Grand Challenges
African Drug Discovery Accelerator

Drug Discovery and Development Course

In vivo pharmacokinetics

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Key objectives

1. What are VD and clearance?
2. How do we estimate them?
3. Are transporters involved?
4. What is DDI?
5. Some renal physiology and excretion points to remember.

Why study PK?

The roots of our ADME  **worries!**

"Good" Pharmacokinetics

→ Convenient Dosing Regimens

→ Higher Patient Compliance

→ Better Efficacy

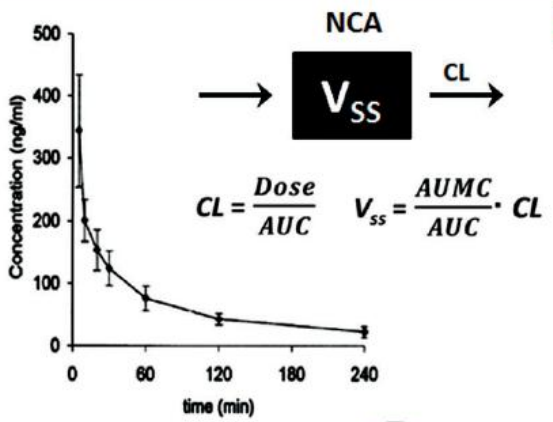
→ Competitive Advantage

One *definition of* ***drug-like*** *could be "pharmacokinetics friendly" as we have to deliver these drugs to humans...therefore we need to reason about pharmacokinetics. (Zhao, DDT,* ***2011****, 64, 158-163).*

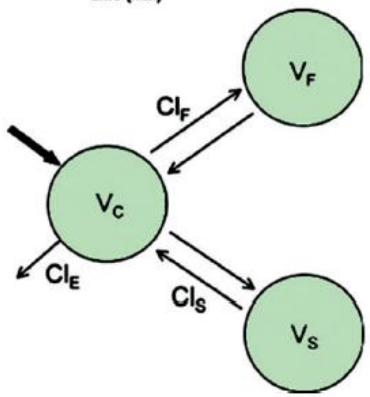
How do we study it? Well…with models!

Array of PK Models

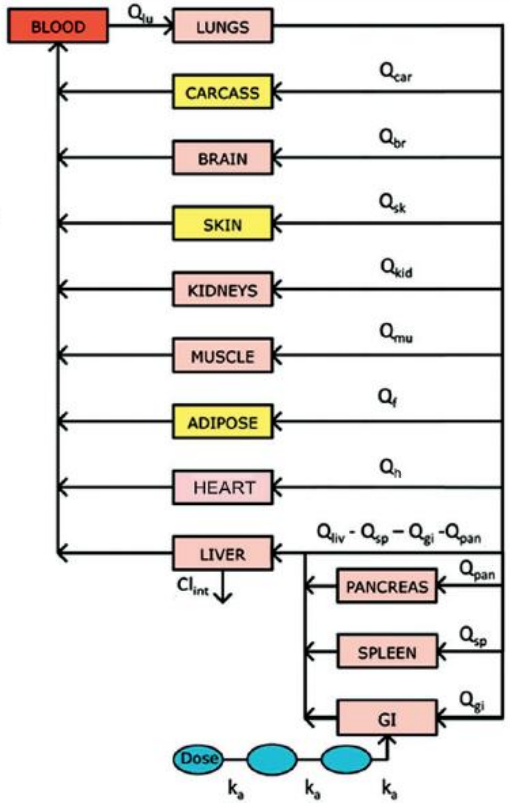
Non-compartmental



Compartmental



Physiologically-based PK



REVIEW of PK/PD

Jusko, *J. Pharm. Sci.* **2013**, 102, 2930-2940.

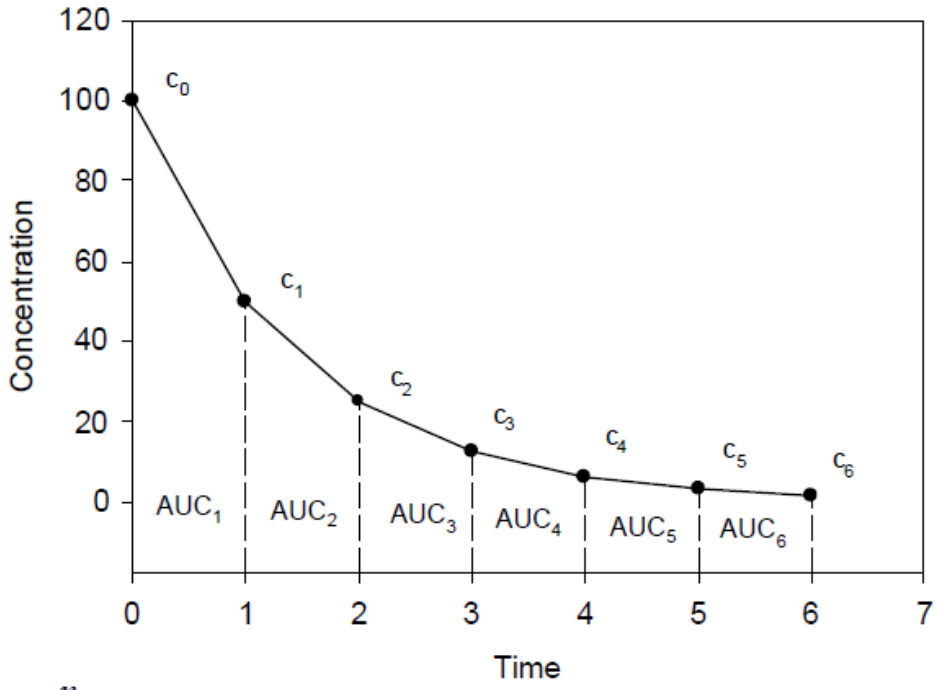
TWO OTHER REFS 2023. IN ADDITIONAL INFORMATION?

Fan J, de Lannoy IA, Pharmacokinetics *Biochem Pharmacol.* **2013**, *87*, 93-120

Nice and condensed review of PK and PD

Non-Compartmental Analysis

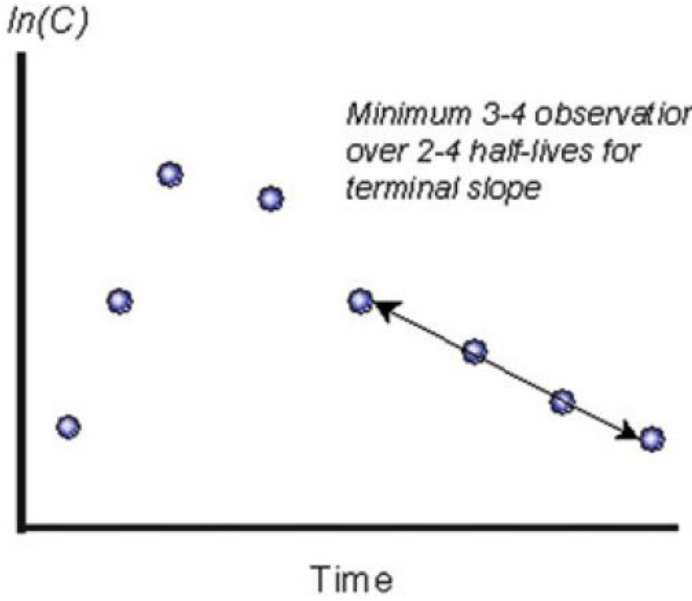
Trapezoidal Rule for AUC calculation
(Typical plot for an iv dose)
(C_o is not measured experimentally but estimated by regression)



$$AUC_0^{t_{last}} = \sum_{i=1}^{n} \frac{C_i + C_{i+1}}{2} \cdot \Delta t,$$

Linear trapezoids (shown) or log-linear trapezoids

(Typical plot for an extravascular dose)

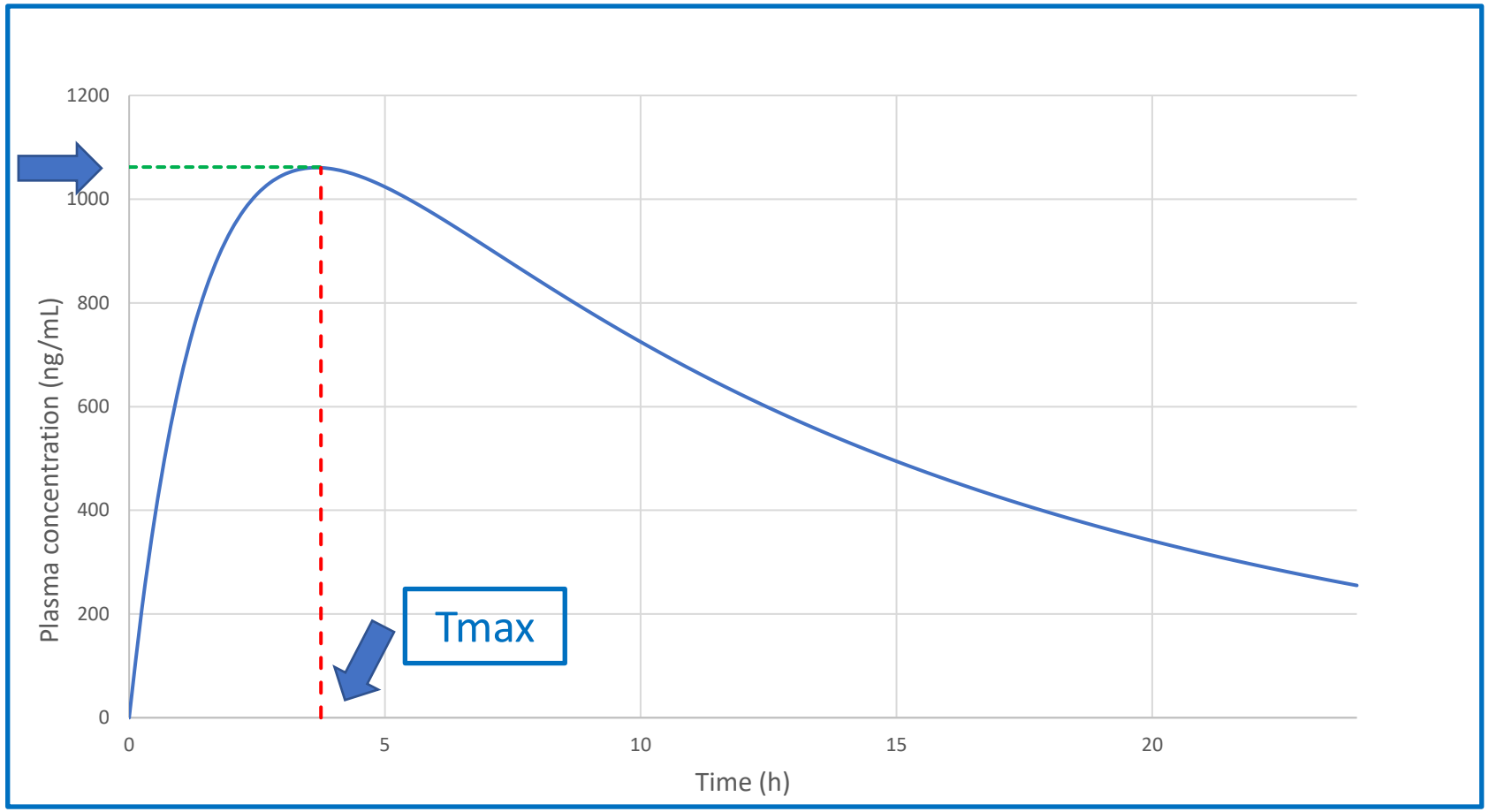


$$AUC_{Clast-\infty} = \frac{C_{last}}{k_{el}}$$

Generally, C_{last}

$k_{(el)}$ is elimination rate constant which is calculated via linear regression of the terminal phase of PK curve.

Extravascular administration: rates and phases



Rate of absorption > rate of elimination

Rate of absorption ≈ rate of elimination

Rate of absorption < rate of elimination

Rate of absorption ≈ 0 (no drug left in GI tract or site of administration) rate of elimination dominates this region

Absorption and bioavailability

Absorption and **B**ioavailability are not interchangeable

Bioavailability (BA or F) is defined as:

$$F = AUC_{route1} / AUC_{route2} * Dose_{route2} / Dose_{route1}$$

if route 2 (reference) is intravenous then the BA is defined as "absolute"

If not, then we talk about relative BA or F, and F is generally defined as the result of

$$F = f_a * f_g * f_h$$

Where f_a, f_g and f_h are, respectively, the fraction absorbed, the fraction escaping gut metabolism and the fraction escaping first-pass liver metabolism.

***B**ioavailability may also be influenced by food either negatively (Cmax and/or AUC ↓ w/food) or positively (Cmax and /or AUC ↑ w/food)*

A single species cannot generally be considered predictive of human, watch for dose, formulation as well as particle size and species-specific factors. Musther et al. *Eur. J. Pharm. Sci.* **2014**, *57*, 280-291. However, we generally consider an average of multiple species F as a reasonable approximation.

Getting…M.A.D.

- The **M**aximum **A**bsorbable **D**ose is a useful (and logical) ***tool*** to rank compounds

$$\mathbf{MAD\ (mg) = S_{6.5} \times k_a \times IFV \times SITT}$$

- $S_{6.5}$ = Solubility @ pH 6.8 (mg/mL) or at similar pH.
- k_a = Absorption rate constant (min^{-1} or h^{-1}, from SPIP* in rat or P_{eff} scaling).
- IFV = Small intestine fluid volume (250 mL given with dose).
- SITT = Small intestine transit time (taken as 270 min or 4.5 h).

*Single Pass Intestinal Perfusion

$\log P_{eff}$ = 0.829*$\log P_{app,(MDCK, A-B)}$ -1.30 (Gertz et al, 2011)

$\log P_{eff}$ = 0.493*$\log P_{app,(Caco-2)}$ -0.145 (Sun et al, 2002)

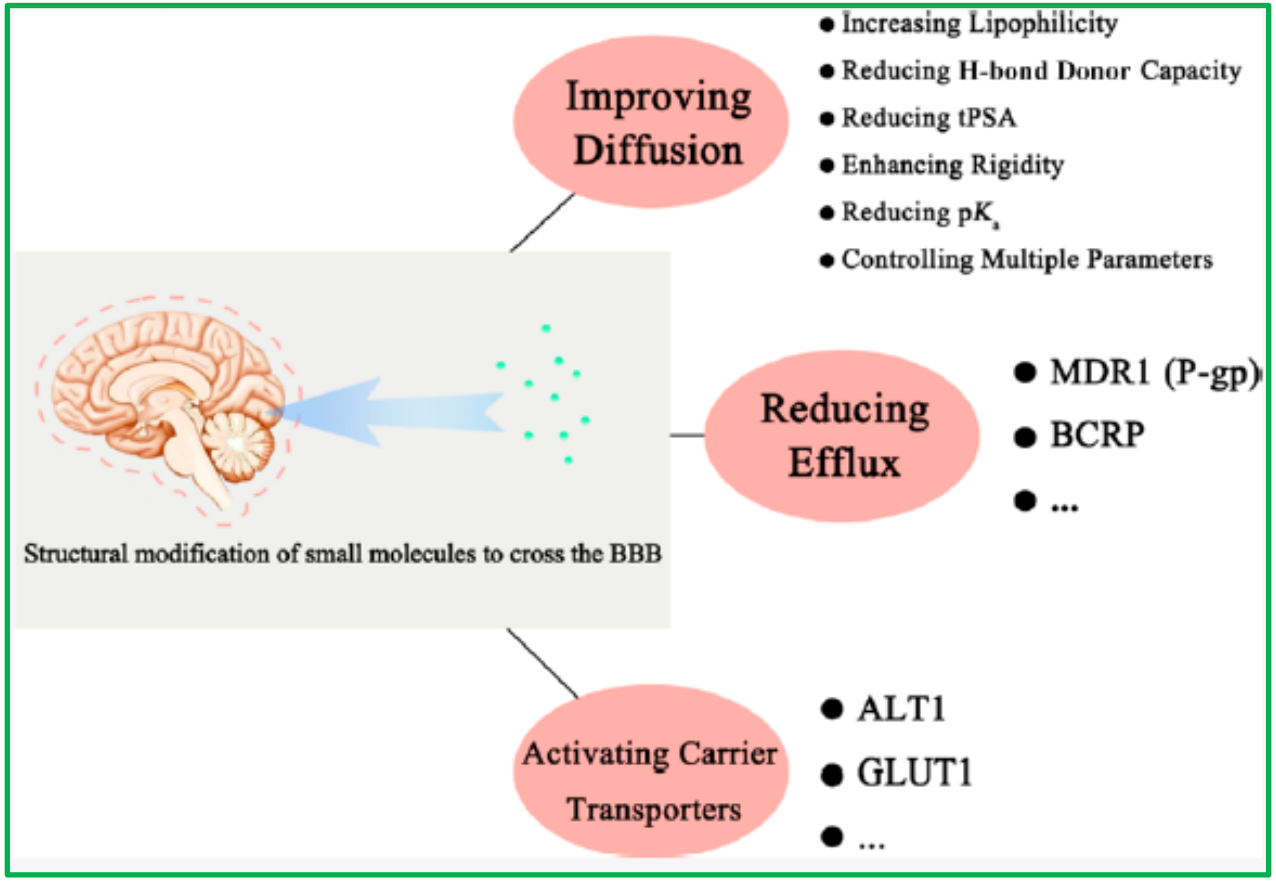
NOT "UNIVERSAL"…DATA DEPENDENT

$k_a = \frac{2 * P_{eff}}{r_{SI}}$ **(in human)** (Chiou et al, 1994)

MAD: K. C. Johnson et al. *Pharm. Res.* **1996**, *13*, 1795-1798; Hintz and Johnson Int. J. Pharm. **1989**, *51*, 9-17; W. Curatolo, *PSTT*, **1998**, *1*, 387-393. (A very good "applied" description).

P_{app}, **P_{eff}** and **k_a**: Chiou, W. L., *Biopharm. Drug Disp.* **1994**, *15*, 709-717; Sun D et al. *Pharm. Res.* **2002**, *19*, 1400-1416; Lennernas H., *Xenobiot.* **2007**, *37*, 1015-1051; Gertz M. et al. *Drug Metab. Dispos.* **2011**, *39*, 1633-1642

The quest for CNS drugs



Strategies for Structural Modification of Small Molecules to Improve Blood–Brain Barrier Penetration: A Recent Perspective
Xiong et al, *J. Med. Chem.* **2021**, *64*, 13152−13173

Loryan et al. Unbound Brain-to-Plasma Partition Coefficient, $K_{p,uu,brain}$-a Game Changing Parameter for CNS Drug Discovery and Development. *Pharm Res.* **2022** 39, 1321-1341

Loryan et al. Brain Distribution of Drugs: Pharmacokinetic Considerations. *Handb Exp Pharmacol.* **2022**, *273*, 121-150.

de Lange ECM et al. Understanding the Blood-Brain Barrier and Beyond: Challenges and Opportunities for Novel CNS Therapeutics. *Clin Pharmacol Ther.* **2022**, *4*, 58-773.

Kettle J et al. Discovery of AZD4747, a Potent and Selective Inhibitor of Mutant GTPase KRASG12C with Demonstrable CNS Penetration *J. Med. Chem.* **2023**, *66*, 9147−9160

Other references in back up slides

Some words of wisdom about $K_{p,uu}$ and BBB

*How Much is Enough? Impact of Efflux Transporters on Drug delivery Leading to Efficacy in the Treatment of Brain Tumors
Wenjuan Zhang · Ju-Hee Oh · Wenqiu Zhang · Sneha Rathi · Jiayan Le · Surabhi Talele · Jann N. Sarkaria · William F. Elmquist
Pharm. Res. **2023**, *40*, 2731–2746

"In another words, is there a numerical cutoff value for $K_{p,uu}$ for potentially effective treatment of brain tumors? The short answer is NO."

Sato, et al. Translational CNS Steady-State Drug Disposition Model in Rats, Monkeys, and Humans for Quantitative Prediction of Brain-to-Plasma and Cerebrospinal Fluid-to-Plasma Unbound Concentration Ratios. *AAPS J*, **2021**, *23*: 81

"These results suggest that the use of $Kp_{,uu,CSF}$ as a surrogate of $_{Kp,uu,brain}$ in rats, monkeys, and humans will have to be reconsidered especially under which conditions Kp,uu,CSF can be assumed to equal Kp,uu,brain."

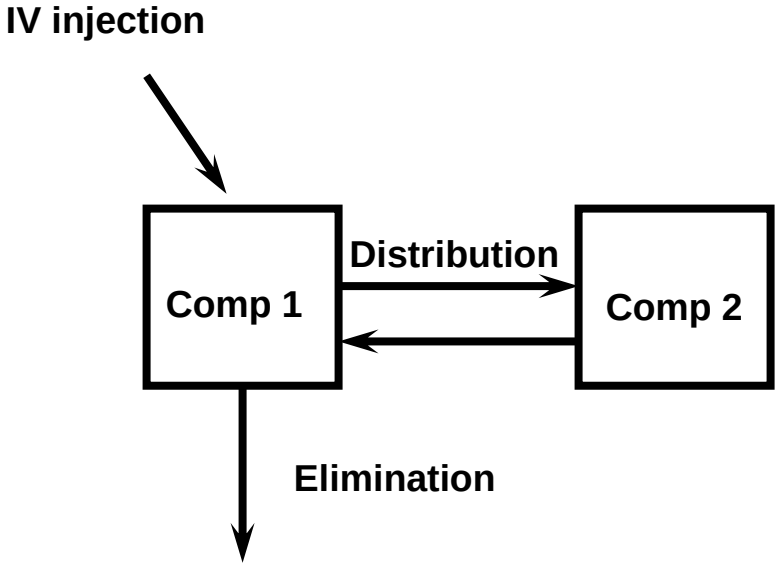
"In addition, the MDCK-MDR1 cell line was selected to determine the in vitro efflux activity for MDR1 in these models because we previously observed that MDCK-MDR1 had superior sensitivity in efflux ratio and correlated well with the brain disposition of different compounds."

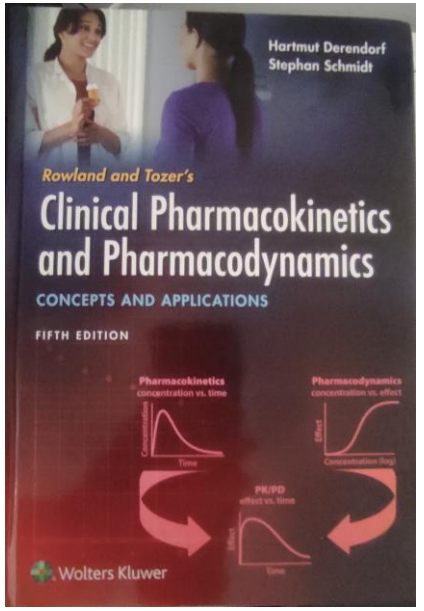
Main points

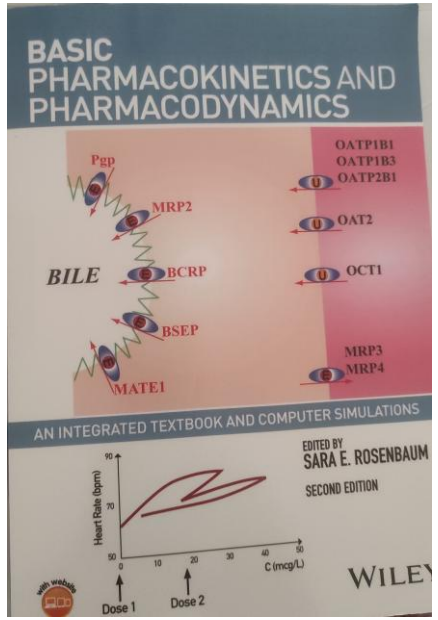
- K_p(total) is not useful and *may be misleading*.
- There is no ABSOLUTE threshold for $K_{p,uu}$ what is your potency?*
- $K_{p,uu\ CSF}$ may be appealing as it is measurable in human, but it is typically higher (some 3-fold) than $K_{p,uu,brain}$.
- **DO NOT "CONDEMN"** a compound o the basis of BCRP and/or MDR1 efflux. Think of risperidone and paliperidone!
  Doran et al, *Drug Metab Dispos* **2005**, *33*, 165–174.

Compartmental Pharmacokinetic Analysis

Mathematical treatment of a two compartment PK model from iv dosing







Mathematical equations associated with a two-compartment PK model *(most often used)*

(sometimes there is the need to use a more complex three compartment PK model)

Differential equation

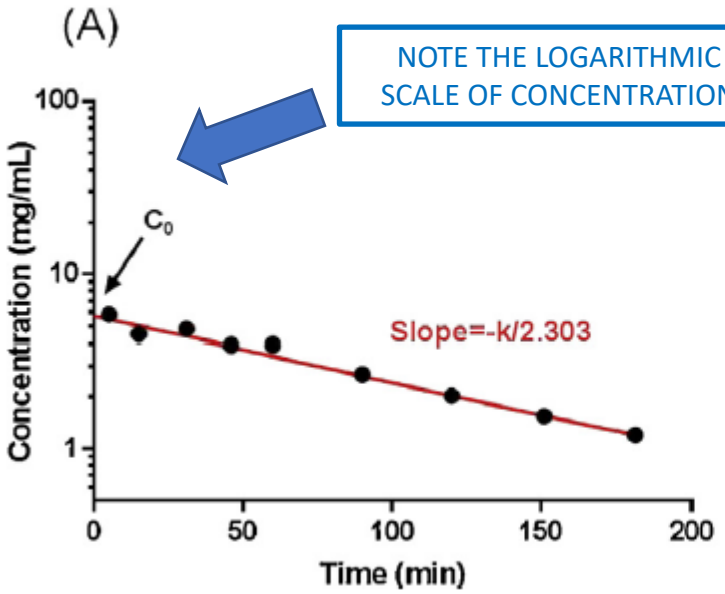
$$\frac{dX1}{dt} = -kel \bullet X1 - k12 \bullet X1 + k21 \bullet X2$$

Integral equation

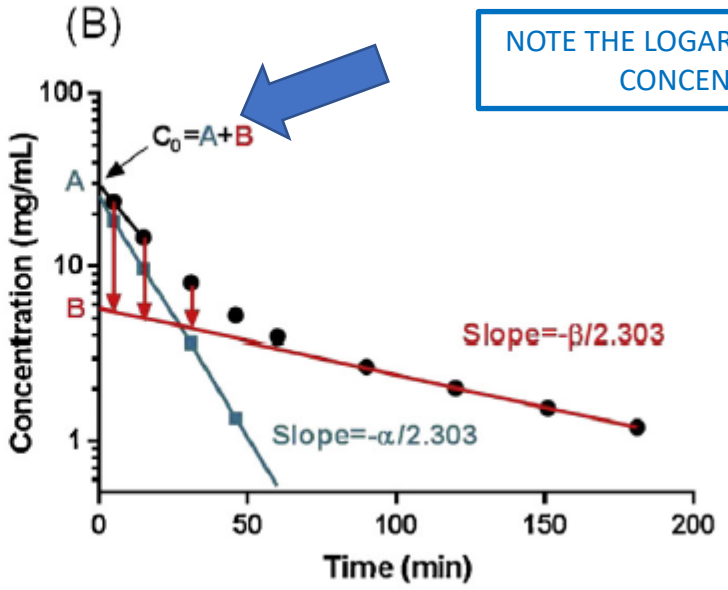
$$Cp = A \bullet e^{-\alpha \bullet t} + B \bullet e^{-\beta \bullet t}$$

Compartmental Pharmacokinetic Analysis

Example of a compound with one compartment PK model



Example of a compound with two compartment PK model



PBPK-Models

Lin, Chen et al. *Pharm Res* **2022**, *39* 1701-1731

They mentioned Gastroplus ®, SIMCYP ® and PK-SIM ® and offered a thorough discussion of challenges and opportunities

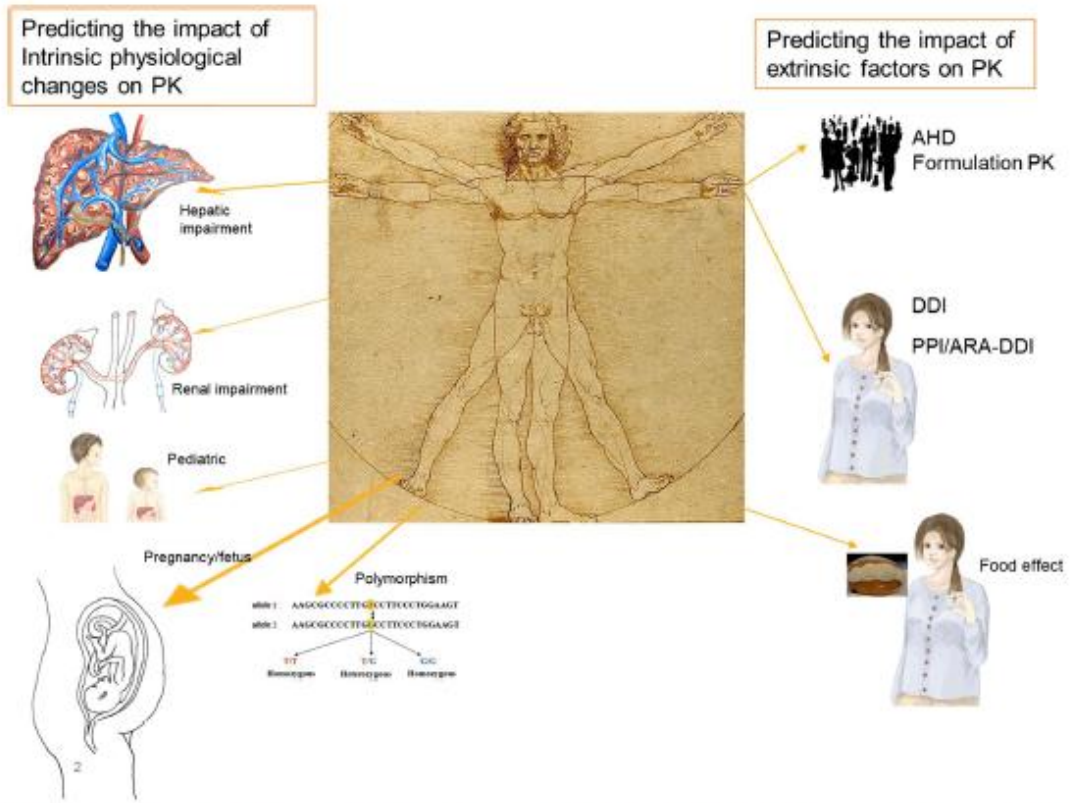


Fig. 1 Selected PBPK modeling applications. AHD: anticipated human dose or dosing regimen; DDI: drug-drug interaction, PPI: proton pump-inhibitor, ARA: acid reducing agent.

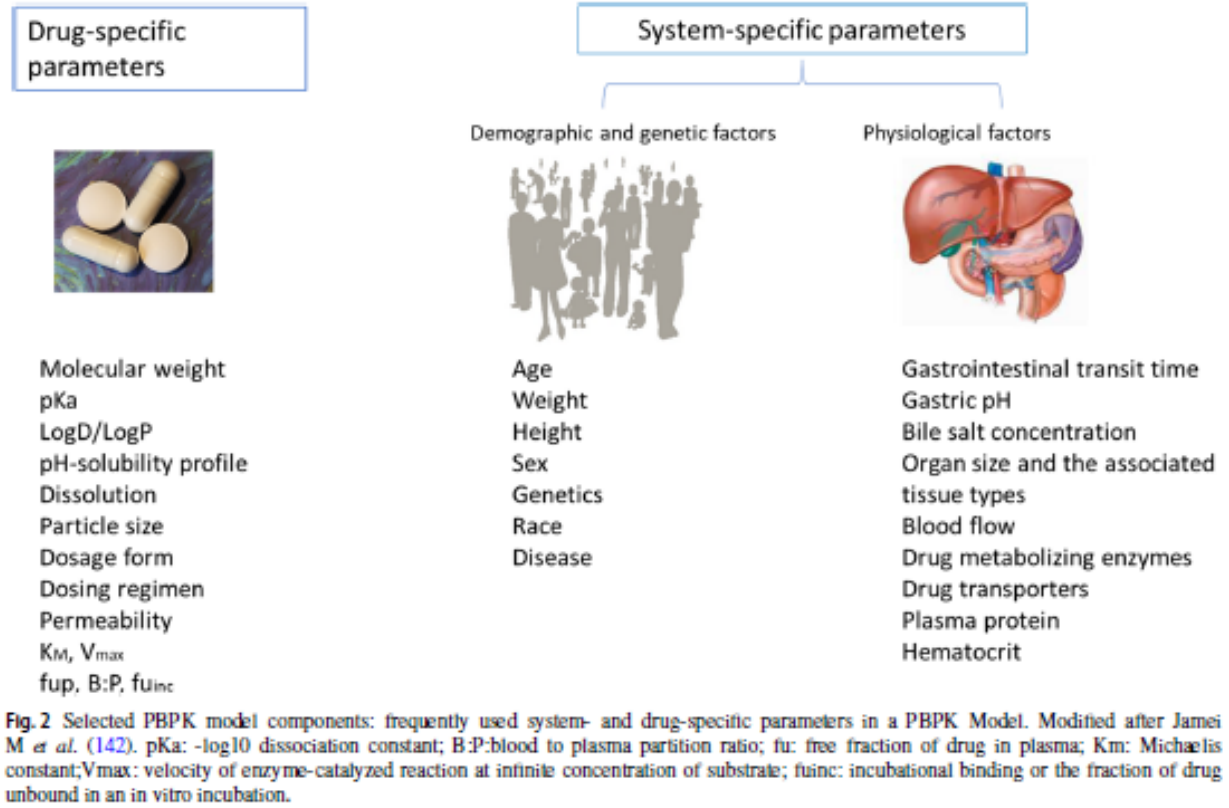


Fig. 2 Selected PBPK model components: frequently used system- and drug-specific parameters in a PBPK Model. Modified after Jamei M *et al.* (142). pKa: -log10 dissociation constant; B:P:blood to plasma partition ratio; fu: free fraction of drug in plasma; Km: Michaelis constant;Vmax: velocity of enzyme-catalyzed reaction at infinite concentration of substrate; fuinc: incubational binding or the fraction of drug unbound in an in vitro incubation.

https://www.simulations-plus.com/software/**gastroplus**/pbpk-software/

PBPK-Models

- Mathematical model that integrates compound specific data (e.g., lipophilicity, particle size, permeability etc.) with physiology (system specific parameters such as blood flow, tissue volumes etc.) to model, fit and predict PK of compounds

- PBPK models composed of many compartments corresponding to the different tissues of the body, e.g., adipose, bone, brain, gut, heart, kidney, liver, lung, muscle, skin, and spleen, which are connected by the circulating blood system (arterial and venous)

See for example Jones HM et al, *AAPS J*, **2009**, *11*, 155-166

However,

The complexity of PBPK models, which include many adjustable parameters, mandates the definition of a ***consistent*** *model building strategy and best practice guidance.*

We believe that a consistent PBPK strategy for FIH predictions, based on best practices and experience across companies, should increase the confidence of regulatory agencies in this application.

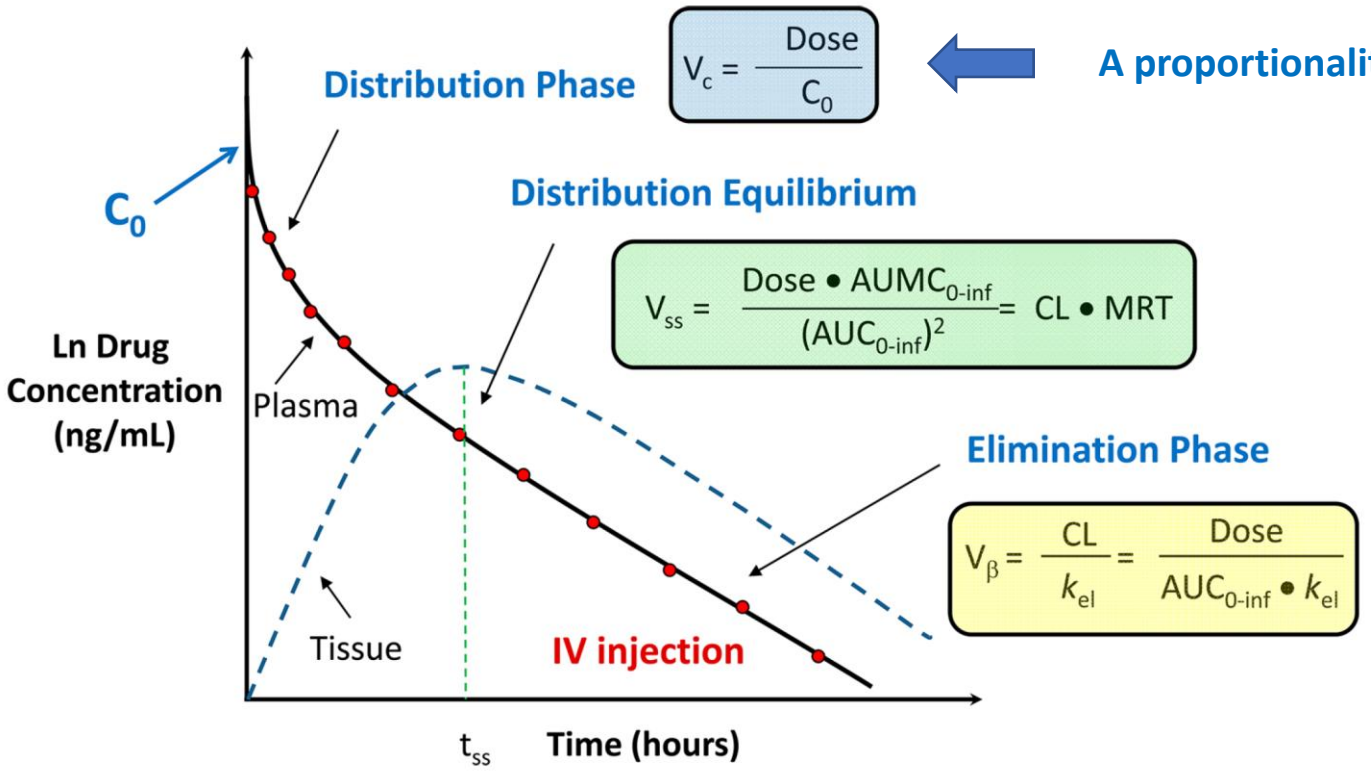
Miller NA et al. *Clin. Pharmacokin.* **2019**, *58*, 727-746

See also: PBPK Analyses, Format and Content Guidance for Industry, FDA, August **2018**

For a very recent discussion and retrospective analysis on Genentech compounds see :
Mao et al. *Biopharm. Drug Dispos* **2023**, *44*, 315-334

PK Parameters: Volume of Distribution

The various types of VD and their determination



A proportionality constant between dose and concentration

In vivo: from plasma data.

Reproduced from: Smith, Beaumont, Maurer and Di. Volume of Distribution in Drug Design *J. Med. Chem.* 2015, *58*, 5691-5698.

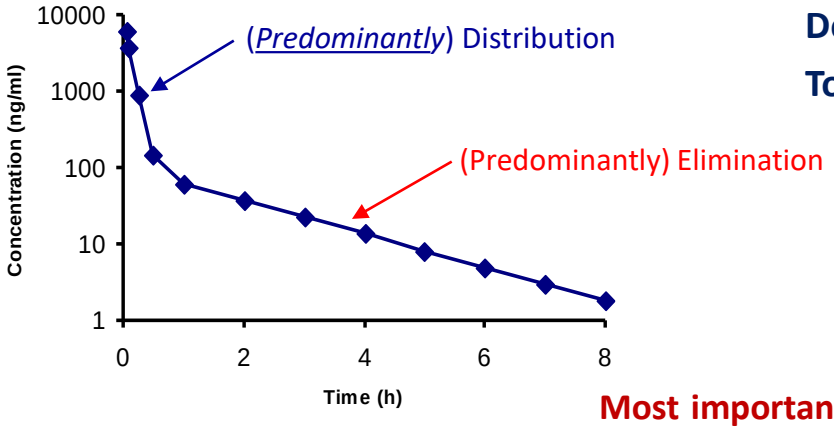
"Accurate V_{ss} prediction is still an evolving field."
Mathew et al. *J. Pharm Sci* **2021**, *110*, 1799-1823

Hsu, Chen and Broccatelli *Drug Metab Disp* **2021**, *49*, 330-336.

Berellini and Lombardo, *Drug Metab Disp* ***2019****, 47,* 1380-1387

Lombardo, Bentzien, et al. *J. Pharm. Sci.* ***2021,*** *110,* 500-509

PK Parameters: Clearance



Dose - After dosing directly into blood stream (e.g., i.v.)

Total exposure - Area under the concentration *vs.* time curve

$$CL_{TOT} = \frac{\text{Dose}}{\text{Area Under the curve}}$$

Most important PK parameter and is a measure of drug elimination from the body. Defined as the volume of biological fluid that is cleared of drug per unit of time (unit of mL/min or L/hr) or per unit of time and unit of weight (mL/min/kg).

CL is measured after systemic (IV) administration of a compound. CL following non-systemic administration (PO, SC, IM or IP) is called apparent CL (CL/F)

Clearance is additive!

$$Cl_{TOT} = Cl_{met} + Cl_{ren} + Cl_{biliary} + \ldots..$$

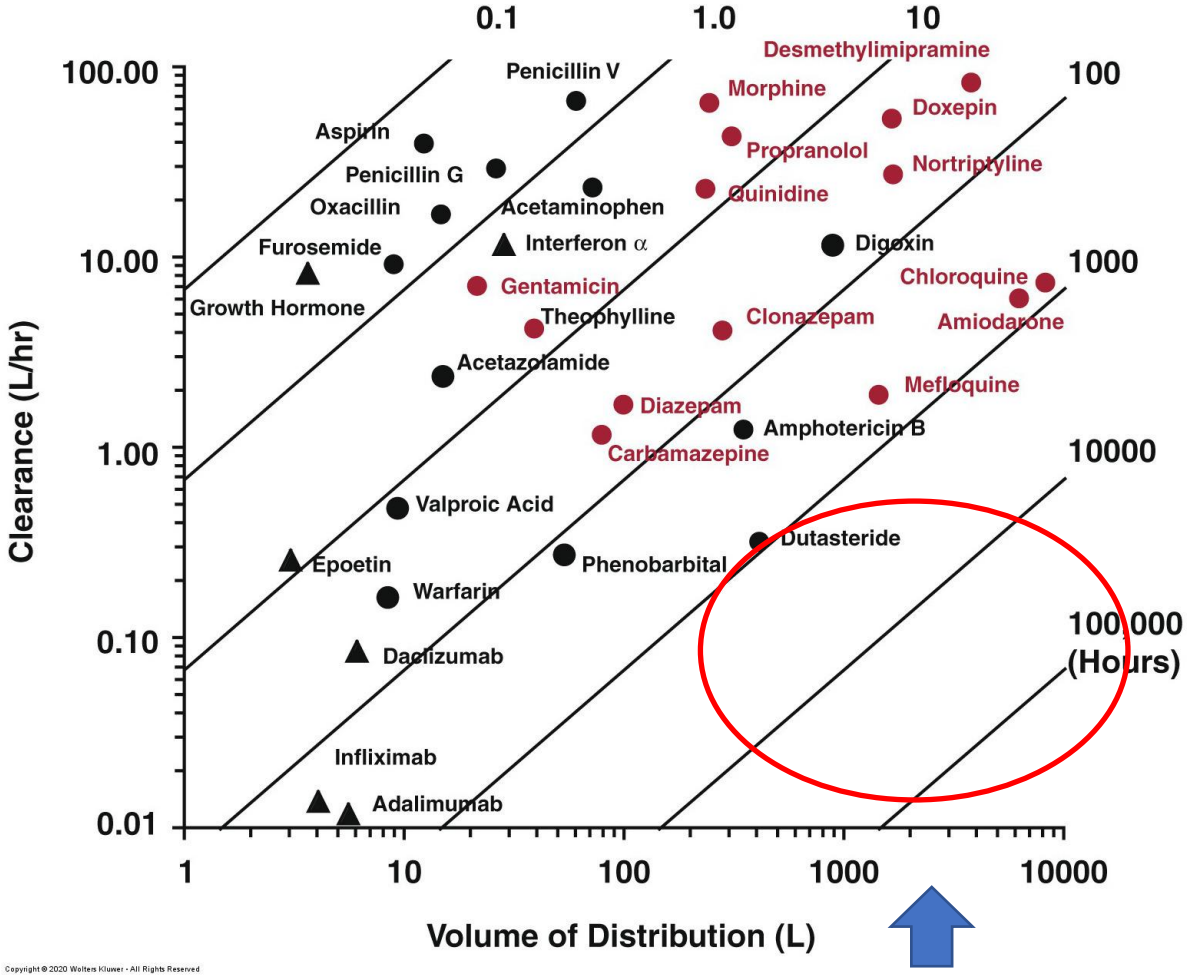
Rate of elimination is independent of fluid where it was measured

$$Rate = Cl_{bl} * C_{bl} = Cl_{pl} * Cl_{pl}$$

Some diagnostics possible knowing hepatic blood flow of species (human 20.7 mL/min/kg)

Basic Pharmacokinetics and Pharmacodynamics, 2nd ed. S. Rosenbaum Ed., Wiley, NY 2017.

PK PARAMETERS: HALF-LIFE ($t_{1/2}$)



In blood (or plasma)

$$t_{1/2} = \frac{0.693 \times VD_{\beta}}{CL}$$

$$MRT = \frac{VD_{ss}}{CL}$$

In the body

- Basic compounds
- Acidic compounds
- Proteins

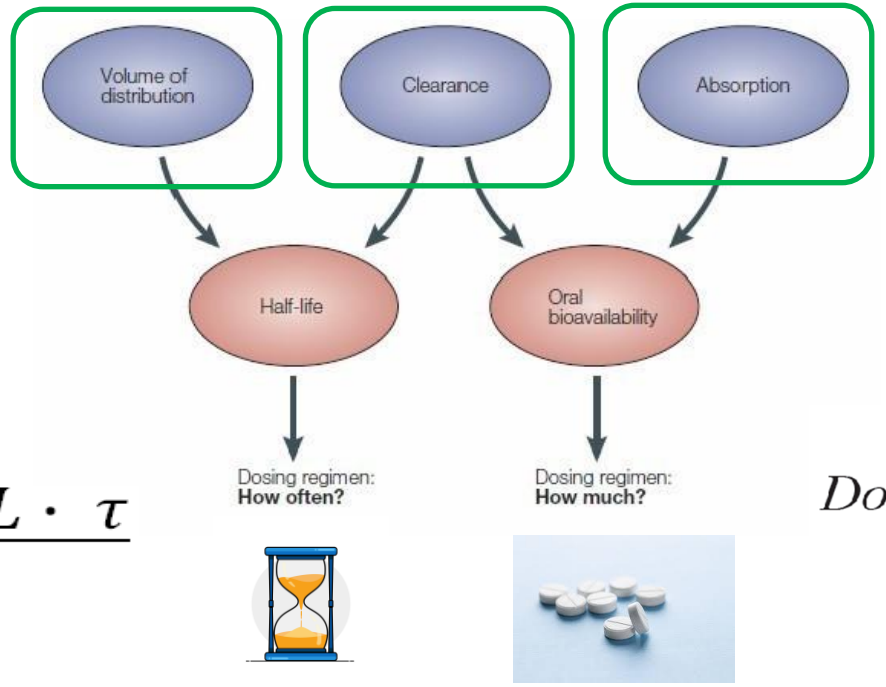
Digoxin is neutral while amphotericin B is zwitterionic

Figure 5-19 in Rowland and Tozer's book. Ch. 5-Elimination

Q: why is this region not populated?

Human PK and dose prediction: the ultimate (lofty) goals!

Upstream or More Downstream? How Early?



$$Dose = \frac{Css \cdot CL \cdot \tau}{F}$$

$$Dose = C_{min} \cdot \frac{Vdss}{F} \cdot \frac{k_a - k_{el}}{k_a} \cdot \frac{1}{\frac{1}{e^{k_{el}\tau} - 1} - \frac{1}{e^{k_a\tau} - 1}}$$

80-90% accurate…..
within a factor of 10!

Equations from: K. M. Page, Validation of Early Human Dose Prediction: A Key Metric for Compound Progression in Drug Discovery. *Mol. Pharm.* **2016**, *13*, 609-620.

M. Wenlock, Profiling the estimated plasma concentrations of 215 marketed oral drugs. *Med. Chem. Comm.* **2016**, *7*, 706-719.

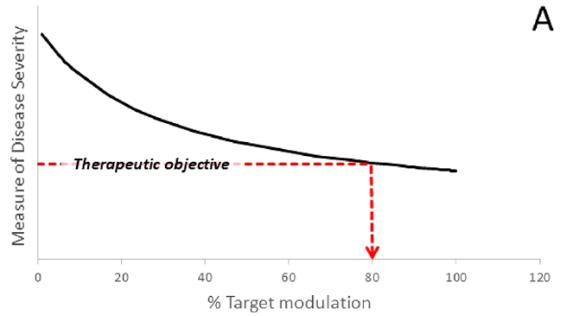
Some simpler estimation methods but…more approximated

Maurer, Smith, Beaumont and Li Dose Prediction in Drug Design

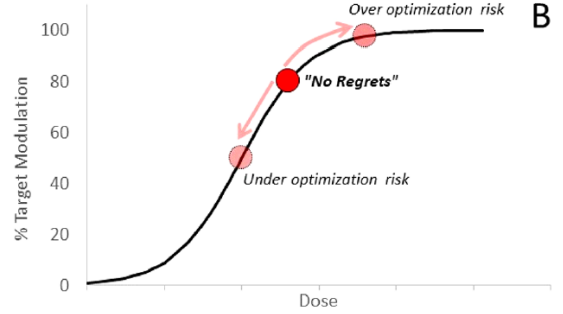
J. Med. Chem. **2020**, *63*, 6423−6435

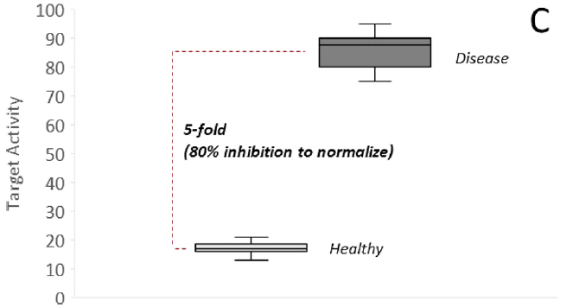
Table 1. One-Compartment Model Equations To Support a Pharmacokinetic-Based Approach to Dose Prediction

parameter	equation
dose for C_{max}	$\text{dose} = \frac{C_{max} V_{ss}(k_a - k_{el})}{F k_a}\left(\frac{e^{-k_{el}T_{max,ss}}}{1 - e^{-k_{el}\cdot tau}} - \frac{e^{-k_a T_{max,ss}}}{1 - e^{-k_a\cdot tau}}\right)^{-1}$
dose for C_{min}	$\text{dose} = \frac{C_{min} V_{ss}(k_a - k_{el})}{F k_a}\left(\frac{1}{1 - e^{-k_{el}\cdot tau}} - \frac{1}{1 - e^{-k_a\cdot tau}}\right)^{-1}$
dose for C_{avg}	$\text{dose} = \frac{C_{avg}\cdot CL\cdot tau}{F}$
$T_{max,ss}$	$T_{max,ss} = \frac{1}{k_a - k_{el}} \ln\left(\frac{k_a(1 - e^{-k_{el}\cdot tau})}{k_{el}(1 - e^{-k_a\cdot tau})}\right)$
k_{el}	$k_{el} = \frac{CL}{V_{ss}}$



Quantifiable reduction in disease severity





Human Dose Prediction: Forgotten Assumptions

- The ultimate goal of discovery project teams is to determine **Human Dose** and **Dosing Regimen** which require ***prediction of human PK parameters***.

- Human PK parameters can be predicted via a ***variety of approaches***.

- Dose is determined utilizing predicted ***human PK parameters and a pharmacologically relevant concentration/target engagement/ target suppression*** which is ***likely*** to yield a beneficial therapeutic effect.

- Important to remember **both the PK and pharmacological assumptions** applied in predicting human dose and ***uncertainty in translation of efficacy*** from pre-clinical studies to human!

Zou, Yu et al. Applications of Human Pharmacokinetic Prediction in First-in-Human Dose Estimation. *AAPS J* **2012**, *14*, 262-281. *(Nice review of approaches to FIH)*

Human Dose Prediction: Forgotten Assumptions

Barrow and Lindsley, *J. Med. Chem.* **2023**, *66*, 4273−4274

Another flawed comparison that is often employed is to administer several compounds in a series (with or without a positive control) at the same dose (e.g., 25 mg/kg) and drawing definitive conclusions. ***PK–PD is about exposure not dose!***

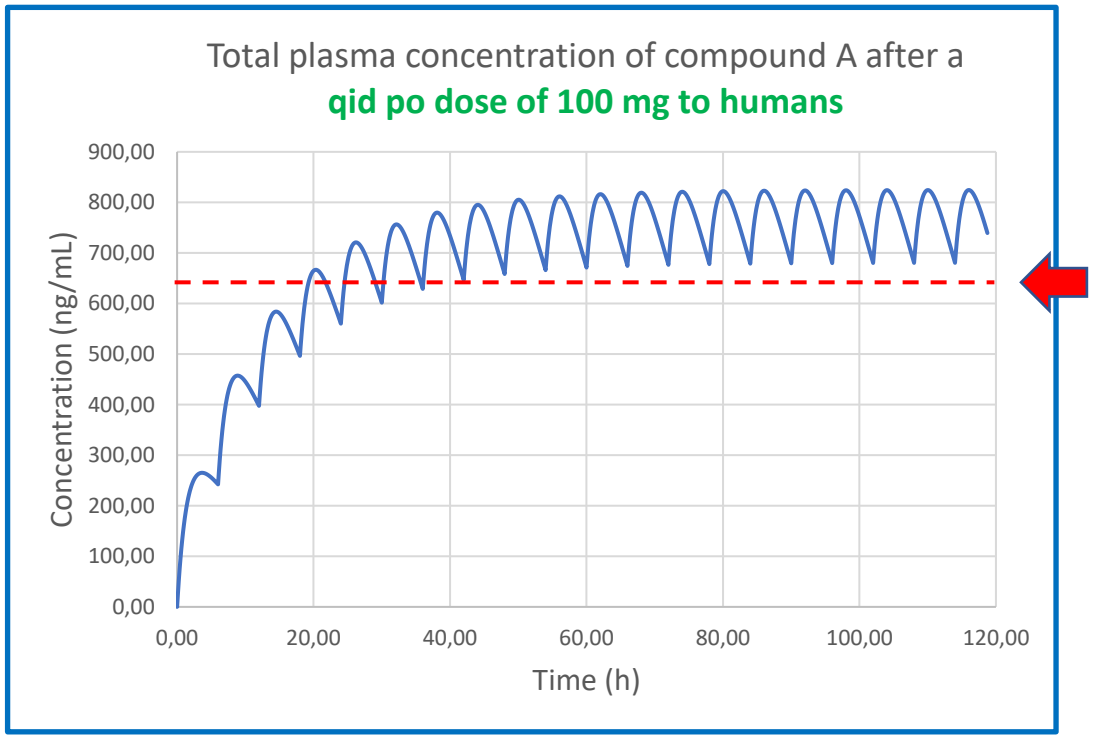
The goal is to administer compounds so that they achieve comparable exposures and draw comparisons based on exposures relative to the ***EC_{50}/IC_{50}'s free*** *(or, in rare cases total exposure).*

Finally, and while not required, it is very informative to understand if in vivo efficacy is C_{max}-driven or trough-driven, as this informs the necessary PK for a novel mechanism and translation to human use.

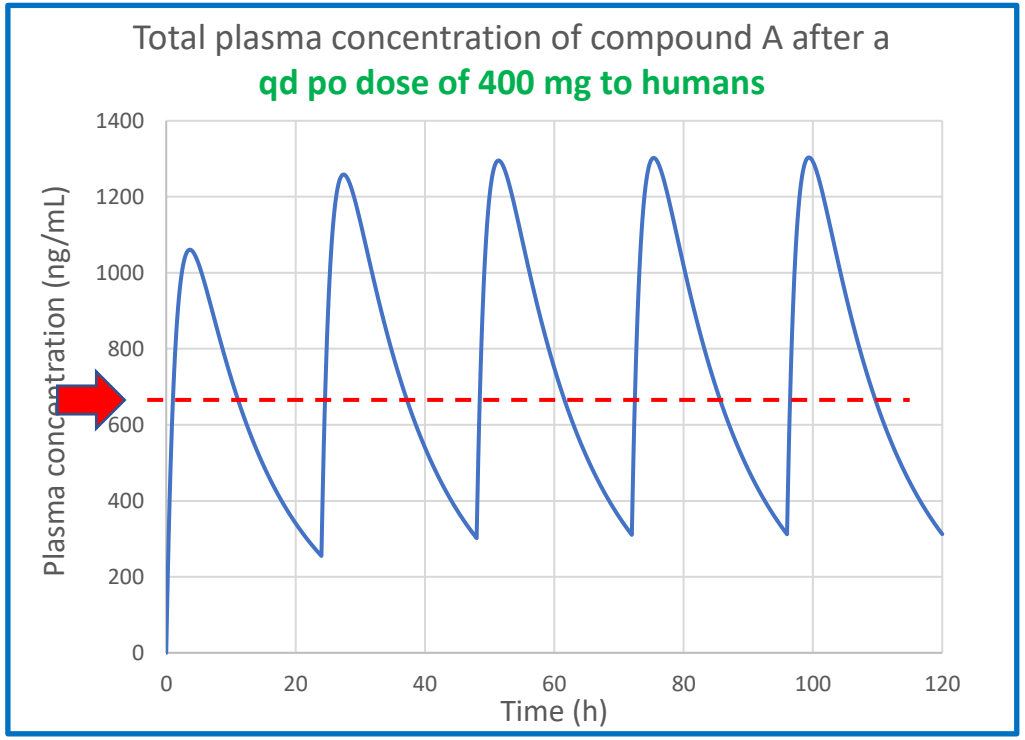
For some of the caveats, and potential errors, in determining IC50 and EC50 see:

Srinivasan and Lloyd, *J. Med. Chem.* **2024**, *67*, 17391-17934

Human Dose Prediction: Dose fractionation and recognition of PK driver



MIC = 650 ng/mL

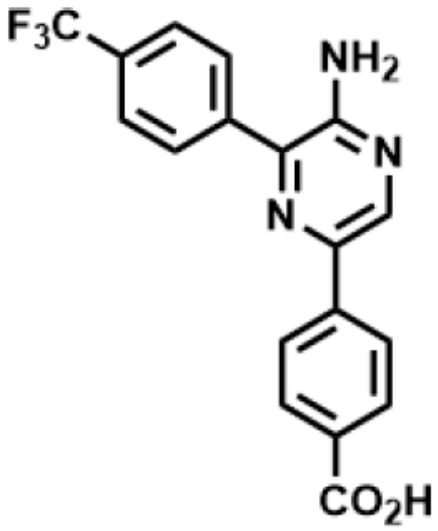


TOTAL $C_{max,ss}$ = 824 ng/mL
TOTAL $C_{min,ss}$ = 681 ng/mL

TOTAL $C_{max,ss}$ =1303 ng/mL
TOTAL $C_{min,ss}$ = 312 ng/mL

For a recent example see : Vendeville et al *J. Med. Chem.* **2024**, 67, 21126-21142
50 mg/kg qd less efficacious than 15 mg/kg bid

Dose fractionation and Wajima's human dose prediction method.



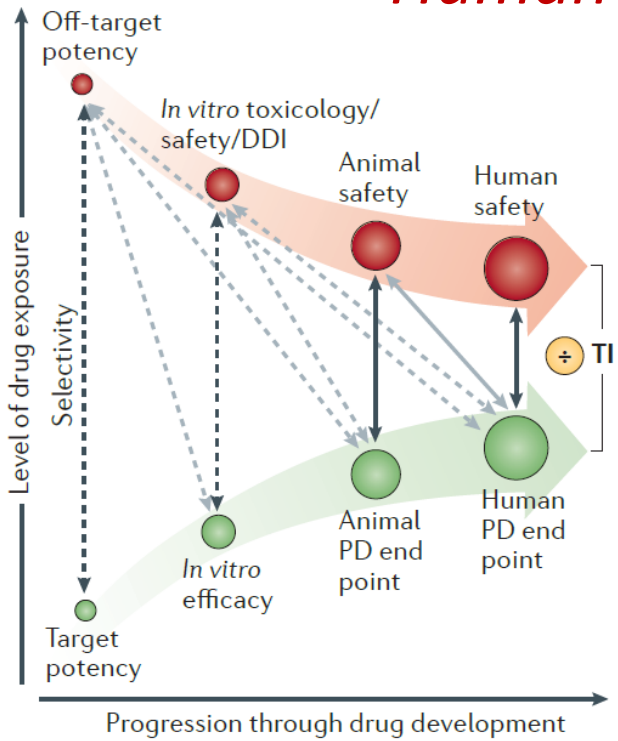
UCT594

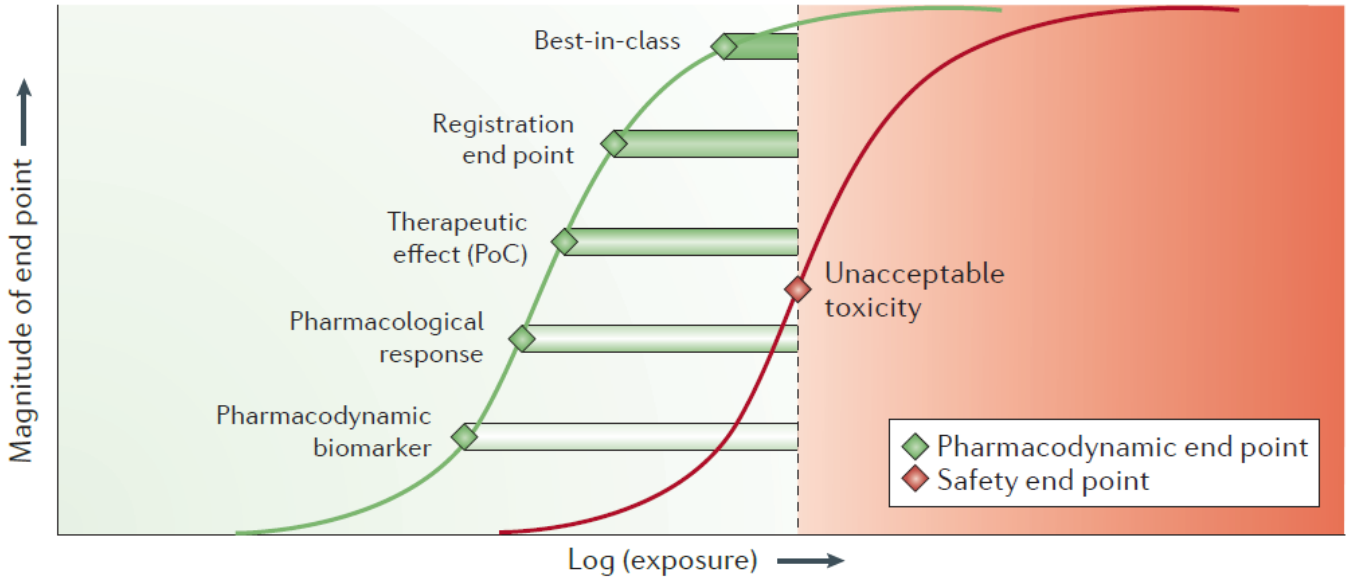
Gibhard L, Njoroge M, Mulubwa M, Lawrence N, Smith D, Duffy J, Le Manach C, Brunschwig C, Taylor D, van derWesthuyzen R, Street LJ, Basarab GS, Chibale K. **2024**.

Dose-fractionation studies of a Plasmodium phosphatidylinositol 4-kinase inhibitor in a humanized mouse model of malaria.

Antimicrob Agents Chemother 68:e00842-24.https://doi.org/10.1128/aac.00842-24

Human Dose Prediction: Forgotten Assumptions





Limitations of exposure data

Muller PY, Milton MN,
Nature Rev. Drug Discov., **2012** , *11*, 751-761

First, plasma exposure is only a surrogate for tissue exposure, which is actually driving most pharmacological and toxicological effects. Using plasma exposure as a surrogate for tissue exposure assumes that free tissue exposure, in equilibrium, is similar to free plasma exposure, which is usually the case for drugs that have reasonable membrane permeability.
Furthermore, in certain cases, plasma levels of a drug may not directly correlate with efficacy.
…..this lack of correlation is because of saturable binding to the target ACE pool

…the dosing regimen and the duration of dosing may considerably affect the pharmacological and/or toxicological effects induced by the drug (for example, an intermittent dosing schedule is sometimes used for oncology drugs to decrease toxicity). This is true particularly for drugs with (predicted) short half-lives in humans and oral drugs with low solubility. A twice a day dosing regimen would also be envisaged in cases when lower doses given more frequently are used in an attempt to reduce (expected) Cmax-driven toxicities

Points to take home

1. Pharmacokinetics models: non-compartmental, compartmental and PBPK

2. Applications and some details: recognizing compartments.

3. Volume(s) of distribution, clearance (additive), AUC, t1/2 and MRT concepts.

4. Routes of administration and absolute vs. relative bioavailability.

5. The blood-brain barrier

6. Dose prediction: target exposure, caveats and assumptions. The Wajima method.