

WHERE
insights LEAD TO
answers



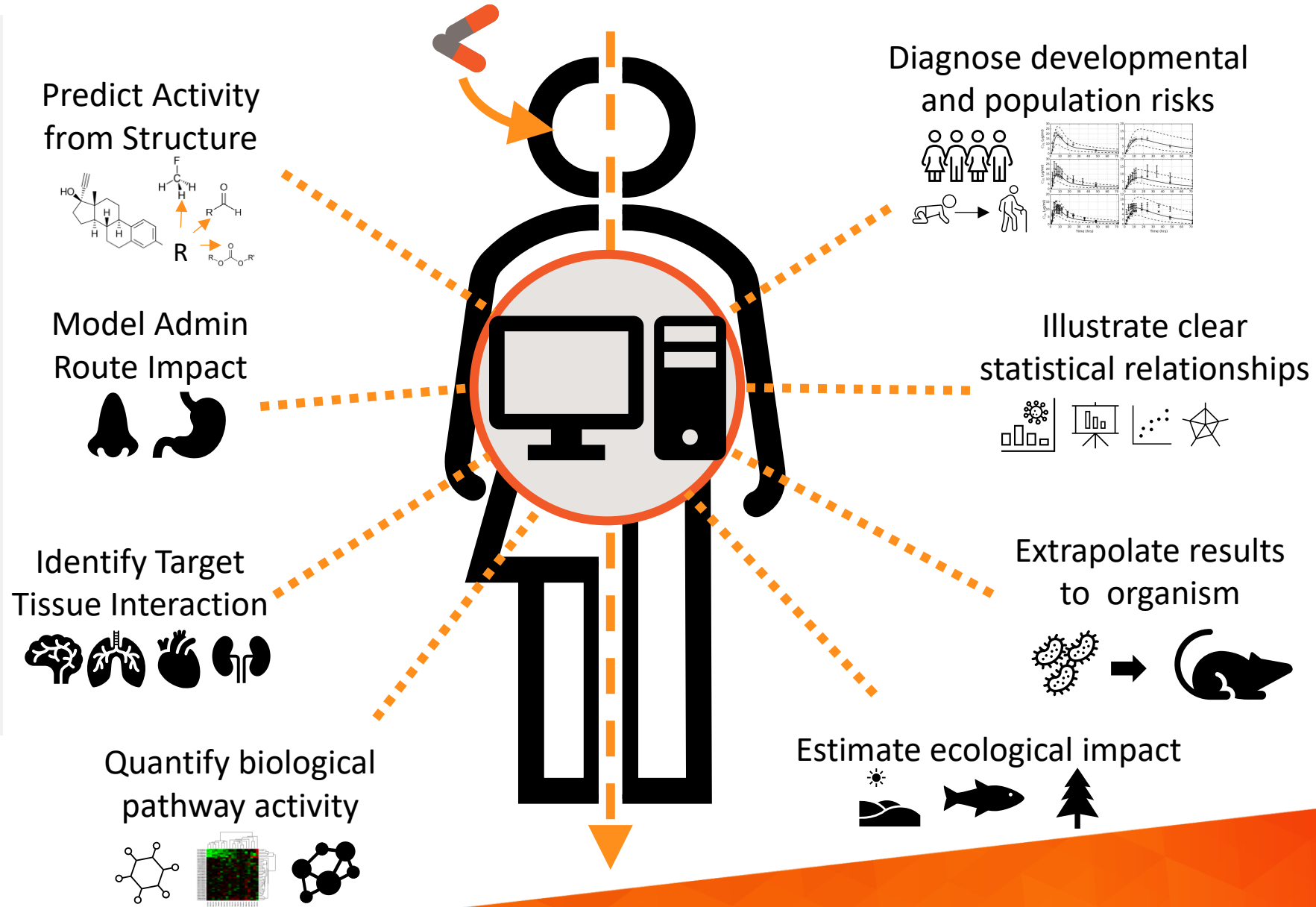
Genetic Toxicology Workshop

Beyond Safety: Predictive Toxicology and Bioinformatics

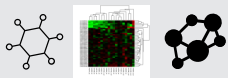
Solutions oriented biology to address complex **Toxicity** and **Bioactivity** questions

The RTP Predictive Biology Platform:

- Leverages modern computational approaches to predict trial outcomes
- Characterizes chemical behavior when in vivo data aren't available or before experimentation to improve confidence
- Quantifies biology to estimate risk and hazard before testing

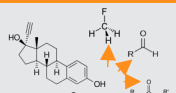


Our expertise applies to key pharma and tox needs



Bioinformatics

- Quantifies biological pathway activity and mechanisms of action (MoA) from biomolecule data
- Results in powerful interaction maps to understand how a chemical acts, both on target and off target



Chemical Structure-Activity Modeling (QSAR) and Cheminformatics

- Predicts biological activity from structural information to estimate target ranges
- Can be used upstream in product design to tailor chemical activity
- Can estimate range of activities with very little information



In silico predictive pharmacokinetics (PK)

- Predicts tissue-level concentrations based on adsorption, distribution, metabolism, and excretion (ADME)
- Can extrapolate across doses, dosing routes, and species
- Can accommodate population variability



In vitro - in vivo Extrapolation (IVIVE/ISVE)

- Leverages PK models
- Maps active concentrations to administered dose
- Can be used to evaluate the effects of population variability



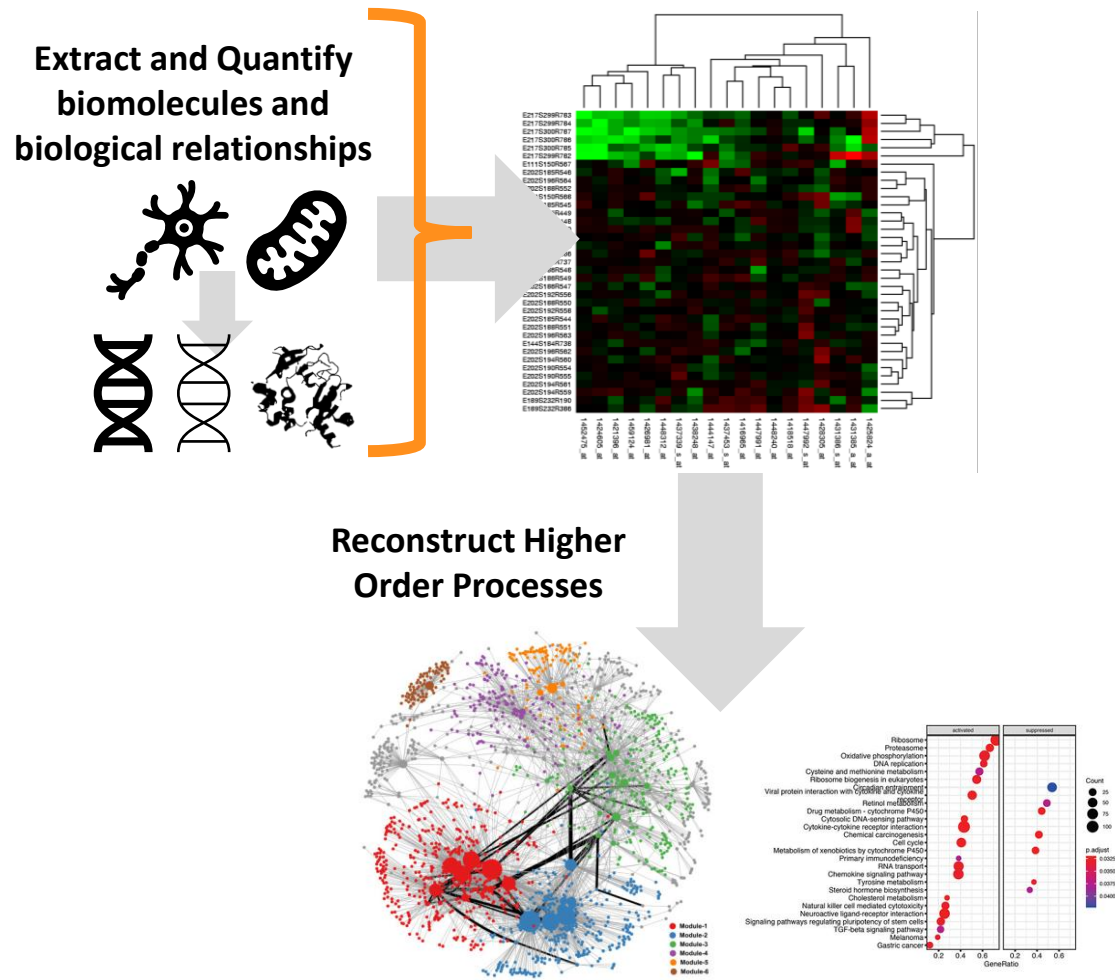
Biostatistics

- Integrates information at all levels to ensure accuracy of predictive platforms
- Clarifies significant responses and supports informatic conclusions
- Available to consult on a range of projects

Bioinformatics adds holistic systems-level context to target research

Bioinformatics measures the expression and activity of different biomolecules to

- Calculate regulatory networks and biological pathway expression (**Protein and RNA**)
- Describe cellular response and trajectory (**RNA, miRNA, and targeted assays**)
- Understand species abundance or mutation frequency (**DNA**)
- Model organ system interaction and health/disease outcomes with modern machine learning



Understand target effects and disease

Gu 10.1186/1752-0509-6-32

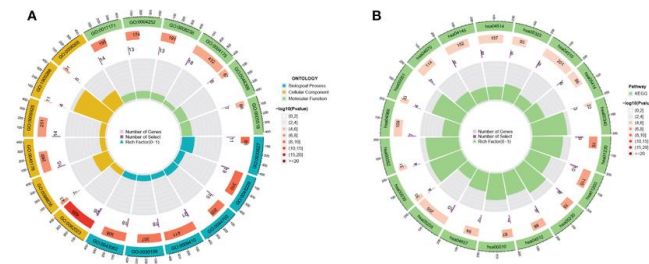
Zhou 10.3389/fonc.2020.01147

Bioinformatics connects different systems

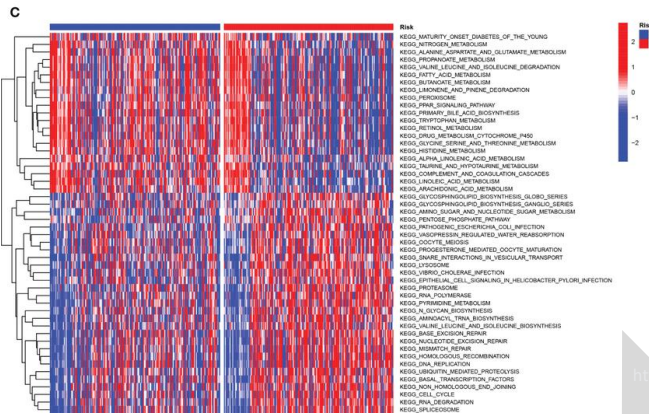
Analysis targets client questions:

- Identify active genes and pathways
- Find off target effects
- Ensure target system efficacy before refined analysis
- Measure all genes and proteins at once
- Understand activity in biological context
- Spatial Single Cell Transcriptomics relates images to biological activity in each visualized cell
- Modern AI and ML bioinformatics integration can streamline existing pipelines by assisting current scientists to increase speed and reduce human burden

Quantify gene/protein/pathway activity

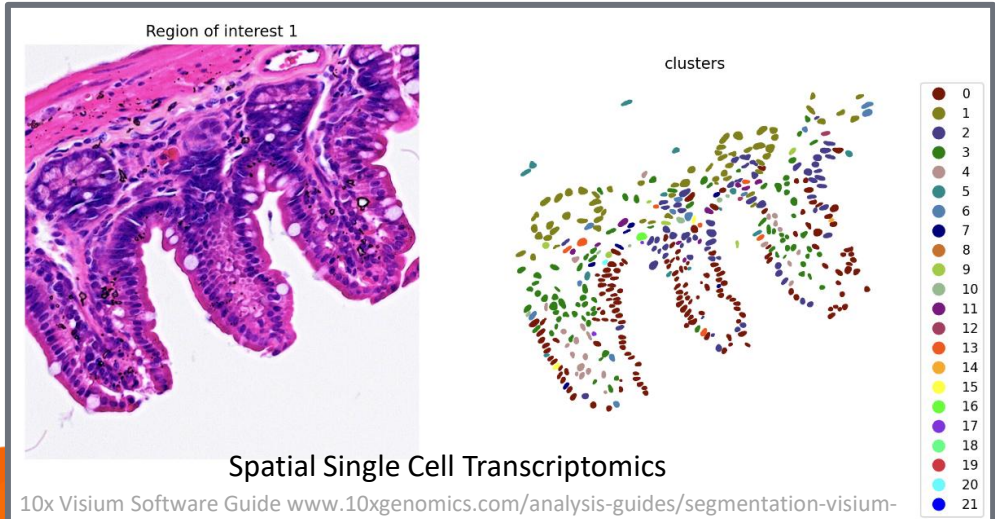


Integrate networks



DOI 10.3389/fimmu.2023.1137025.

Provide in situ context





Computational Chemistry and QSAR

Sherif Farag, PhD - Inotiv-RTP

Pharmacokinetics (PK) predicts drug distribution, clearance and concentration

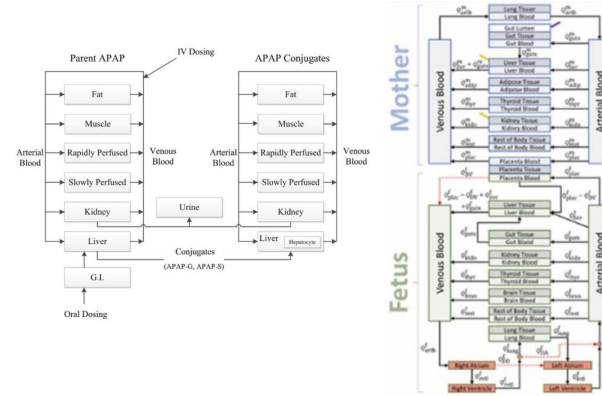
Generates clear understanding of

- drug therapeutic window (efficacy vs toxicity)
- duration of drug action
- effective dosing regiment

PK can be tailored to custom

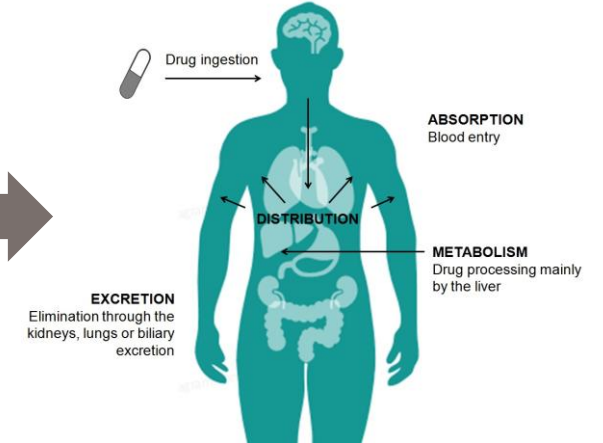
- Population and patient-related factors
- Specific compartments of whole body
- Novel chemical properties
- Scenarios:
 - Mother-Fetus / Elderly / Child

Compartmentalized Kinetic Models



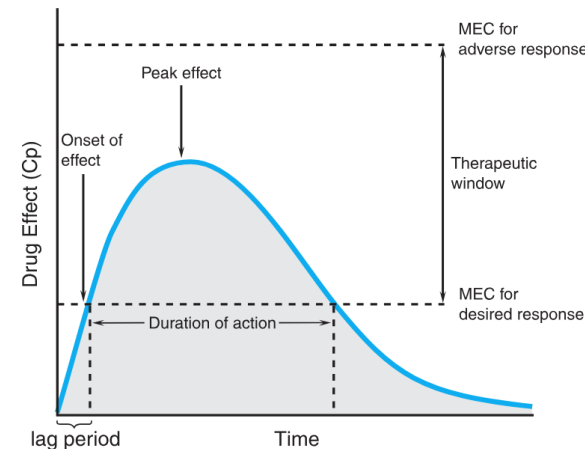
Zurlinden TJ, Reisfeld B. Eur J Drug Metab Pharmacokinet. 2016 Jun;41(3):267-80. Kapraun DF, et al. Reprod Toxicol. 2022 Oct;113:172-188.

Critical drug behavior



Figures: <https://www.sb-peptide.com/process-development/peptide-pharmacokinetics-studies/pharmacokinetic-studies/>

Toxicological and Therapeutic Response



Brunton and Knollman, Goodman and Gilman's, The Pharmacological Basis of Therapeutics, McGraw Hill, 2017

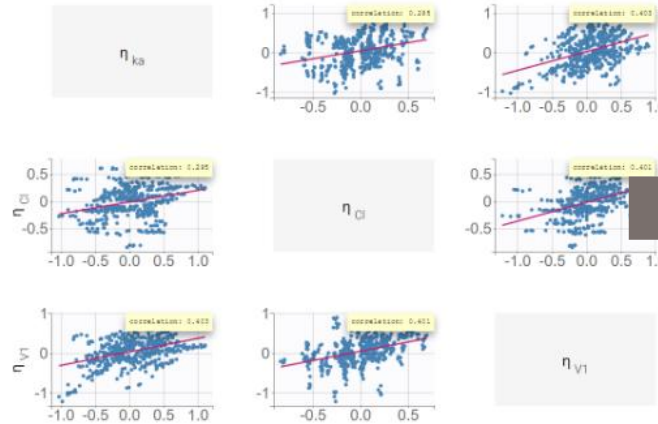
Pharmacokinetics (PK) estimates prospective drug behavior to save clients time and money

Predict drug accumulation and target tissues in silico from lead candidates

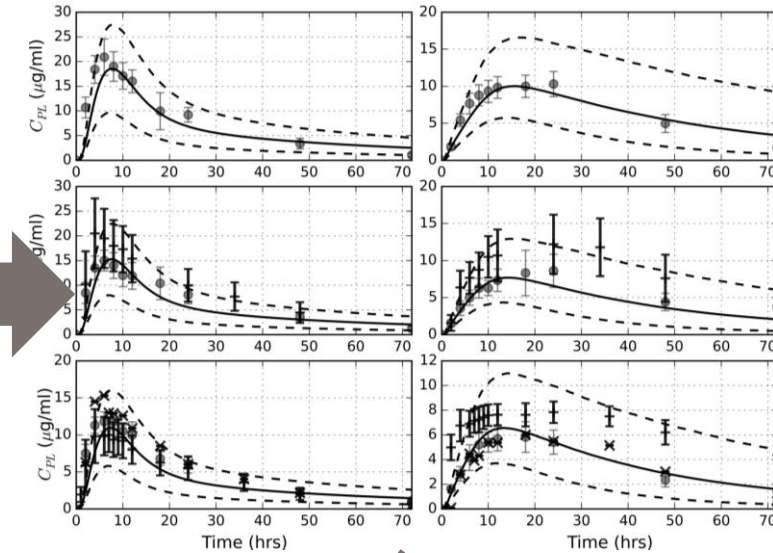
Identify unwanted effects early from structure

Understand dosing strategies and inform formulation design

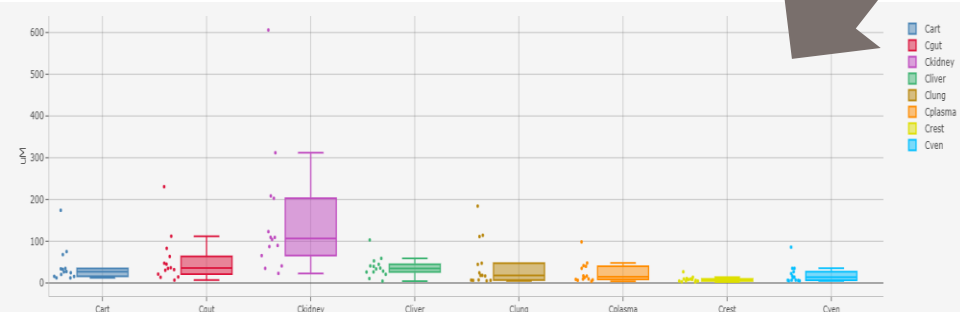
Estimate key physio-chemical Characteristics



Simulate Concentration-Time in Target Tissues

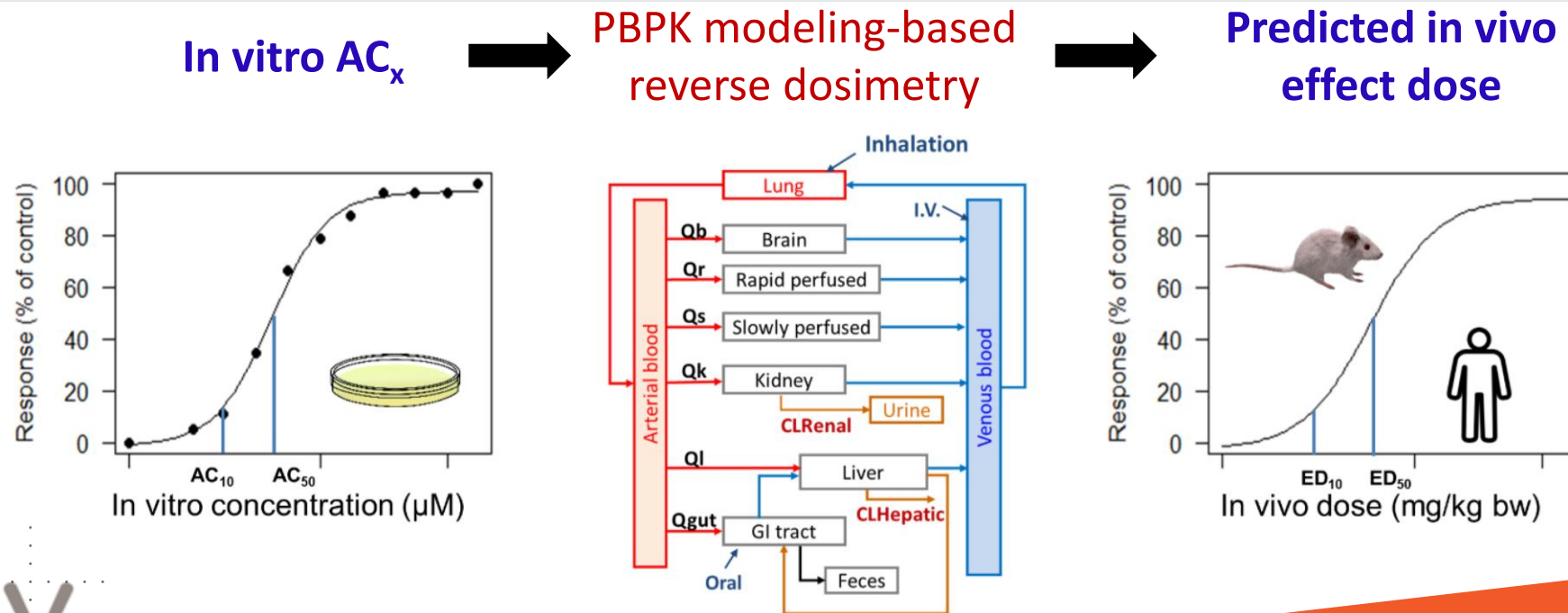


Predict Tissue Accumulation



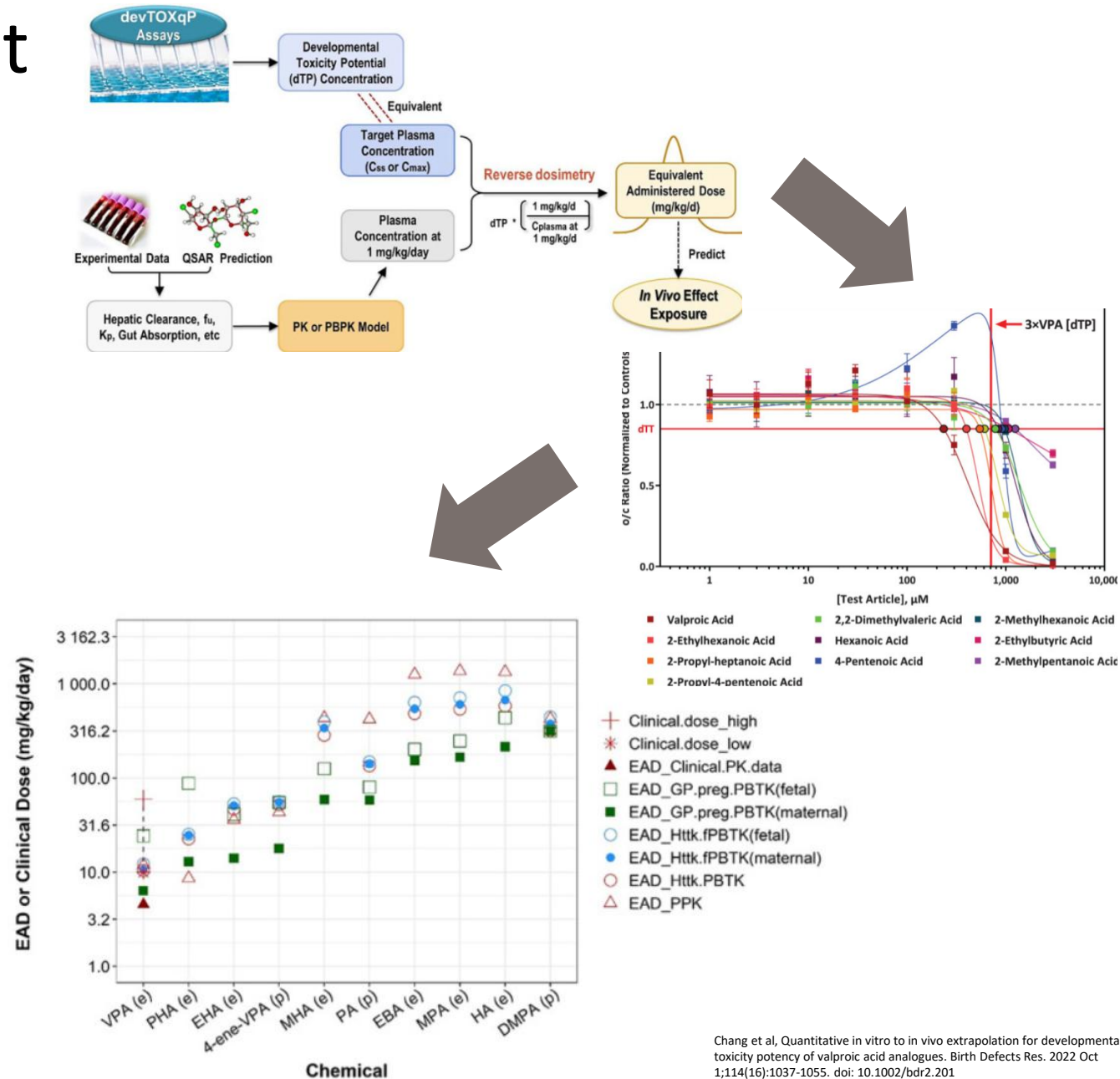
In vitro/in silico to in vivo extrapolation (IVIVE/ISIVE) extends PK to estimate in vivo response from early testing

- Approach that uses **in vitro experimental data** or **in silico predictions** to predict **in vivo phenomena or outcomes** (e.g. effect dose, kinetics, toxicity)
 - > Takes a lab test and extrapolates the result DIRECTLY to target species response (e.g., human)
- Reverse dosimetry: estimation of dose/exposure based on internal concentration



IVIVE makes test data relevant

- Makes in vitro results human (or other species)-relevant
- Promotes use and acceptance of in vitro data in drug development
- Reduces the number of animals used
- Reduces cost, saves time
- Provides mechanistic insights



Biostatistics ensures conclusion quality and uncertainty are understood in total context

- **Informs safe dose levels:** Through dose-response modeling and safety assessments
- **Aids in biological interpretation:** Helps interpret complex biological data and provides context for experimental results
- **Designs robust experiments:** Ensures study designs are statistically sound with appropriate sample sizes and validated methods
- **Regulatory compliance:** Provides necessary statistical analyses for regulatory submissions

Example:

Dose Level (mg/kg/day)	Endpoint 1	Endpoint 2	Endpoint 3	Endpoint 4
	<i>mc1, log</i>	<i>mc2</i>	<i>mc1</i>	<i>mc2, ></i>
0	—			
1	XX			
2	XX*			
3	XX*			
Linear Trend	XX [^]	XX ^v	XX	XX
	<i>sc1</i>	<i>sc2</i>	<i>sc1</i>	<i>sc2, inv, ></i>
EMS	XX*	XX	XX	XX

Two-sided test unless otherwise indicated.

mc1: One-way ANOVA; Dunnett's test and parametric trend test

mc2: Dunn's test and Jonckheere-Terpstra trend test

sc1: T-test

sc2: Mann-Whitney U test

> One-sided test for increase

< One-sided test for decrease

log: log10 transformation applied

sqrt: square root transformation applied

inv: inverse transformation applied

* $p \leq 0.05$

[^] Increasing linear trend, $p \leq 0.01$

^v Decreasing linear trend, $p \leq 0.01$

Biostatistics visualizes the impact of scientific outcomes

Ready-to-go workflows for:

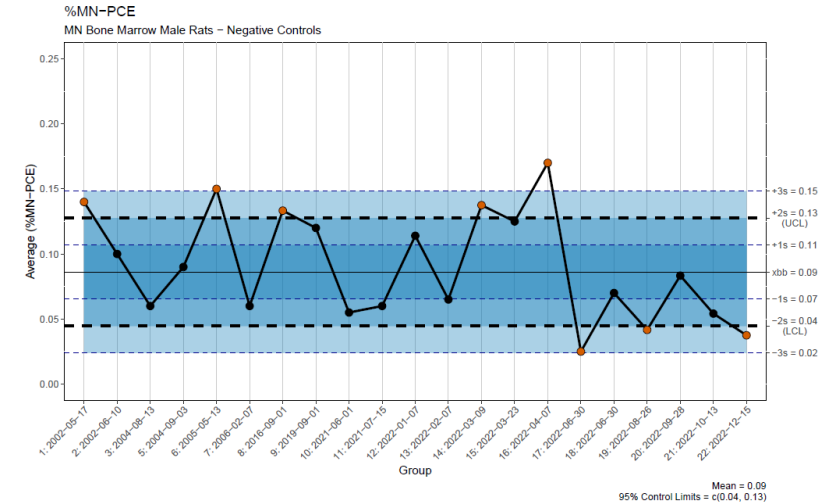
- Trend tests and pairwise comparisons for vehicle control and treatment groups
- Comparisons of vehicle control and positive control
- Assumptions tests for normality, variance, and outliers

We ensure that all statistical methods used are validated, providing confidence in the results.

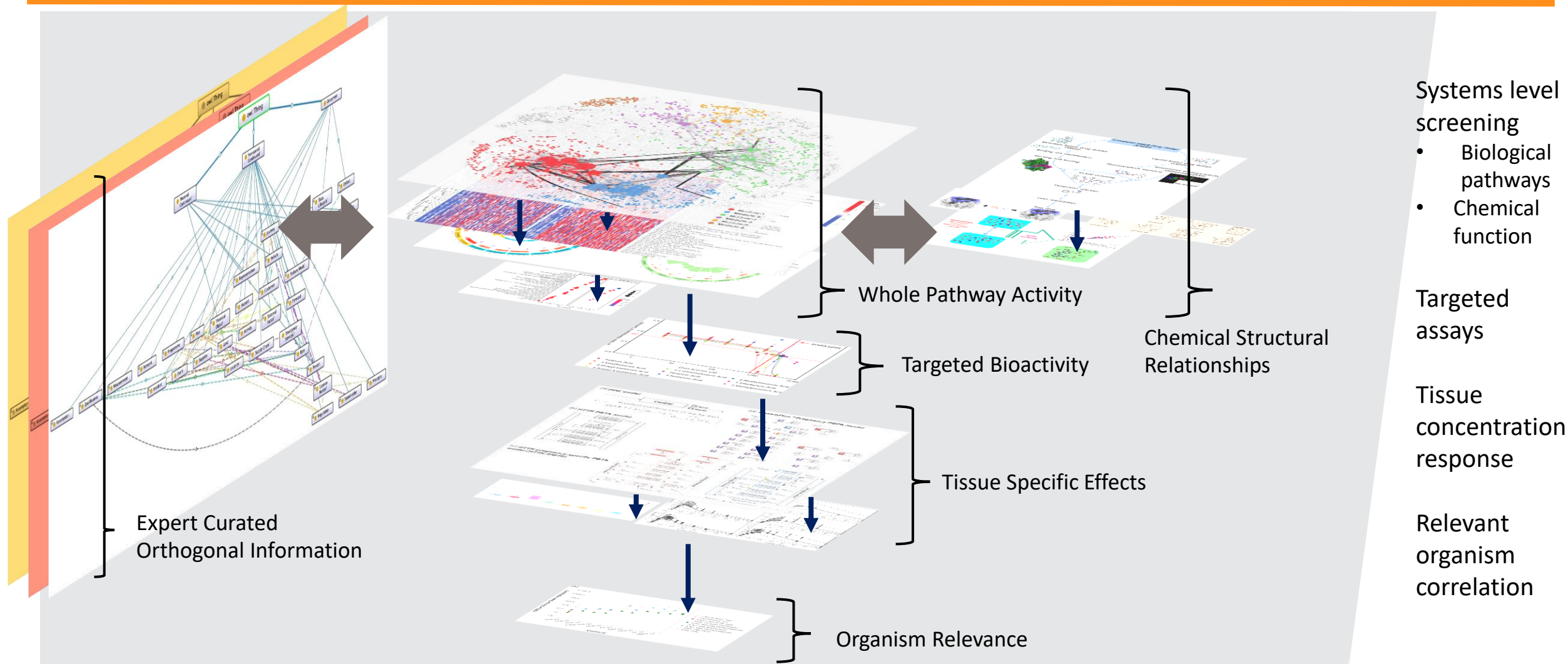
Traceable, reproducible methods

```
title 'STUDY_NUMBER endpoint T-Test';  
footnote 'Username: &SYSUSERID | Run Date: &SYSDATE9 | &SYSPROCESSID';  
  
%macro analyze(dsn, group, depend, side, control, treatment);  
  title2 Variable: &depend | Side: &side;  
  
  data ttest_data;  
    set &dsn;  
    if &group = "&control" then new_group = "ctrl_&control";  
    else if &group = "&treatment" then new_group = "trt_&treatment";  
  
  proc sort data = ttest_data;  
    by descending new_group;  
  
  proc ttest sides = &side data = ttest_data order = data;  
    class new_group;  
    var &depend;  
  run;  
  
%mend analyze;  
  
%analyze(dataset_name, group, endpoint1, side, control, treatment);  
%analyze(dataset_name, group, endpoint2, side, control, treatment);
```

Clean explanatory graphics



Computational Tox integrates screening and targeted informatics alongside expert curated annotation to identify critical therapeutic outcomes



The RTP Predictive Biology Platform streamlines development pipelines with low cost *in silico* screening

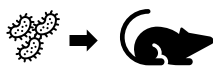
- Model systems to simulate how chemicals act and interact in biological systems
- Predict hazard and risk or target efficacy before work begins
- Estimate chemical exposures
- Generate some cool and informative visuals!
- Apply advanced ML and AI tools to reduced costs, increased speed, improved efficacy

Team members in the subject areas



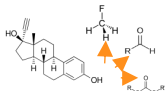
In silico predictive pharmacokinetics (**PK**):

Brad Reisfeld, Aswani Unnikrishnan



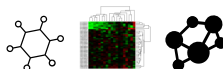
In vitro - in vivo Extrapolation (**IVIVE/ISVE**)

Anna Kreutz, Xiaoqing Chang



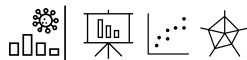
Chemical structure-activity relationships (**QSARs**)

Sherif Farag



Bioinformatics

Bryant Chambers



Biostatistics

Victoria Hull, Maddy Santoso

Explore our Genetic Toxicology Services



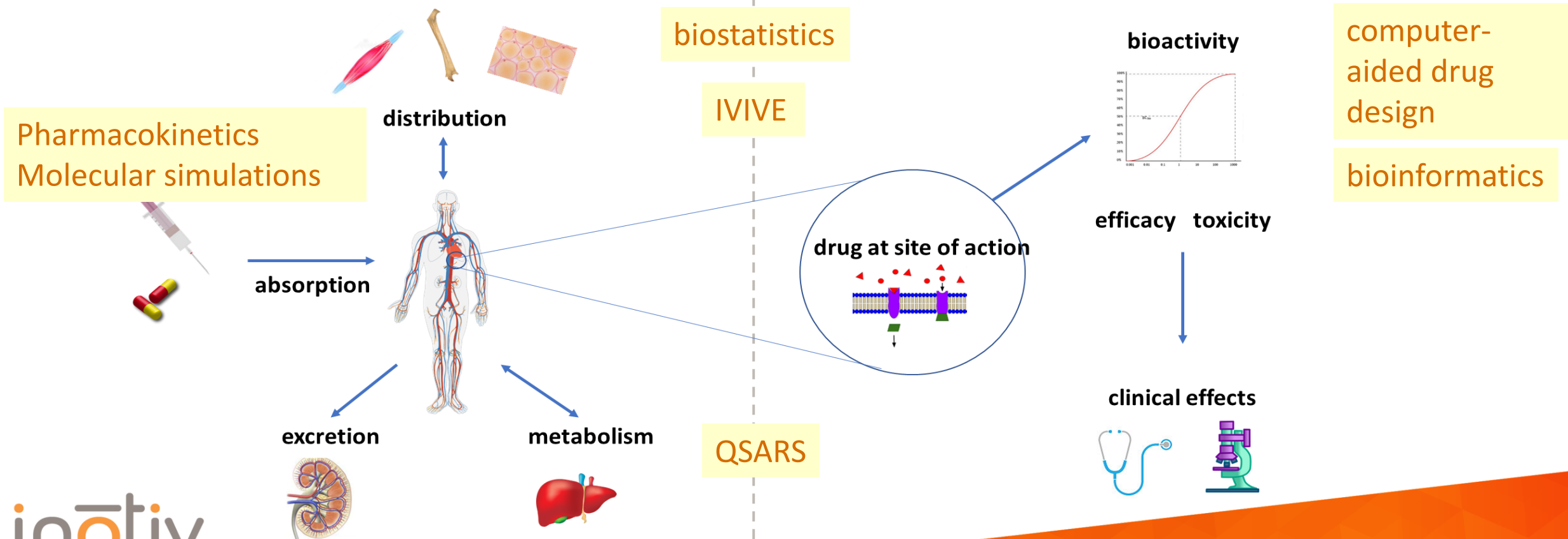
Overview and context: Drug disposition and effects

pharmacokinetics

‘what the body does to the drug’

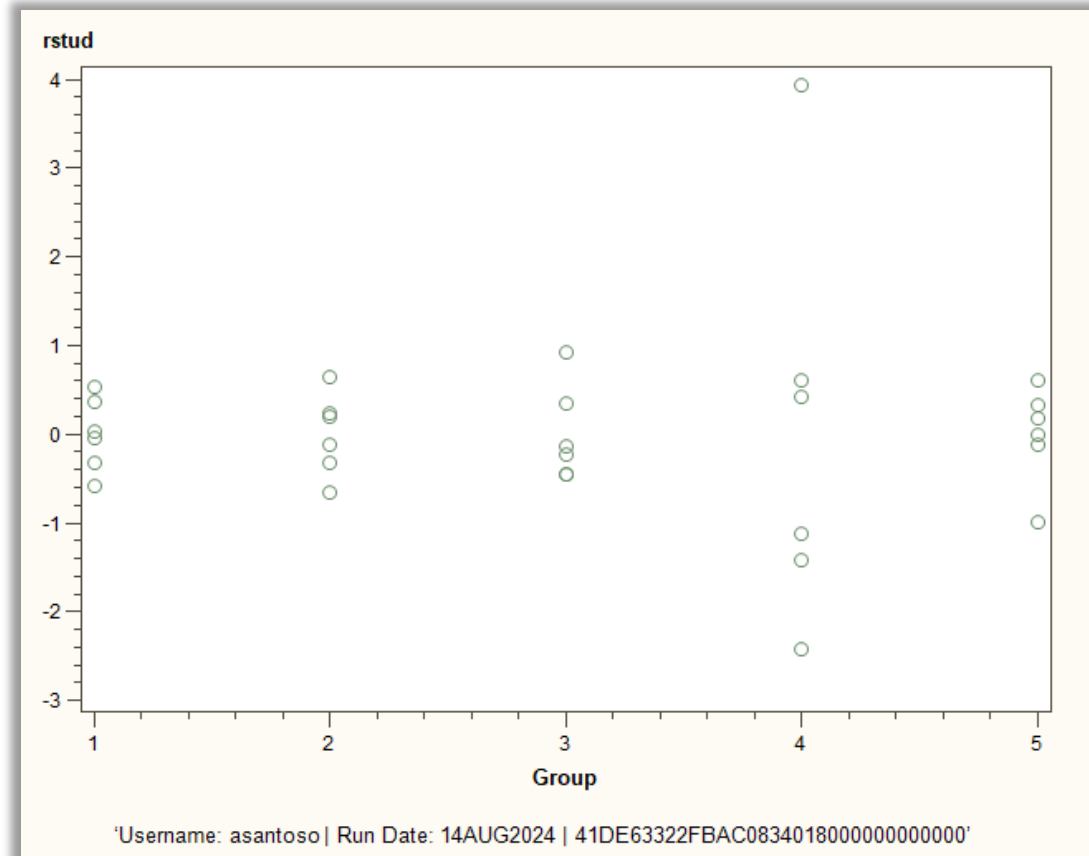
pharmacodynamics

‘what the drug does to the body’



Biostatistics: Data Visualizations

- **Data visualizations** enhance understanding of data and facilitate decision-making.



Biostatistics: Tools

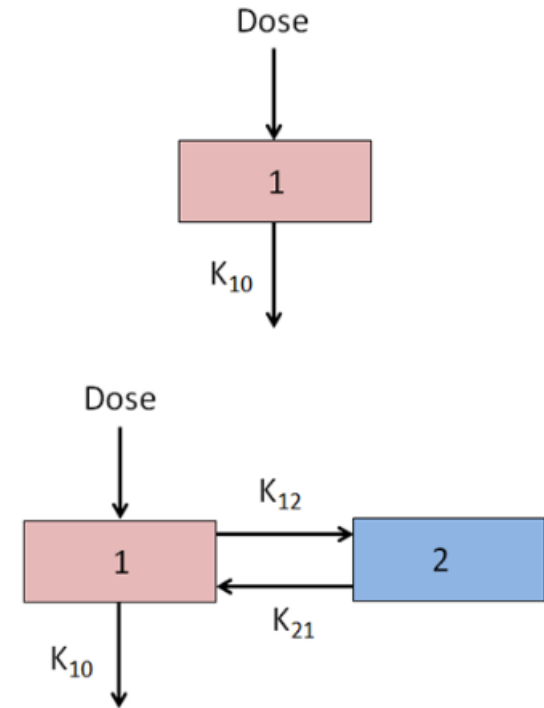
- GLP: Use of **SAS** and **Provantis** ensures regulatory compliance and accuracy in reporting.
 - > **Provantis** is an integrated preclinical software developed by Instem
 - > **SAS** is a statistical software suite developed by SAS Institute.
- Non-GLP: Use of **R** provides flexibility in non-GLP studies, allowing for rapid analysis and exploration of methods.
 - > **R** is a free open-source, open-access software for statistical analysis



Predicting the pharmacokinetics of a drug

‘Simple’ compartmental models

- The body is divided into hypothetical compartments (often one or two).
- The compartments do not represent actual physiological tissues
- Can be used to gain insights into many aspects of a drug’s ADME
- Often applied to characterize PK across multiple studies and observed variability across patients



K represents a rate of elimination or transfer

Pharmacokinetics (PK)

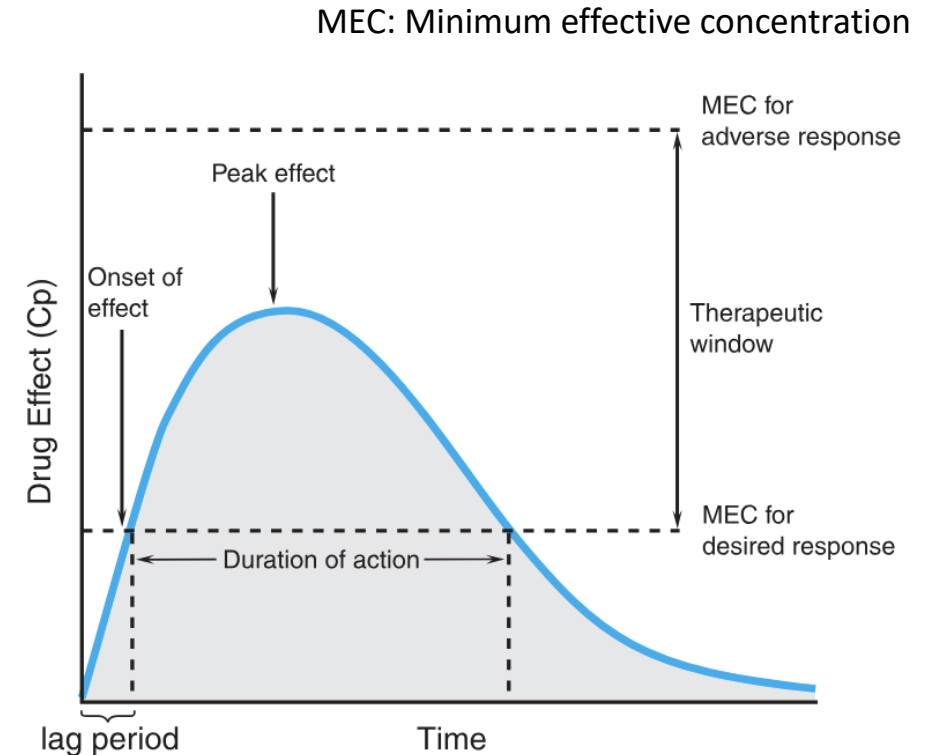
Why does understanding PK matter?

ADME processes determines a drug's

- > biodistribution
- > clearance rate
- > concentration over time

Analysis of PK is necessary to help determine

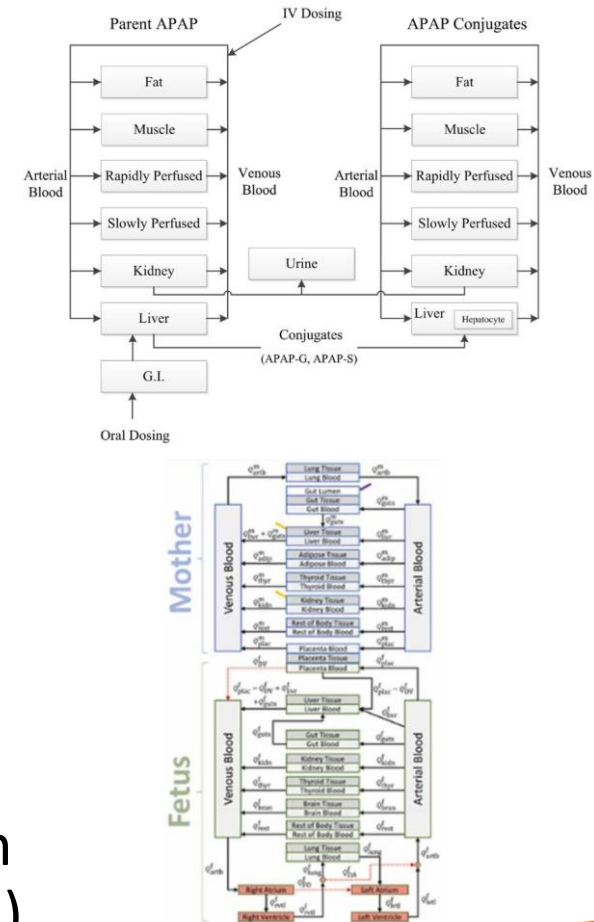
- > the therapeutic window (efficacy vs toxicity)
- > the duration of drug action
- > an effective dosing regimen



Predicting the pharmacokinetics of a drug

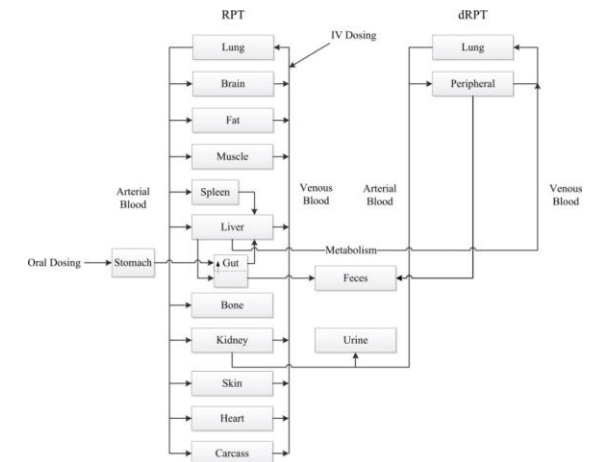
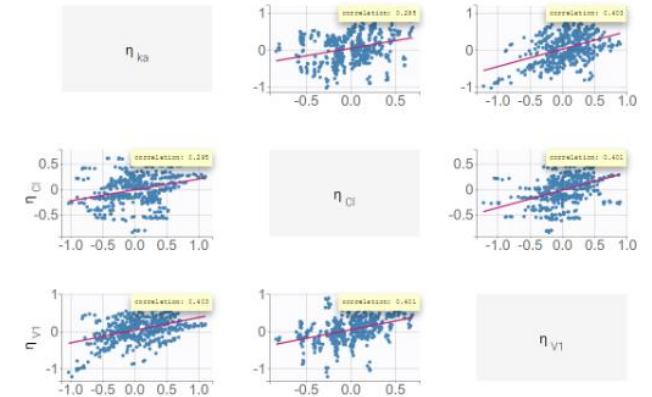
Physiologically-based pharmacokinetic (PBPK) models

- Represents the physiology and biochemistry of the species of interest through appropriate compartment
- Can include mechanistic information related to the specific drug and metabolites
- Allows tissue-specific predictions
- Very useful in extrapolating across doses, routes of administration, and species
- The US FDA supports the use of PBPK modeling results in various drug applications (e.g., INDs, NDAs, BLAs, ANDAs)

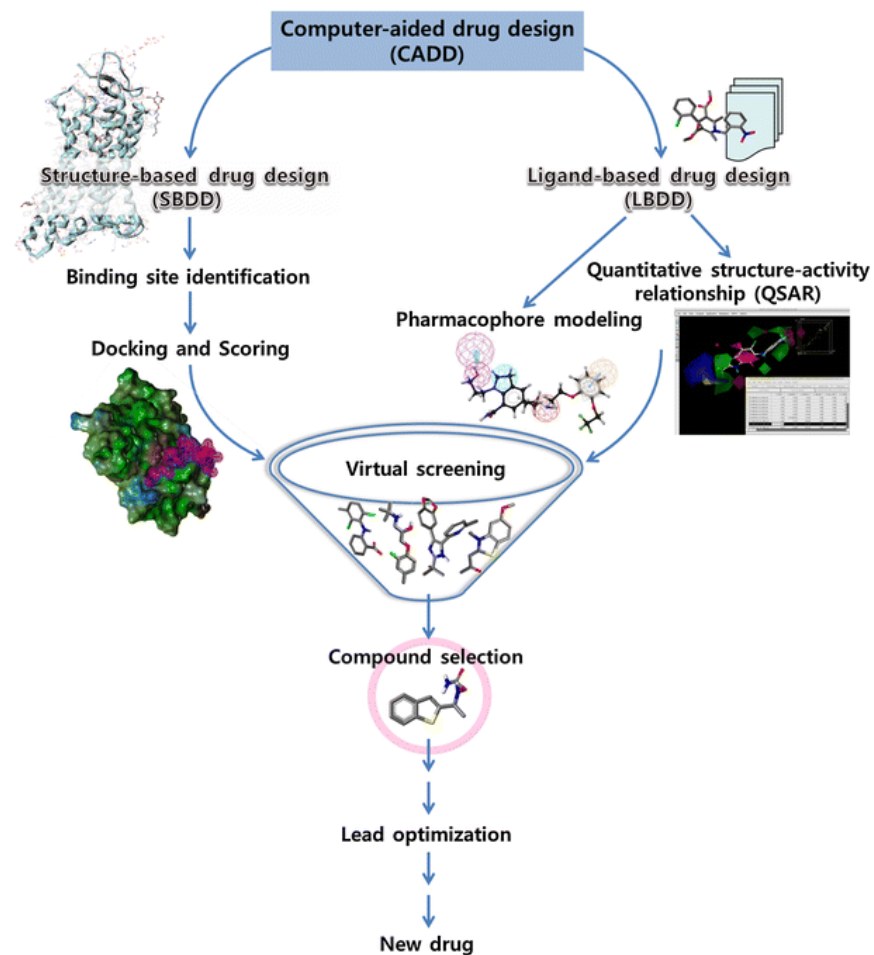


In silico predictive pharmacokinetics: What we can provide

- Simple non-compartmental and compartmental pharmacokinetic model development and application
 - Tools: MonolixSuite - PKanalix, R – mrgsolve, Python - Chi
- Physiologically-based pharmacokinetic (PBPK) model development and application
 - Custom models for chemicals of interest, with inclusion of relevant tissues/compartments, and detailed models of each ADME process
 - Utilize parameters from experiments, the scientific literature, and predictive ADME tools

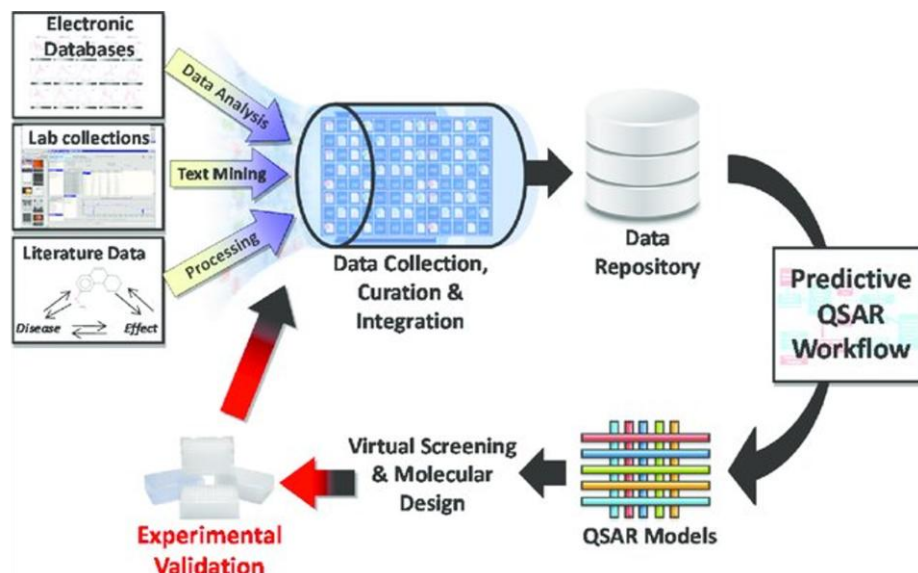


Computer Aided-Drug Design (CADD)



1. Macalino, S.J.Y., Gosu, V., Hong, S. *et al.* Role of computer-aided drug design in modern drug discovery. *Arch. Pharm. Res.* 38, 1686–1701 (2015)

Quantitative Structure Activity Relationship (QSAR)

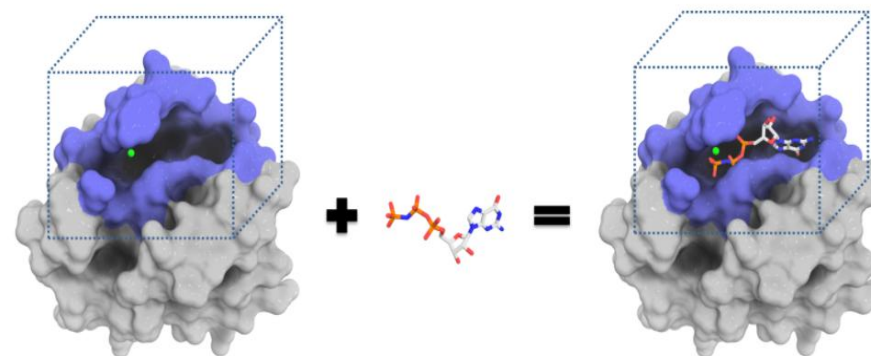


- Compute binding affinity.
- Predict ADME properties.
- Predict toxicity.
- Many other applications.

1. Cherkasov A.; et al. QSAR modeling: where have you been? Where are you going to?. *Journal of medicinal chemistry*. 2014, 57(12): 4977-5010.

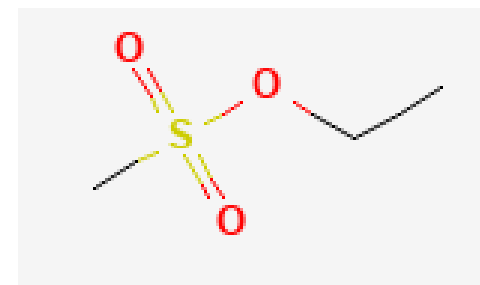
Cheminformatics Tools and Services

- Develop and Apply Ligand and Structure Based Computational Approaches
 - > QSAR Models
 - CASE Ultra by MultiCASE
 - > Docking
 - > Virtual screening (SBVS & LBVS)
 - > Pharmacophore modelling
 - > SAR Analysis
 - > Design Cheminformatics pipelines & workflows
 - Data mining, curation and visualization
- Support Prioritization of Chemical/Biological Testing and Regulatory Processes Through *In Silico* Modeling.



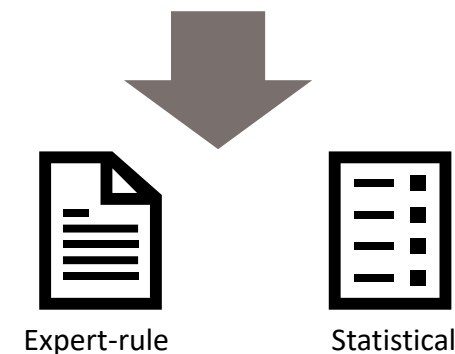
In Silico Testing for Bacterial Mutagenicity

- ICH M7 guideline
 - > Assessment and control of DNA reactive impurities in pharmaceuticals to limit potential carcinogenic risk
- CASE Ultra by MultiCASE: QSAR prediction methodologies that predict the outcome of a bacterial mutagenesis assay
 - > Expert-rule based model
 - > Statistical model



Test Compound

CCOS(=O)(=O)C



Other In silico Prediction Models that are available

- Hepatotoxicity

- > Models for DILI, liver function, bile duct disorders, cholestasis, gall bladder disorders

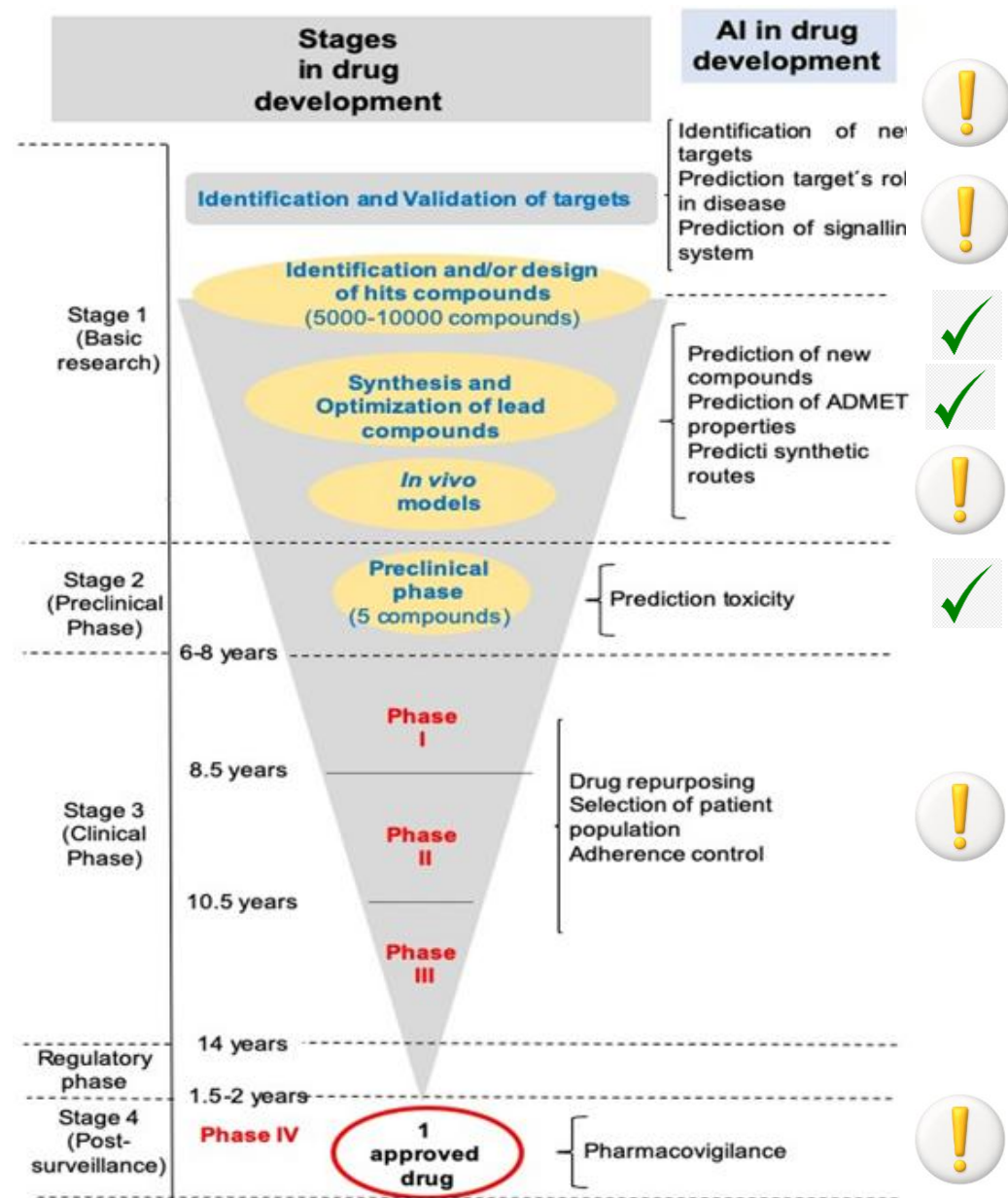
- Cardiotoxicity

- > Models for hERG K⁺ channel blockers and protectors

- ADME

- > Models for extent of metabolism, oral bioavailability, plasma protein binding, urinary excretion, volume of distribution (human)

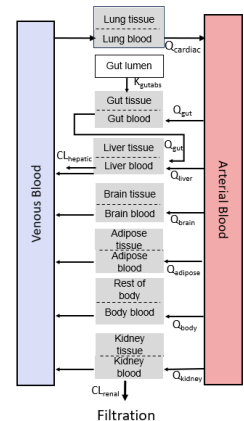
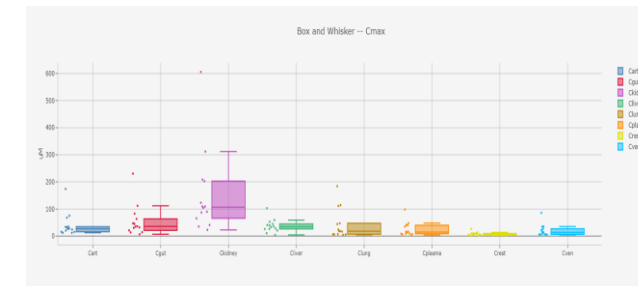
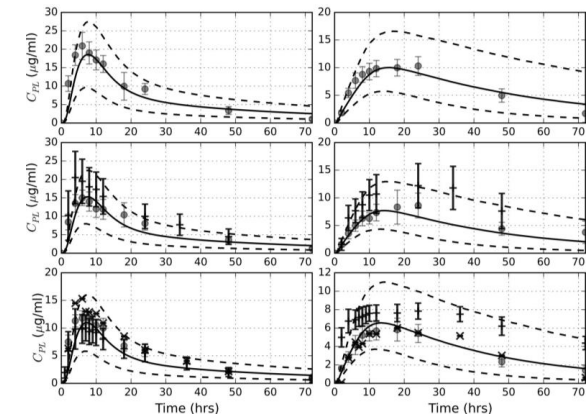
AI in Drug Discovery



Gallego, Victor, et.al. (2021). AI in drug development: a multidisciplinary perspective. Molecular Diversity. 25. 10.1007/s11030-021-10266-8.

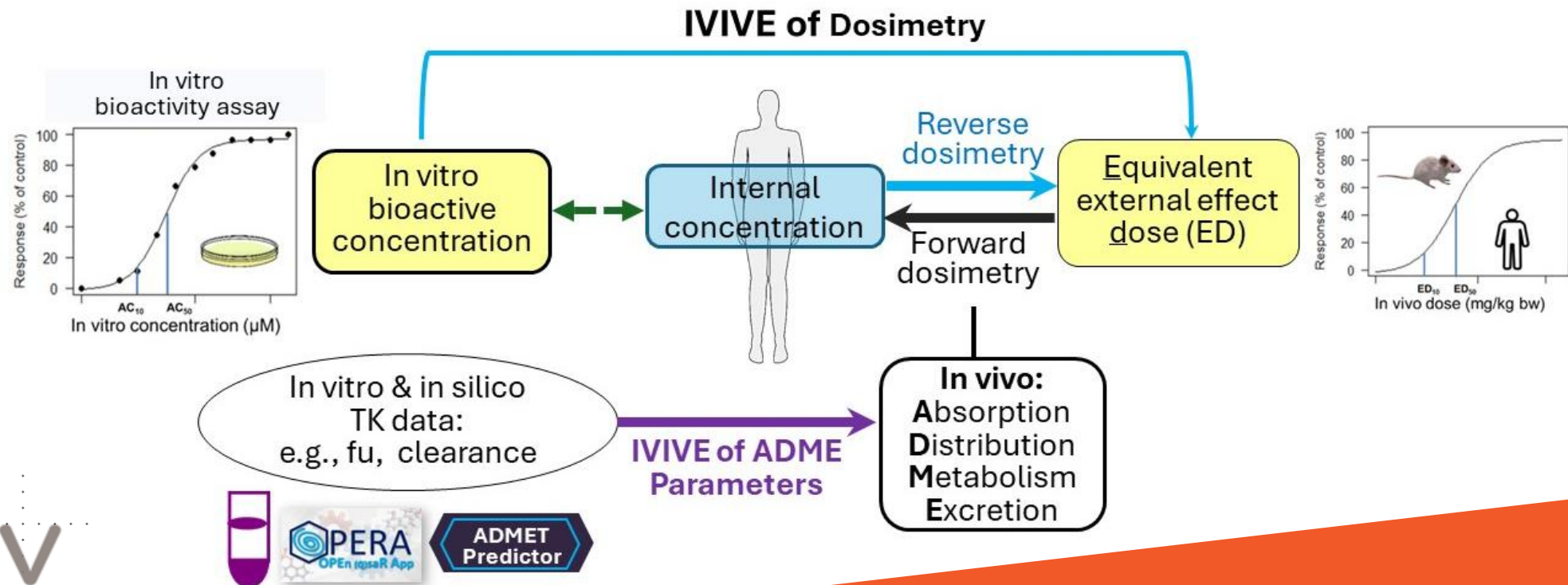
In silico predictive pharmacokinetics: What we can provide

- Physiologically-based pharmacokinetic (PBPK) model development and application
 - > Population/subpopulation models
 - variability in physiological, anatomical, and biochemical measures
 - hierarchical parameter estimation
 - > High-throughput analyses for libraries of chemicals, including predictions of tissue specific concentrations and dose-metrics like C_{max} , C_{ss} , AUC, and $t_{1/2}$
 - > Dose $\rightarrow$ Tissue Concentration, Tissue Concentration $\rightarrow$ Dose
 - > Tools: MCSim, PK-Sim, htk, GastroPlus, Integrated Chemical Environment



In vitro/in silico to in vivo extrapolation (IVIVE/ISIVE) extends PK to estimate in vivo response from early testing

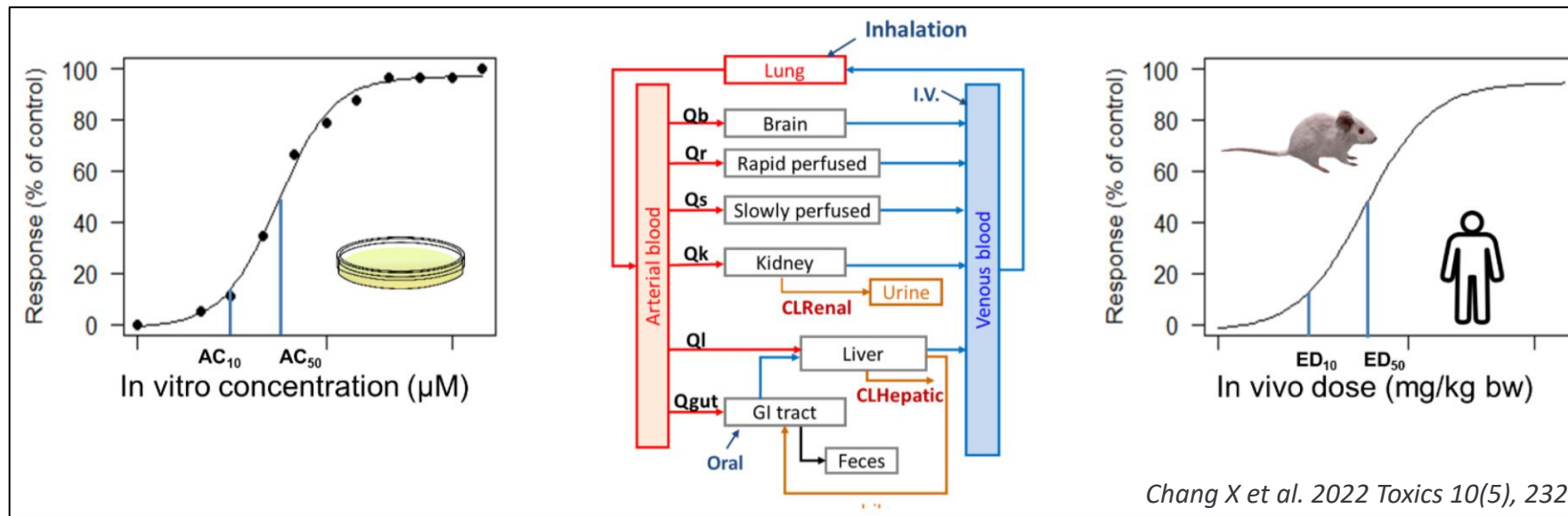
- Approach that uses **in vitro experimental data** or **in silico predictions** to predict **in vivo phenomena or outcomes** (e.g. effect dose, kinetics, toxicity)
 - > Takes a lab test and extrapolates the result DIRECTLY to target species response
- Reverse dosimetry: estimation of dose/exposure based on internal concentration



IVIVE of Dosimetry

- Uses pharmacokinetic (PK) models to translate in vitro activity concentrations (AC_x) into equivalent exposure
- Requires use of PK or physiologically based PK (PBPK) modeling

In vitro AC_x → PBPK modeling-based reverse dosimetry → Predicted in vivo effect dose



IVIVE of ADME parameters

- Translates in vitro experimental data on in silico predictions into in vivo **kinetic** outcomes
- Common in vitro techniques
 - > Rapid equilibrium dialysis (RED) to measure fraction unbound to plasma protein (f_u)
 - > Caco-2 cell assay to predict intestinal absorption
 - > Scaling from in vitro intrinsic clearance ($\mu\text{L}/\text{min}/10^6$ cells) to hepatic clearance (L/h)

