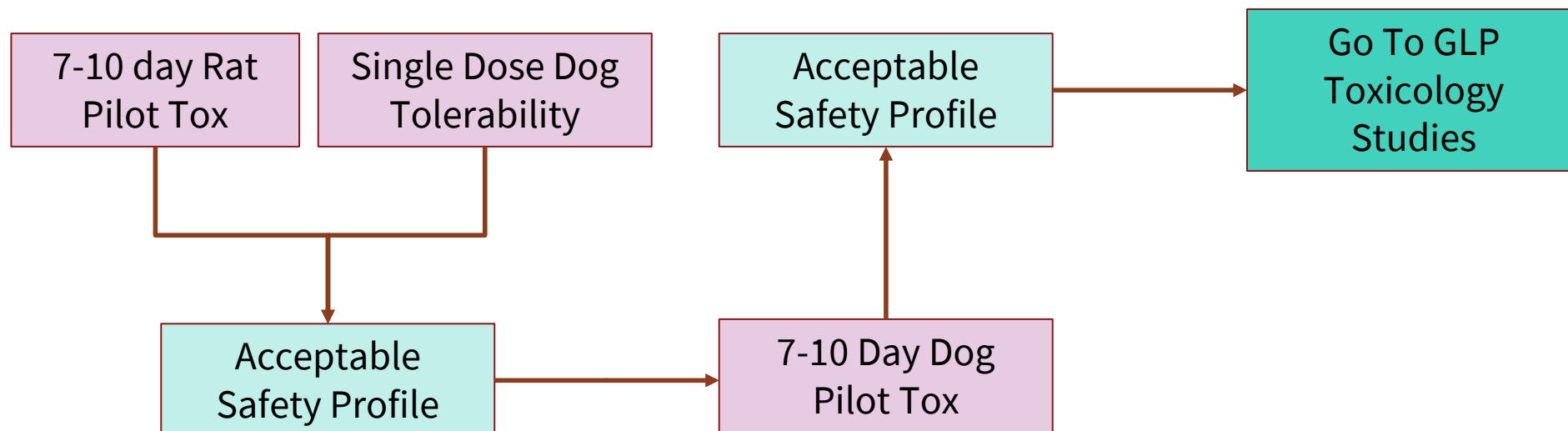
- A. Monkeys are smaller than dogs and use less test material.
- B. Pharmacological relevance in both toxicology species is a regulatory requirement.
- C. Rodents are commonly used for biotherapeutic safety testing.
- D. Metabolite coverage in one toxicology species is required.



# Conceptual Animal Study Flow Prior to "Go To GLP-Tox" Decision



- First animal studies are short in duration, limited number of animals, non-GLP
- Typically terminal studies in dog/monkey are gated on “go” results from rodent studies
- These early animal studies are referred to as “pilot tox”, “dose-range finding” or “exploratory” studies
- Goal of pilot toxicology studies:
  - Establish initial safety profile of molecule and target organs of toxicity, explore mechanism
  - Initial understanding of safety margins
  - Inform design of FIH-Enabling GLP Tox studies



# Typical Design of IND-Enabling GLP Toxicology Studies

Standard dosing duration : 28 days			
Dose	Number of Animals		Target exposure multiples
	Rodent	Non-rodent	
1. Vehicle	10 per sex	5 per sex	N/A
2. Low	10 per sex	5 per sex	1-3X
3. Medium	10 per sex	5 per sex	5-15X
4. High dose	10 per sex	5 per sex	30-50X +

## Dose Selection Considerations

- PK and Tolerability in pilot tox studies
- Desirable to identify toxicity at the high dose
- Targeted multiples of projected human efficacious exposure

## Endpoints Included

**Pharmacokinetics:** drug concentration in plasma and other tissues; AUC, Cmax, Tmax

**Clinical Observations:** physical signs or behaviors

**Clinical Pathology:** collect/analyze blood and serum:

- Hematology: red cell count, white cell count, platelet counts
- Serum chemistry: liver/kidney function, electrolytes, serum proteins and lipids

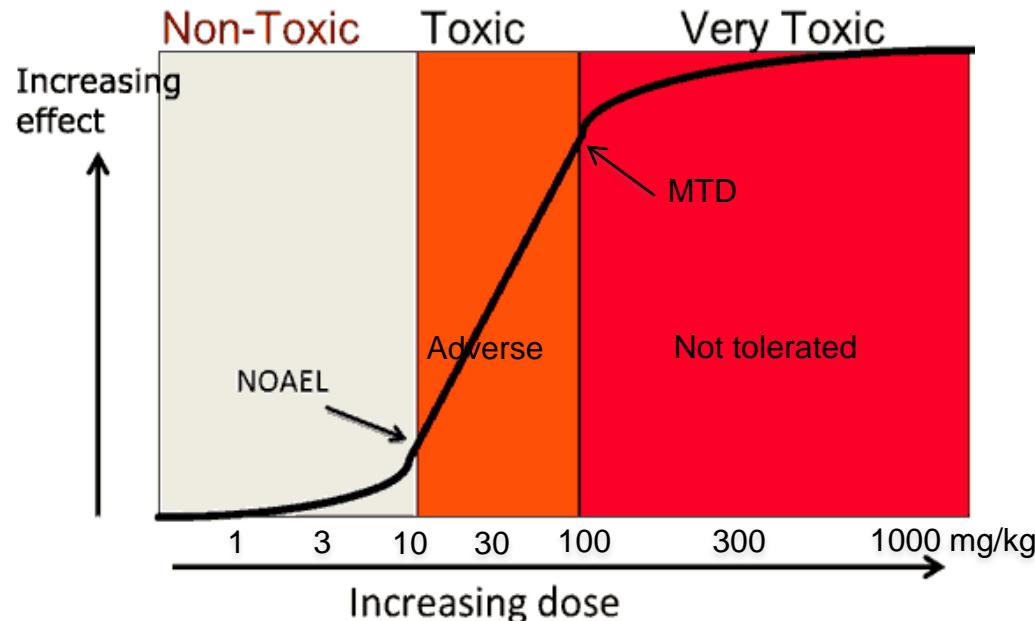
**Necropsy:** macroscopic evaluations internal organs and tissues; weigh major organs

**Histopathology:** microscopic evaluation of tissues:

- Bone, brain, heart, intestine, kidneys, liver, lungs, lymph nodes, ovaries, peripheral nerves, pancreas, skeletal muscle, skin, spleen, testes.

**Additional endpoints** based on findings in pilot tox studies or any other concerns

# Understanding “Exposure-effect Relationship” is the Foundation of Toxicity Evaluation In Vivo



- **Adverse** = change [...] that results in an **impairment of functional capacity** [...] or the capacity to compensate for additional stress (*Keller et al. 2012; ToxSci*).
- **NOAEL** = **No Observed Adverse Effect Level**
- **MTD** = **Maximum Tolerated Dose**
- **MABEL**: Minimum Anticipated Biological Effect Level

- ⑩ Typically, drugs become more toxic with increasing dose (or exposure)
- ⑩ Toxicology studies examine effects over a range of doses/concentrations
  - ❖ What is the highest dose at which no adverse effects are observed?
    - NOAEL = 10 mg/kg
  - ❖ What is the highest dose that is tolerated?
    - MTD = 100 mg/kg. Dose-limiting-toxicities likely occur here.
  - ❖ What is the shape of the dose-response curve? Steep, shallow?
  - ❖ Are different organ systems impacted at different dose levels?

# Good Laboratory Practices (GLP)

- ⑩ **Quality system** concerned with the process and the conditions under which non-clinical health and environmental safety studies are planned, performed, monitored, recorded, archived and reported.
- ⑩ FDA and other HAs require most studies be performed in compliance with GLP (**Good Laboratory Practice**) or equivalent
- ⑩ Introduced in the US in 1978 in response to the Industrial BioTest Labs scandal
- ⑩ Goal: ensure and promote **safety, consistency, high quality, and reliability** of chemicals in the process of non-clinical and laboratory testing
- ⑩ Compliance to GLP is not required for discovery, basic research, screening or other studies where the safety of a product is not being assessed. GLP is required for extrapolation to humans.
- ⑩ Non-GLP studies can be of high quality, and oftentimes non-GLP studies are submitted to HAs (supportive, mechanistic studies)
- ⑩ GLP does not ensure study is well designed!



# Guidances for Regulatory Packages and Conduct of Studies: ICH

## ICH (International Council for Harmonization)

- ⑩ US, EU, Japan (generally accepted worldwide)
- ⑩ Brings together health authorities and pharmaceutical industry to create guidelines around scientific and technical aspects of drug approval.
- ⑩ Framework of expectations for different aspects of safety assessment

The ICH topics are divided into four categories and ICH topic codes are assigned according to these categories.

- Q Quality Guidelines**  
Harmonisation achievements in the Quality area include pivotal milestones such as the conduct of stability studies, defining relevant thresholds for impurities testing and a more flexible approach to pharmaceutical quality based on Good Manufacturing Practice (GMP) risk management.
- S Safety Guidelines**  
ICH has produced a comprehensive set of safety Guidelines to uncover potential risks like carcinogenicity, genotoxicity and reprotoxicity. A recent breakthrough has been a non-clinical testing strategy for assessing the QT interval prolongation liability: the single most important cause of drug withdrawals in recent years.
- E Efficacy Guidelines**  
The work carried out by ICH under the Efficacy heading is concerned with the design, conduct, safety and reporting of clinical trials. It also covers novel types of medicines derived from biotechnological processes and the use of pharmacogenetics/genomics techniques to produce better targeted medicines.
- M Multidisciplinary Guidelines**  
These are the cross-cutting topics which do not fit uniquely into one of the Quality, Safety and Efficacy categories. It includes the ICH medical terminology (MedDRA), the Common Technical Document (CTD) and the development of Electronic Standards for the Transfer of Regulatory Information (ESTRI).

<https://www.ich.org/page/safety-guidelines>

ICH harmonisation for better health

HOME ABOUT ICH WORK PRODUCTS MEETINGS TRAINING NEWSROOM Search...

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### Safety Guidelines

ICH has produced a comprehensive set of safety Guidelines to uncover potential risks like carcinogenicity, genotoxicity and reprotoxicity. A recent breakthrough has been a non-clinical testing strategy for assessing the QT interval prolongation liability: the single most important cause of drug withdrawals in recent years.

Stakeholders are invited to report Safety Guideline issues at [safety@ich.org](mailto:safety@ich.org).

- S1A - S1C Carcinogenicity Studies**
- S2 Genotoxicity Studies**
- S3A - S3B Toxicokinetics and Pharmacokinetics**
- S4 Toxicity Testing**
  - > **S4 Duration of Chronic Toxicity Testing in Animals (Rodent and Non Rodent Toxicity Testing)**
- S5 Reproductive Toxicology**
- S6 Biotechnological Products**
  - > **S6(R1) Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals**
- S7A - S7B Pharmacology Studies**
- S8 Immunotoxicology Studies**
- S9 Nonclinical Evaluation for Anticancer Pharmaceuticals**
  - > **S9 Nonclinical Evaluation for Anticancer Pharmaceuticals**
  - > **S9 Q&As Questions and Answers: Nonclinical Evaluation for Anticancer Pharmaceuticals**
- S10 Photosafety Evaluation**
- S11 Nonclinical Paediatric Safety**
- S12 Non-clinical Biodistribution Studies for Gene Therapy Products**

# Guidances for Regulatory Packages and Conduct of Studies: FDA

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① Nonclinical Safety Evaluation of the Immunotoxic Potential of Drugs and Biologics Guidance for Industry	PDF (136.29 KB)	02/19/2020	Center for Drug Evaluation and Research Center for Biologics Evaluation and Research	Pharm/Tox	Draft	No	04/20/2020
① Investigational Enzyme Replacement Therapy Products: Nonclinical Assessment: Guidance for Industry	PDF (95.62 KB)	10/03/2019	Center for Drug Evaluation and Research	Pharm/Tox	Final	No	01/02/2020
① Osteoporosis: Nonclinical Evaluation of Drugs Intended for Treatment Guidance for Industry: Guidance for Industry	PDF (94.69 KB)	08/15/2019	Center for Drug Evaluation and Research	Clinical - Medical, Pharm/Tox	Final	No	
① Oncology Therapeutic Radiopharmaceuticals: Nonclinical Studies and Labeling Recommendations Guidance for Industry	PDF (167.51 KB)	08/02/2019	Center for Drug Evaluation and Research	Pharm/Tox	Final	No	
① Pathology Peer Review in Nonclinical Toxicology Studies: Questions and Answers	PDF (57.38 KB)	08/01/2019	Center for Drug Evaluation and Research	Pharm/Tox	Draft	No	09/30/2019
① Oncology Pharmaceuticals: Reproductive Toxicity Testing and Labeling Recommendations Guidance for Industry: Guidance for Industry	PDF (118.32 KB)	05/10/2019	Center for Drug Evaluation and Research	Pharm/Tox	Final	No	
① Severely Debilitating or Life-Threatening Hematologic Disorders: Nonclinical Development of Pharmaceuticals Guidance for Industry	PDF (121.8 KB)	03/15/2019	Center for Drug Evaluation and Research	Pharm/Tox	Final	No	
① Testicular Toxicity: Evaluation During Drug Development	PDF (180.41 KB)	10/25/2018	Center for Drug Evaluation and Research	Clinical - Medical, Pharm/Tox	Final	No	

<https://www.fda.gov/regulatory-information/search-fda-guidance-documents>

N=34 documents in total

# Toxicology Strategy Driven by the Clinical Development Plan

## Goal of Toxicology Strategy

- Determine if drug is **safe to proceed into humans**
- Help establish **clinical monitoring plan**
- Help establish a **safe human starting dose**



## Risk Management

- Toxicology findings in animals inform the monitoring plan in human trials
- Concerning findings in animals need to be deemed **monitorable, manageable, reversible** in humans and fit within the risk tolerance of the patient population (if not, exposure limit may be needed)



## Clinical Development Plan Drives Tox Strategy

- **Understand patient population** to assess risk tolerance.
  - I.e. life-threatening vs non-life-threatening
- **Tox studies must match/exceed duration** of dosing in early human trials
- **Same route of administration** as planned for human trials

## Risk Communication

- Findings are shared with Clinical Investigators and Regulatory Authorities via the **Investigator's Brochure (IB)**
- Findings are shared with patients via the **Informed Consent Form**

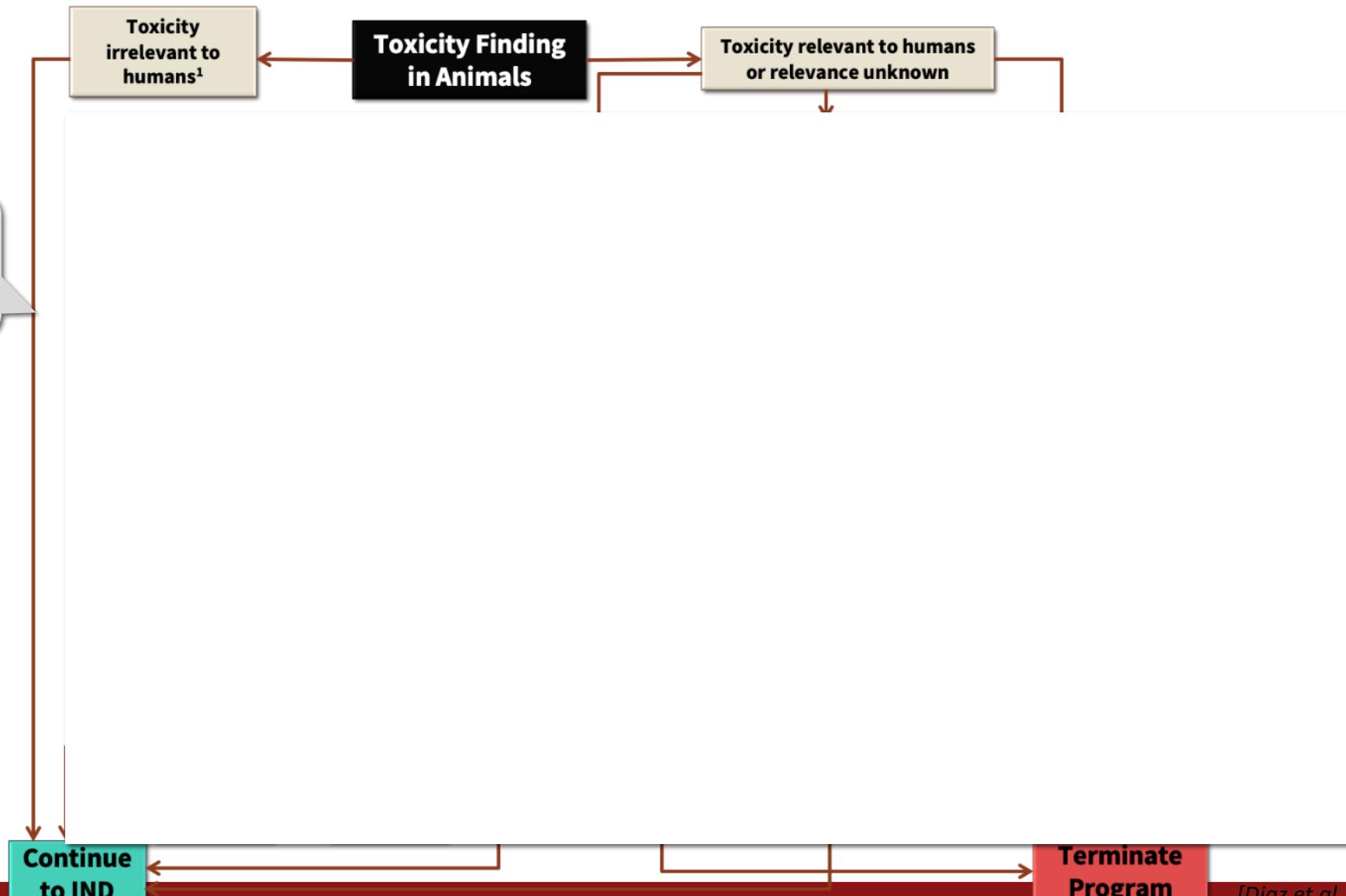


# Typical Toxicology Packages to Enable FIH Dosing

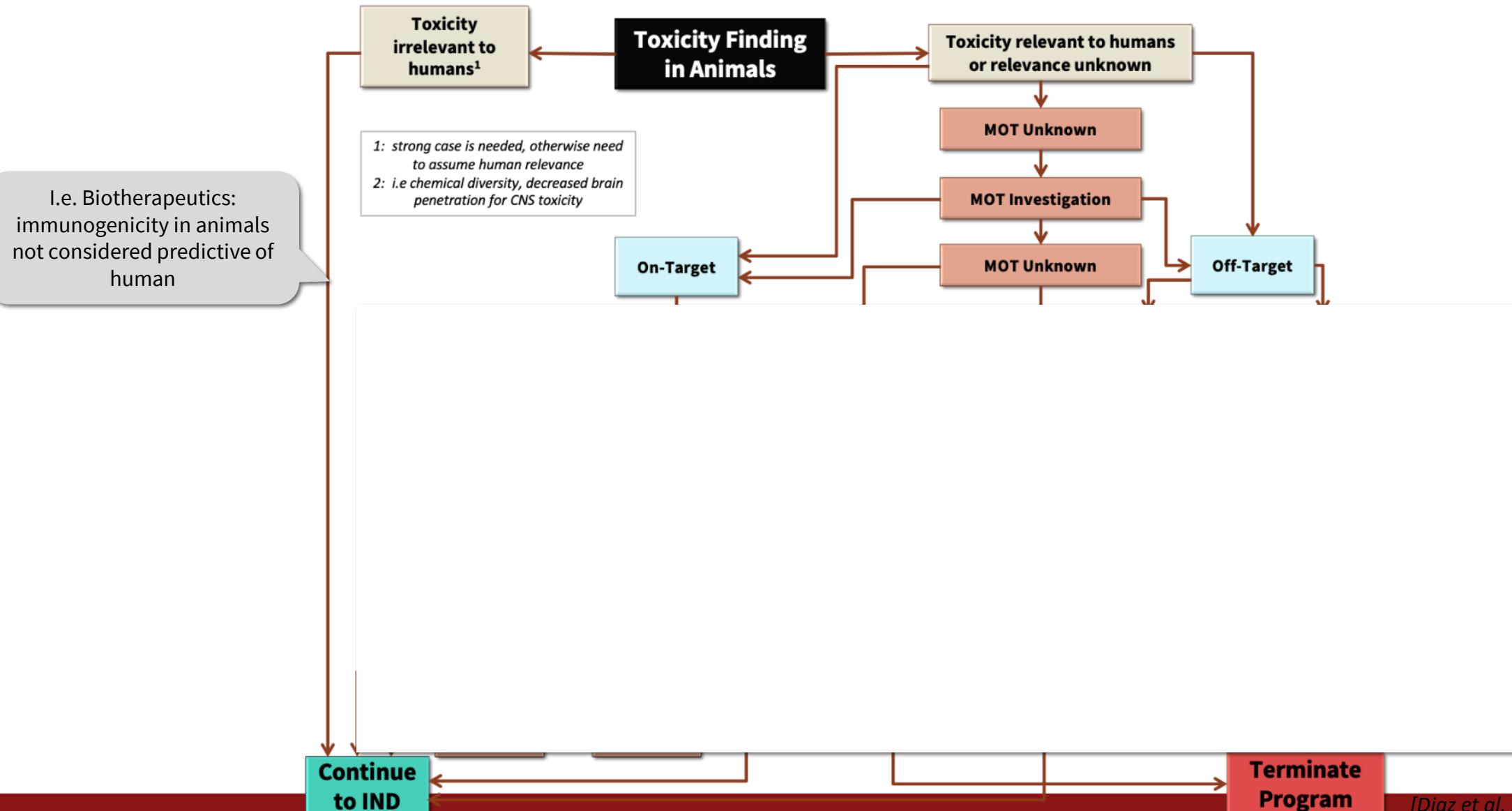
Non GLP  
GLP

Small Molecule	<p>Pilot Toxicity Studies</p> <p>Screening Studies</p> <p>Mechanistic Studies</p> <p>1-3 mo Repeat-Dose Rodent</p> <p>1-3 mo Repeat-Dose Non-Rodent</p> <p>Single-Dose Cardiovascular Study in Telemeterized non-rodent</p> <p>Single-Dose Respiratory study in rodent</p> <p>In-Vivo rodent Micronucleus Test</p> <p>In-Vitro Micronucleus Test (clastogenicity)</p> <p>Ames Assay (Mutagenicity Assay)</p> <p>hERG assay</p> <p>In-Vitro Phototoxicity Test</p>
Biotherapeutic	<p>Pilot Toxicity Studies</p> <p>Mechanistic Studies</p> <p>1-3 mo Repeat Dose Rodent* (weekly dosing)</p> <p>1-3 mo Repeat Dose Non-Rodent (weekly dosing)</p> <p>Tissue Cross Reactivity (human, nonrodent, and/or rodent as relevant)</p> <p>*Only if pharmacologically relevant</p>

# How Toxicologists Think about Molecule Progression

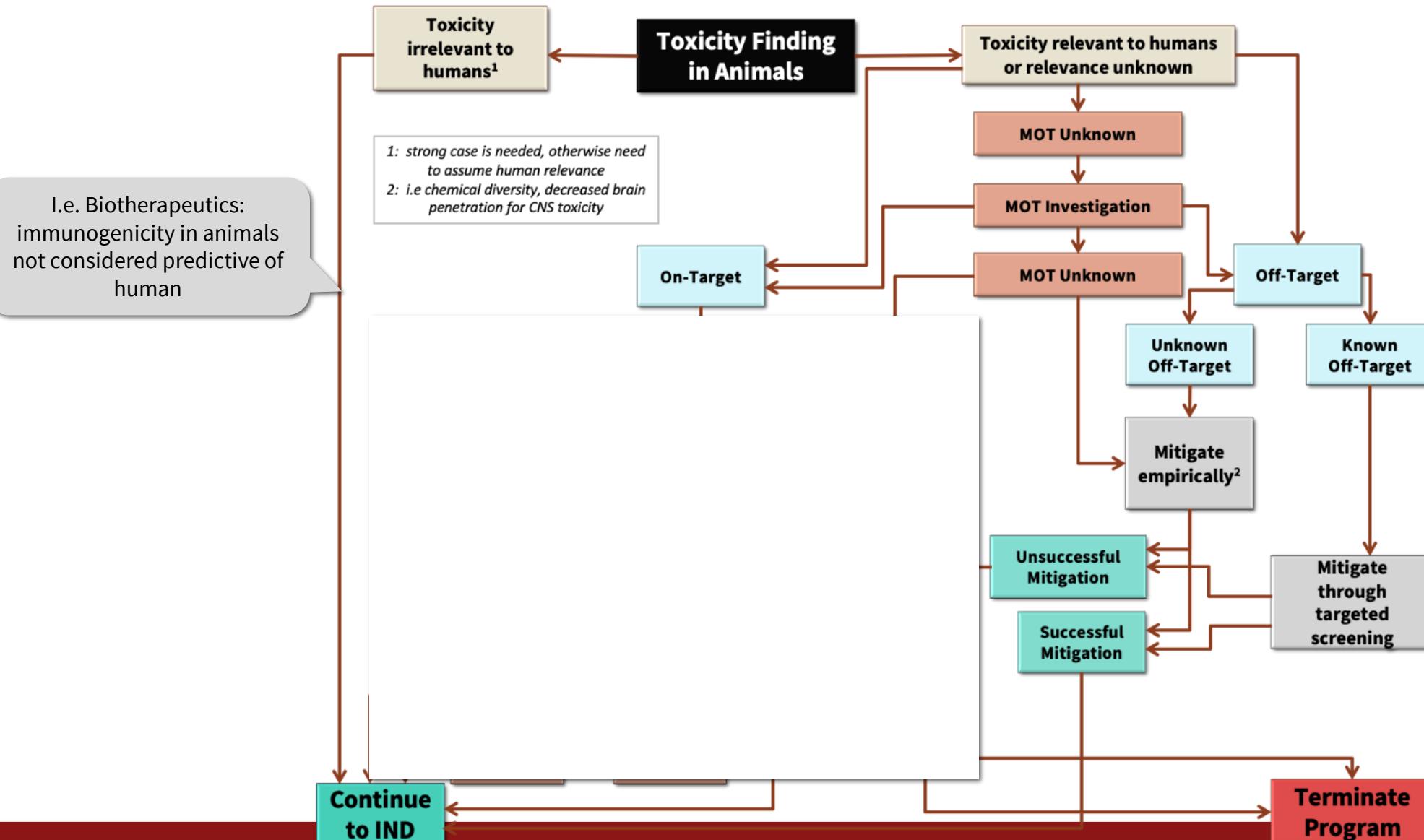


# How Toxicologists Think about Molecule Progression

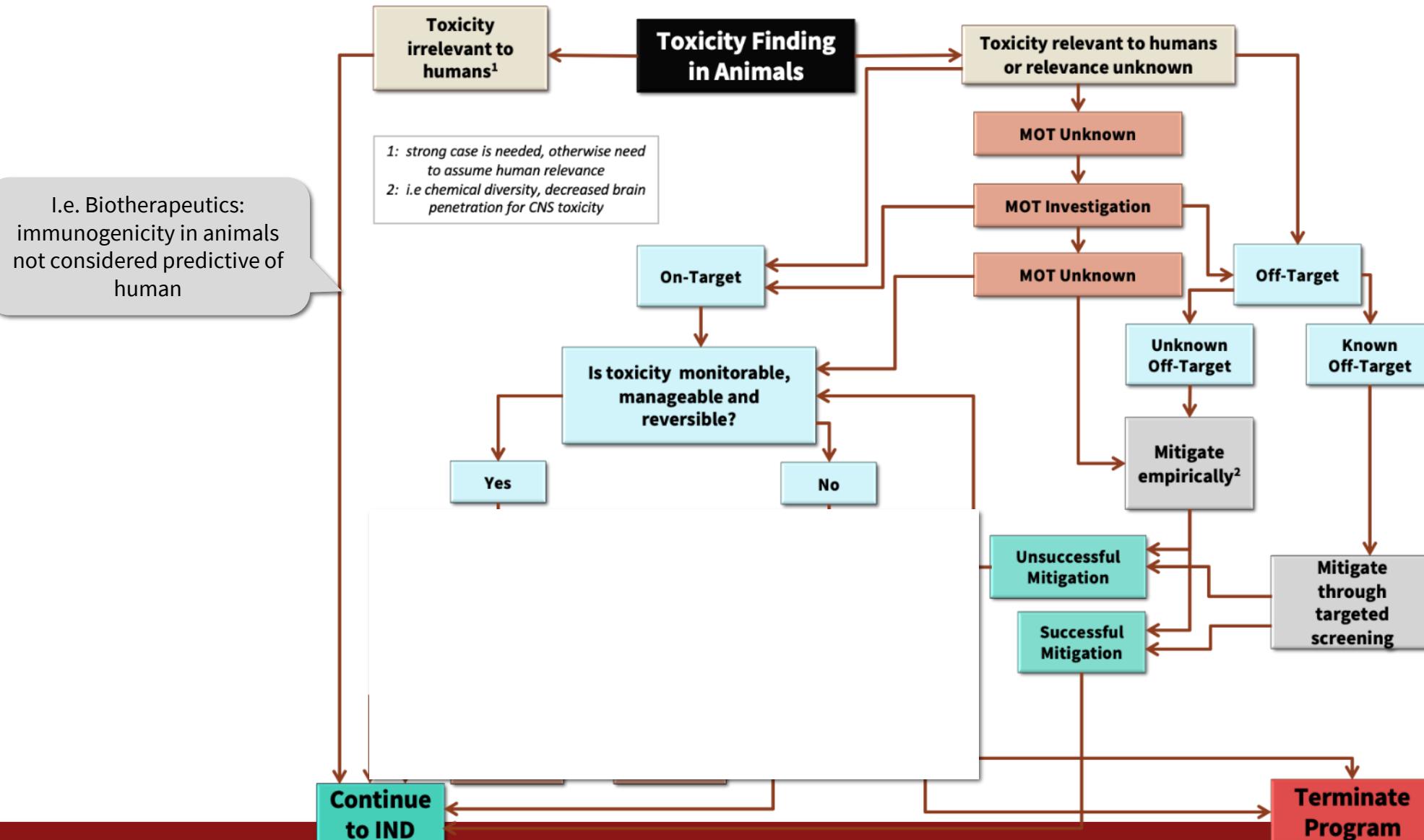


[Diaz et al, 2016]

# How Toxicologists Think about Molecule Progression

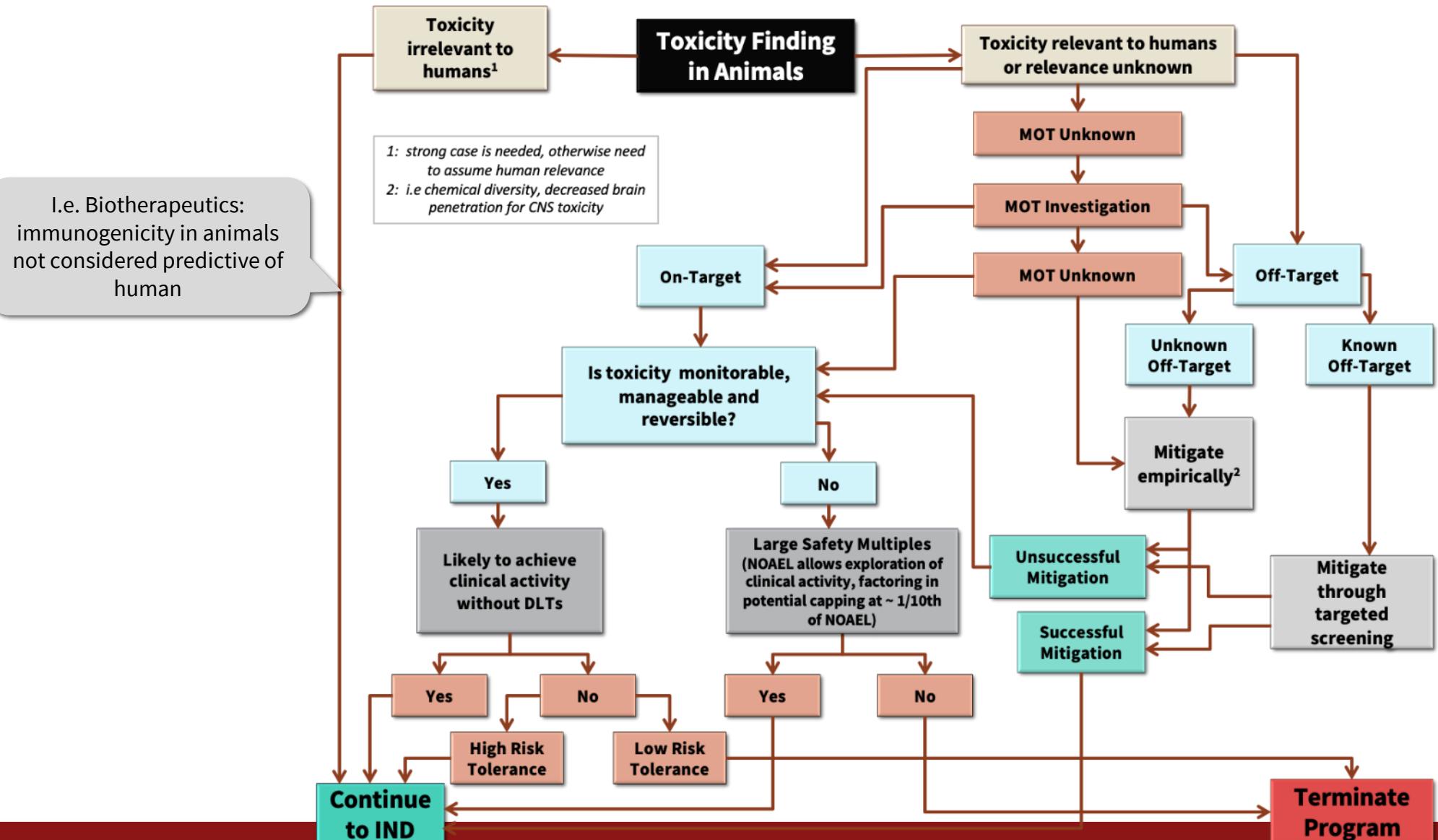


# How Toxicologists Think about Molecule Progression

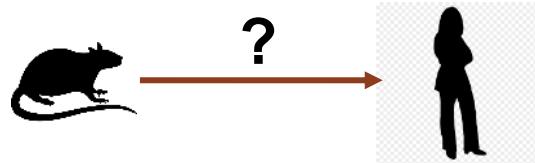


[Diaz et al, 2016]

# How Toxicologists Think about Molecule Progression



# Decision to proceed with human testing (clinical entry)



## Relevant Questions

**Sufficient nonclinical information to evaluate potential human risks?**

**Sufficient nonclinical information to design the clinical protocol?**

**Effective risk management in the clinical protocol?**

**Overall risk-benefit supports clinical entry?**  
Healthy volunteers, patients?

Acceptable quality for administration to humans (i.e. impurities)

Any potential issues that would prevent proceeding into **later development** for the patient population?

## Early Clinical Trials have a Good Safety Track Record

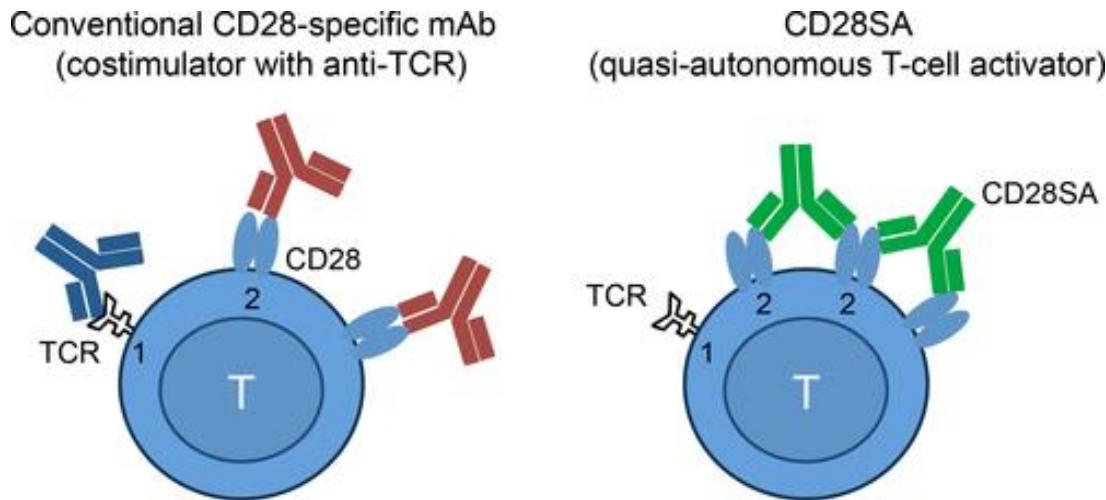
Clinical trials are still the best tool for providing the evidence needed for drug approval and appropriate clinical practice. Phase 1 trials are generally safe; there have been only two trials with very severe adverse events affecting several volunteers among the 14,700 studies (3100 first-in-human studies) involving 305,000 participants that have been conducted in the EU since 2005. It is hoped that the revised EMA guidelines, when available, will enhance strate-

(Bonini and Rasi, NEJM, 2016)

- BIA 10-2474 (Bial), 2016
- SM FAAH inhibitor
- 1 subject died, 3 others had severe neurological effects
- Likely reason: poor selectivity against other lipase enzymes and suboptimal PK awareness

## Another Exception: TGN1412 (TeGenero)

- Strong agonist of CD28 in T Cells for leukemia (super agonist)
- 6 volunteers had severe inflammatory reaction, cytokine storm
- After first dose of 0.1 mg/kg, 500-fold below dose that was safe in animals



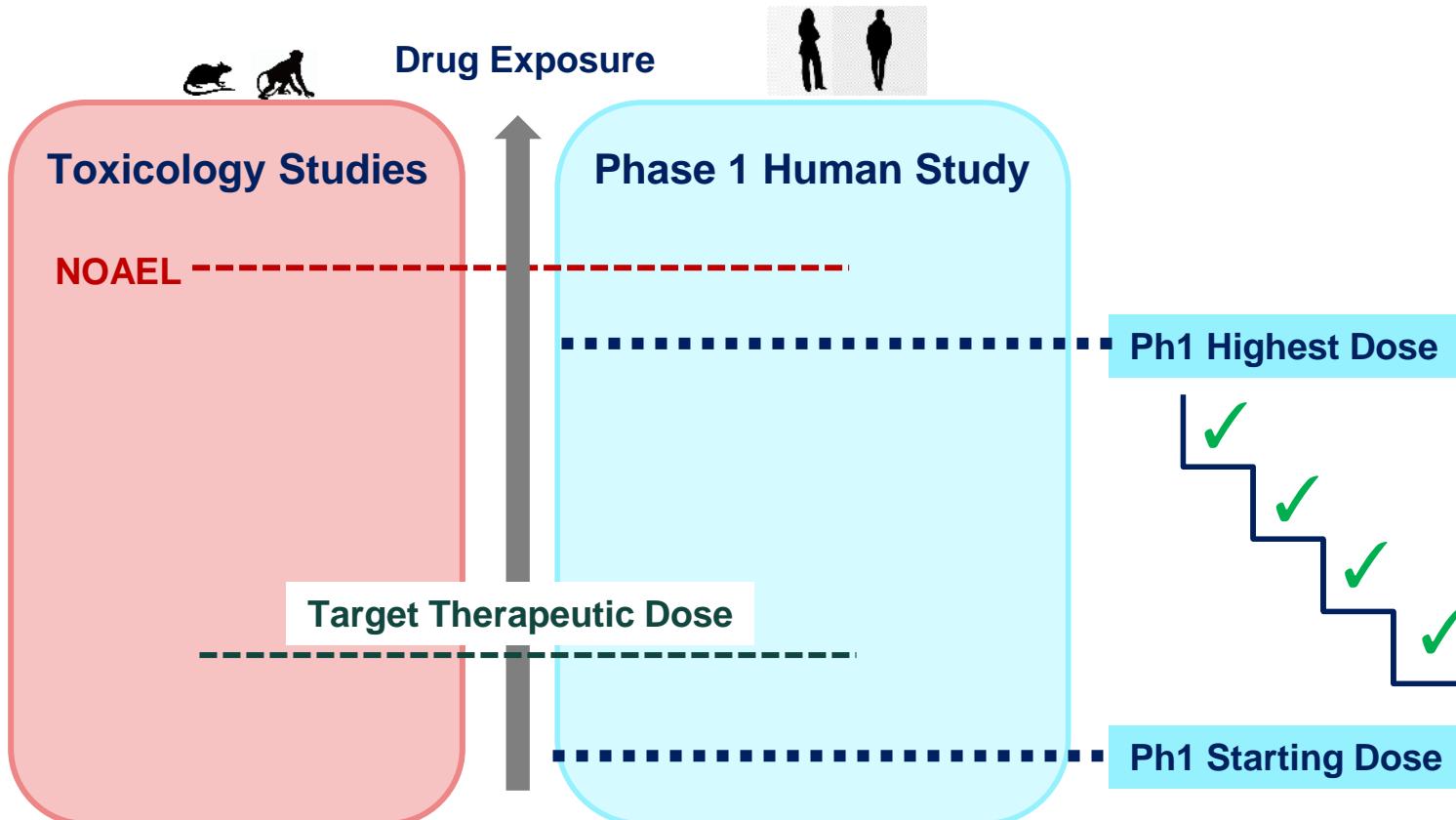
### Conclusion

- Followed all protocols, but monkey lacked CD28 expression in CD4 Memory T Cells
- In addition, despite the low dose used, dose was expected to trigger maximum pharmacology

### Learnings

- New approaches to immune activators, including relevant models (i.e. *in vitro* systems that can detect cytokine storm)
- Starting dose: use of “Minimal Anticipated Biological Effect Level” (MABEL) which considers receptor binding and occupancy data
  - Particularly for immune activators
  - Sometimes used for novel mechanism drugs

# Starting Human Dose and Dose Escalation



## Monitoring Plan

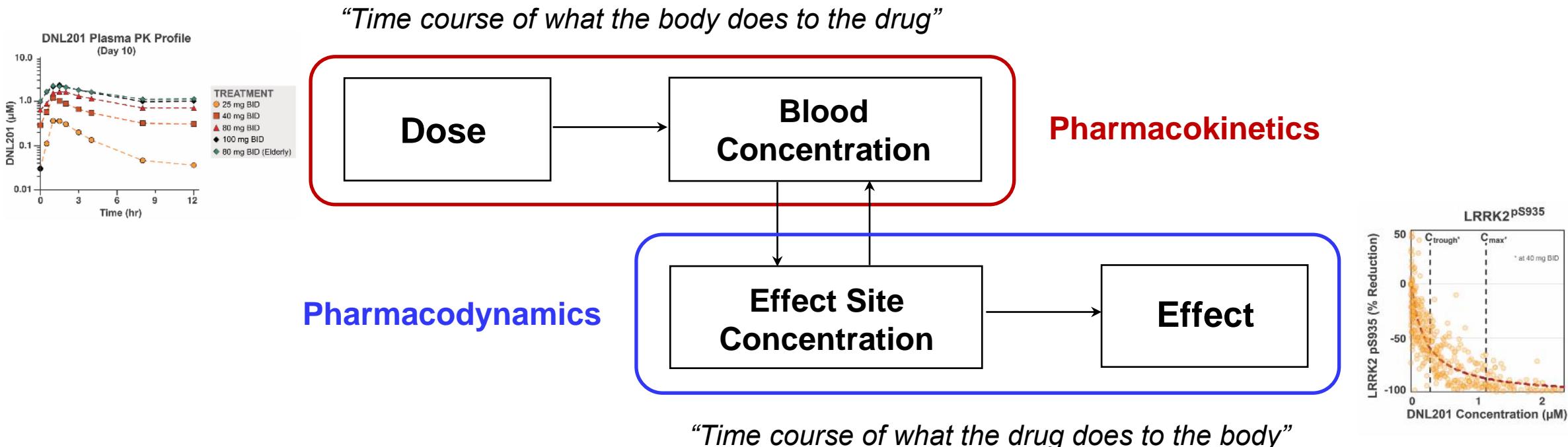
- Subjects are closely monitored, typically in clinical units
- Standard monitoring includes frequent vitals, CV function, hematology and blood chemistry
- Additional monitoring based on preclinical safety profile

- Typically the first human cohort will receive < 1/10 of the NOAEL (from most sensitive tox species) after correcting for body surface area differences between animal and human
- Immune activators or drugs with novel MoA may be expected to start even lower (i.e. Minimum Anticipated Biological Effect Level; MABEL)



# FIH Trials Determine Safety, PK and Early Exposure Response

- Nonclinical PK/PD relationships support translational efforts to select Phase 1 starting doses
- Emerging clinical PK/PD relationships **can inform dose escalation decisions in real time**



# Toxicology in the Drug Development Process



## Clinical Development

**GOAL:** Determine if molecule is safe for long term dosing and as a marketed product (inform label)

- Continue to characterize toxicity: long term tox studies, reproductive and developmental tox, etc
- Toxicity assessments beyond the drug itself: impurities, metabolites, exposures during manufacturing

# Toxicology Studies to Support Phase 2/3 Trials and registration

## Chronic Toxicology Studies

### Key Scientific Questions to Address

**Do toxicity findings from shorter duration studies progress? Do any new findings occur?**  
6-month rodent, and a 6 or 9-month non-rodent study

## Development and Reproductive Toxicology (DART)

**Is there an impact to fertility and embryo-fetal development?**  
Typically: Fertility, Embryo-fetal development and pre and postnatal development in rats and rabbits

## Juvenile Toxicity (pediatric safety risks)

**Are there any unique toxicities in juvenile age animals that might be relevant to pediatric patients?**  
Animals are dosed at young ages that match the development stages of the intended pediatric

## Metabolites in Safety Testing\* (MIST)

**Major circulating human metabolites? Do nonclinical tox species produce these at similar levels? Do these have potential for toxicity?**  
Existing tox studies can cover these, or dedicated studies may be needed

## Abuse Liability Assessment\* (ALA)

**Is there a potential for abuse? Does the drug need to be classified as a scheduled substance?**  
Studies in rats for drug discrimination, self administration and physical dependence

## Carcinogenicity\* (cancer risk)

**Is there is potential for carcinogenicity?**  
Tox assessments may include a 2-year rodent assay and a 6-month transgenic mouse

\*Almost exclusively small molecules

# POLL QUESTION 5

**Which statement is CORRECT regarding toxicology studies to support Phase 2/3 studies:**

- A. Abuse liability studies studies are usually conducted in primates
- B. Juvenile toxicity studies are usually conducted in primates
- C. Carcinogenicity studies are usually performed in 1 rodent and 1 non-rodent
- D. Development and Reproductive toxicity studies are usually conducted in rats and rabbits





# Considerations for Small Molecules vs Biotherapeutics

**Table I** Difference between innovator products and small-molecule drugs

	<b>Small-molecule drugs</b>	<b>Biologic drugs</b>
<b>Product-related differences</b>	<ul style="list-style-type: none"><li>Produced by chemical synthesis</li><li>Low molecular weight</li><li>Well-defined physiochemical properties</li><li>Stable</li><li>Single entity, high chemical purity, purity standards well established</li><li>Administered through different routes of administration</li><li>Rapidly enters systemic circulation through blood capillaries</li><li>Distribution to any combination of organ/tissue</li><li>Often specific toxicity</li><li>Often non-antigenic</li></ul>	<ul style="list-style-type: none"><li>Biotechnologically produced by host cell lines</li><li>High molecular weight</li><li>Complex physiochemical properties</li><li>Sensitive to heat and shear (aggregation)</li><li>Heterogeneous mixture, broad specification which may change during development, difficult to standardize</li><li>Usually administered parenterally</li><li>Larger molecule primarily reach circulation via lymphatic system, subject to proteolysis during interstitial and lymphatic transit</li><li>Distribution usually limited to plasma and/or extracellular fluid</li><li>Mostly receptor mediated toxicity</li><li>Usually antigenic</li></ul>
<b>Manufacturing differences</b>	<ul style="list-style-type: none"><li>Completely characterized by analytical methods</li><li>Easy to purify</li><li>Contamination can be generally avoided, is easily detectable and removable</li><li>Not affected by slight changes in production process and environment</li></ul>	<ul style="list-style-type: none"><li>Difficult to characterize</li><li>Lengthy and complex purification process</li><li>High possibility of contamination, detection is harder and removal is often impossible</li><li>Highly susceptible to slight changes in production process and environment</li></ul>

(Singh-Sekhon and Saluja, 2014)

# 10 Minute Q&A, Break



**C E R S I**  
U C S F - S t a n f o r d

# CASE STUDY #1

CERSI-1 is a PAK1,2,3 inhibitor, and it is being explored as a potential treatment for oncology indications. When administered at an oral dose of 25 mg/kg BID (twice a day), it imparted 60% tumor growth inhibition in a relevant mouse xenograft model. Unfortunately, doses of 40 mg once a day (QD) were associated with death in the majority of study animals within 2–4 h after dosing. Investigative work revealed that the deaths were due to acute cardiac failure.

In addition to CERSI-1, two other PAK inhibitors also caused similar findings, and below is the selectivity data for these 3 molecules (CERSI-1 = 28):

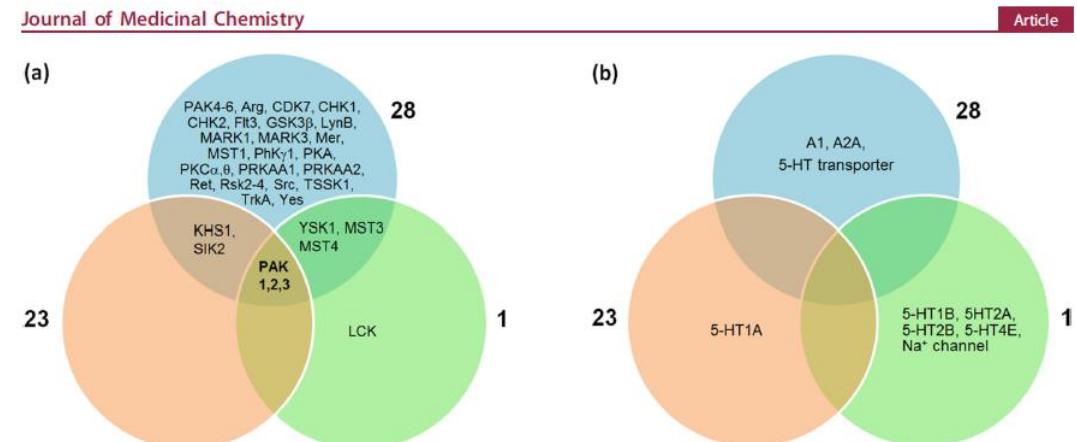


Figure 4. (a) Venn diagram showing the kinases inhibited at >75% at a test concentration of 0.1  $\mu$ M (compound 1) and 1  $\mu$ M (compounds 23 and 28). The kinase screening panel contained 237 kinases for compound 1, 96 kinases for compound 23, and 145 kinases for compound 28. The panel used for 1 included all the 96 kinases tested for 23 and all 145 kinases tested for 28. All kinases that were inhibited by compound 1 at >75% were included in the panels of the other two compounds. (b) Venn diagram showing the targets from a secondary pharmacology screening panel modulated at >75% at a test concentration of 10  $\mu$ M (52 targets tested overall). Details are provided in the Supporting Information.

# CASE STUDY #1 (CONT'D)

**Question #1:** Do these data support the toxicity in mice being on or off target and why? Do you feel the data are conclusive in either direction and why?

**Question #2:** Which path/s forward would you recommend for this molecule and why?

- a. Continue development since the data support the existence of a therapeutic index and the indication is oncology
- b. Stop development and investigate mechanism of toxicity
- c. Continue development and investigate mechanism of toxicity to apply learnings to a follow-on molecule.

**Question #3:** Which approach/es would you pursue to further investigate mechanism of toxicity (on vs. off target)?

- a. Introduce chemical modifications to the molecule and test if the toxicity is mitigated
- b. Synthesize an inactive chemical analog of the molecule and investigate if the toxicity is mitigated?
- c. Evaluate if there is a correlation between target potency and toxicity?
- d. Generate KO mice and evaluate their phenotypes and test the molecule in this model?

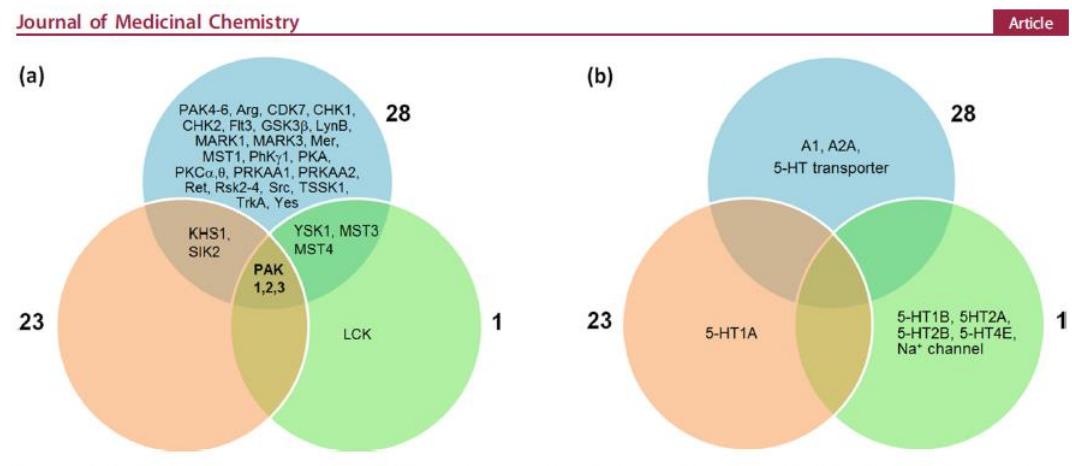
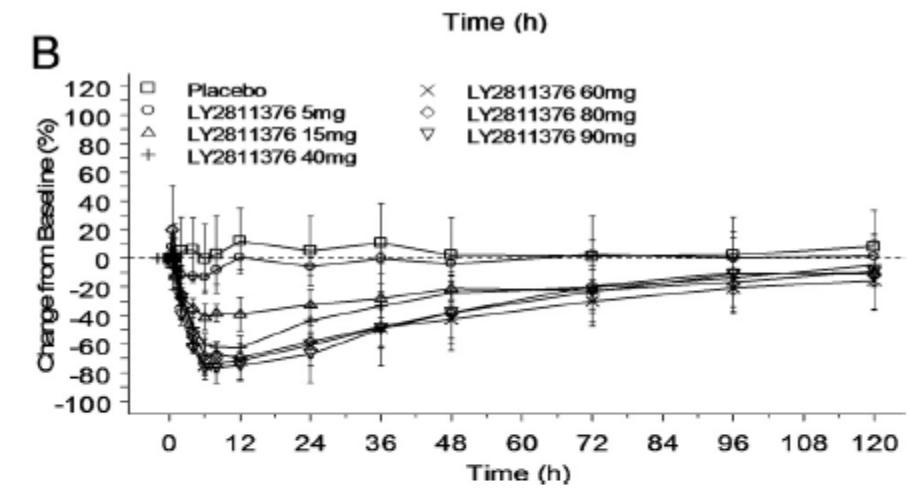


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# CASE STUDY #2

- DRUG-A is a potent small molecule BACE1 inhibitor developed for the treatment of Alzheimer's disease (AD). The molecule had an acceptable preclinical safety profile in rats and dogs in the IND-enabling 28-day toxicology studies and it proceeded to first-in-human studies. In single dose studies in healthy volunteers DRUG-A showed robust pharmacodynamic effects of Abeta reduction in cerebrospinal fluid (CSF) (see graph below), and it was well tolerated.
- In parallel to the phase 1 studies in healthy volunteers, a 3-month rat toxicology study was performed to prepare for phase 2 clinical trials. DRUG-A caused photoreceptor degeneration within the retina, a toxicity that is irreversible and it leads to blindness. This happened at an exposure similar to the exposure achieved in humans at the 40 mg dose. A subsequent study using DRUG-A in BACE1 KO mice demonstrated that the findings observed in the retinal epithelium were unrelated to the BACE1 pharmacological target (i.e. **Off-target mechanism**).



**Figure 4.** SAD study in healthy volunteers. Mean plasma  $\text{A}\beta_{1-40}$  (A) and  $\text{A}\beta_{1-x}$  (B) change from baseline after single doses of LY2811376. After single doses of LY2811376 between 5 and 90 mg, plasma concentrations of both  $\text{A}\beta_{1-40}$  and  $\text{A}\beta_{1-x}$  decreased, reached a nadir, and then slowly returned to their predose baseline values. The time at which the nadir occurred ranged from a mean of 6–12 h and appeared to be independent of dose. The magnitude of the decrease in plasma  $\text{A}\beta_{1-40}$  and  $\text{A}\beta_{1-x}$ , as measured by either the nadir or the average reduction over the first 24 h tended to increase with increasing doses of LY2811376. Plasma concentrations of  $\text{A}\beta_{1-40}$  and  $\text{A}\beta_{1-x}$  after the highest dose of 90 mg did not fully return to their predose baseline values within the 120 h sampling period of the study. Plasma  $\text{A}\beta_{1-40}$  and  $\text{A}\beta_{1-x}$  PD response provided guidance for dose selection to the second part of the trial looking at PD effect in CSF.

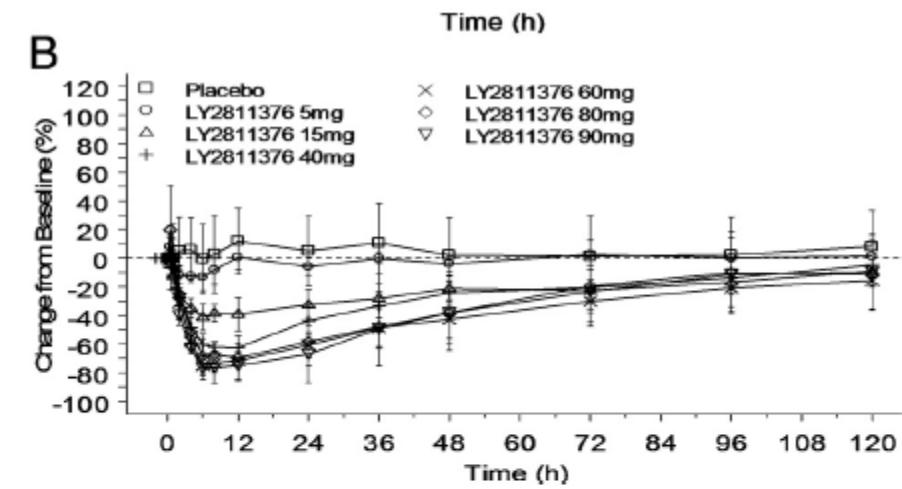
# CASE STUDY #2 (CONT'D)

**Question #1:** To reach this conclusion of 'off-target', what do you think were the results in the BACE1 KO mouse study?

**Question #2:** If you were the company, how would you proceed with the clinical study?

- Would you continue with the study?
- Would you modify the study?
- What assessments would you conduct in the healthy volunteers?
- The company investigated the mechanism of toxicity and it concluded that it was due to the inhibition of the protease CatD. This protease resides in the lysosomes of the retinal cells that showed the toxicity, where it degrades photoreceptors during their turnover. Inhibition of CatD by DRUG-A caused accumulation of undegraded photoreceptor fragments leading the retinal cell degeneration over time.
- However, the researchers were puzzled that the selectivity of DRUG-A for CatD was 100-fold (i.e. DRUG-A was 100-fold more potent for BACE1 compared to CatD), and such high selectivity is typically sufficient to avoid toxicities driven by off-target effects.

**Question #3:** What is your hypothesis as to why DRUG-A was still toxic in rats despite this high selectivity?



**Figure 4.** SAD study in healthy volunteers. Mean plasma  $\text{A}\beta_{1-40}$  (A) and  $\text{A}\beta_{1-x}$  (B) change from baseline after single doses of LY2811376. After single doses of LY2811376 between 5 and 90 mg, plasma concentrations of both  $\text{A}\beta_{1-40}$  and  $\text{A}\beta_{1-x}$  decreased, reached a nadir, and then slowly returned to their predose baseline values. The time at which the nadir occurred ranged from a mean of 6–12 h and appeared to be independent of dose. The magnitude of the decrease in plasma  $\text{A}\beta_{1-40}$  and  $\text{A}\beta_{1-x}$ , as measured by either the nadir or the average reduction over the first 24 h tended to increase with increasing doses of LY2811376. Plasma concentrations of  $\text{A}\beta_{1-40}$  and  $\text{A}\beta_{1-x}$  after the highest dose of 90 mg did not fully return to their predose baseline values within the 120 h sampling period of the study. Plasma  $\text{A}\beta_{1-40}$  and  $\text{A}\beta_{1-x}$  PD response provided guidance for dose selection to the second part of the trial looking at PD effect in CSF.

## CASE STUDY #2 (CONT'D)

- The company developed DRUG-A.1, a drug with 1,000 fold selectivity against CatD. This molecule still caused retinal toxicity in rats, but in this case at higher doses/exposures. In the rat toxicology study, these were the results:

Dose	AUC ( $\mu\text{M}^*\text{h}$ )	Retinal Toxicity	PD Effects (Abeta reduction in CSF)
10 mg/kg	10	Absent	100%
30 mg/kg	30	Absent	100%
100 mg/kg	100	Present	100%

**Question #4:** Would you proceed to clinical testing with this molecule and why?

**Question #5:** If you proceed to the clinic, what exposure would you recommend as the maximum exposure to be explored in humans?