Trends in Chemical Biology and Drug Discovery:

Principles of Drug Discovery, Preclinical Development and PKPD

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My Educational and Professional Journey

1995-2001

University of Perugia

BA/Master in Organic & Computational Chemistry

1998

University of Grenoble

Erasmus Program Advanced Organic Chemistry

2002-2003

University of Ferrara

Master in Science, Technology & Management

2003-2006

University of Geneva

PhD Laboratory of ADME profiling and Molecular Modelling.

2017

Diplomate American Board Toxicology

2024

University of Oxford

Venture Finance Program

2001-2002

Inpharmatica & Arrow Therapeutics

London

Molecular Modeler

2007-2008

Merck Serono (Geneva)

DMPK Scientist

2008-2011

Novartis (Basel)

Research Investigator in Metabolism & Pharmacokinetics

2011-2018

Roche (Basel & Shanghai)

Global Head of PK & DMPK project leaders
Site Head of Nonclinical Development
Lab Head DMPK profiling

2018- present

Ridgeline/Versant (Basel)

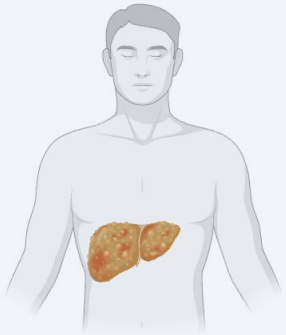
Head R&D
Operating Partner

Principles of Drug Discovery, Preclinical Development and PKPD

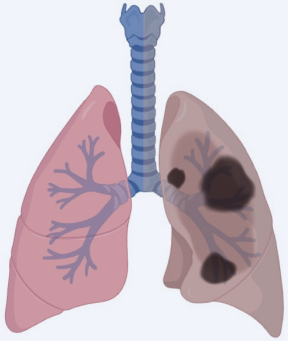
Agenda

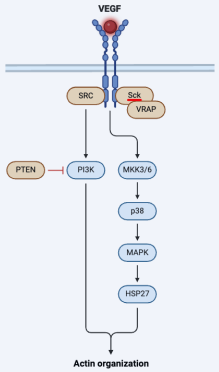
- Introduction to Drug Discovery and Pre-Clinical Development
- Small molecules and monoclonal antibodies: two key drug modalities in Drug Discovery
- Principles of Pharmacokinetics
- Principles of Pharmacodynamics and PKPD models

Drug Discovery: a complex and exciting journey

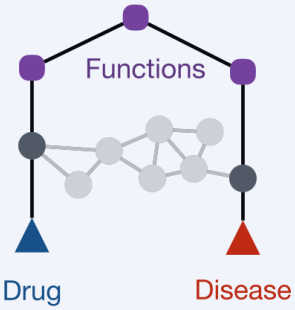


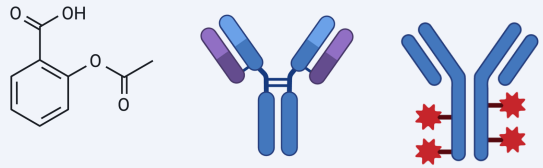
1) Study the disease





2) Identify novel disease mechanisms and druggability

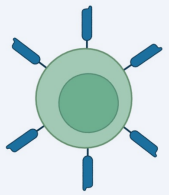




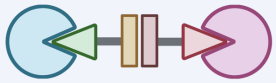
Small molecule mAb ADC

3) Select the adequate modality

Antisense Oligos



CAR-T

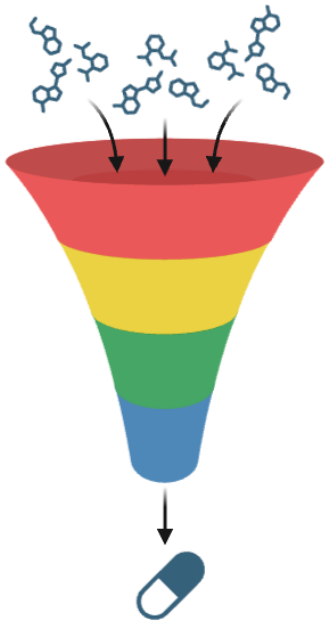


Glue/PROTAC

4) Start Drug Hunting

Drug Discovery: a complex and exciting journey

Drug hunters have to find discrete areas (=galaxies) where the space occupied by biological active molecules (druggable) matches the space of drug-like (PK-friendly) and safe molecules.



When the desired properties require conflicting molecular features, some properties might need to be in the suboptimal zone to maximize the overall molecular quality

Attrition causes for stopping molecules in preclinical development:

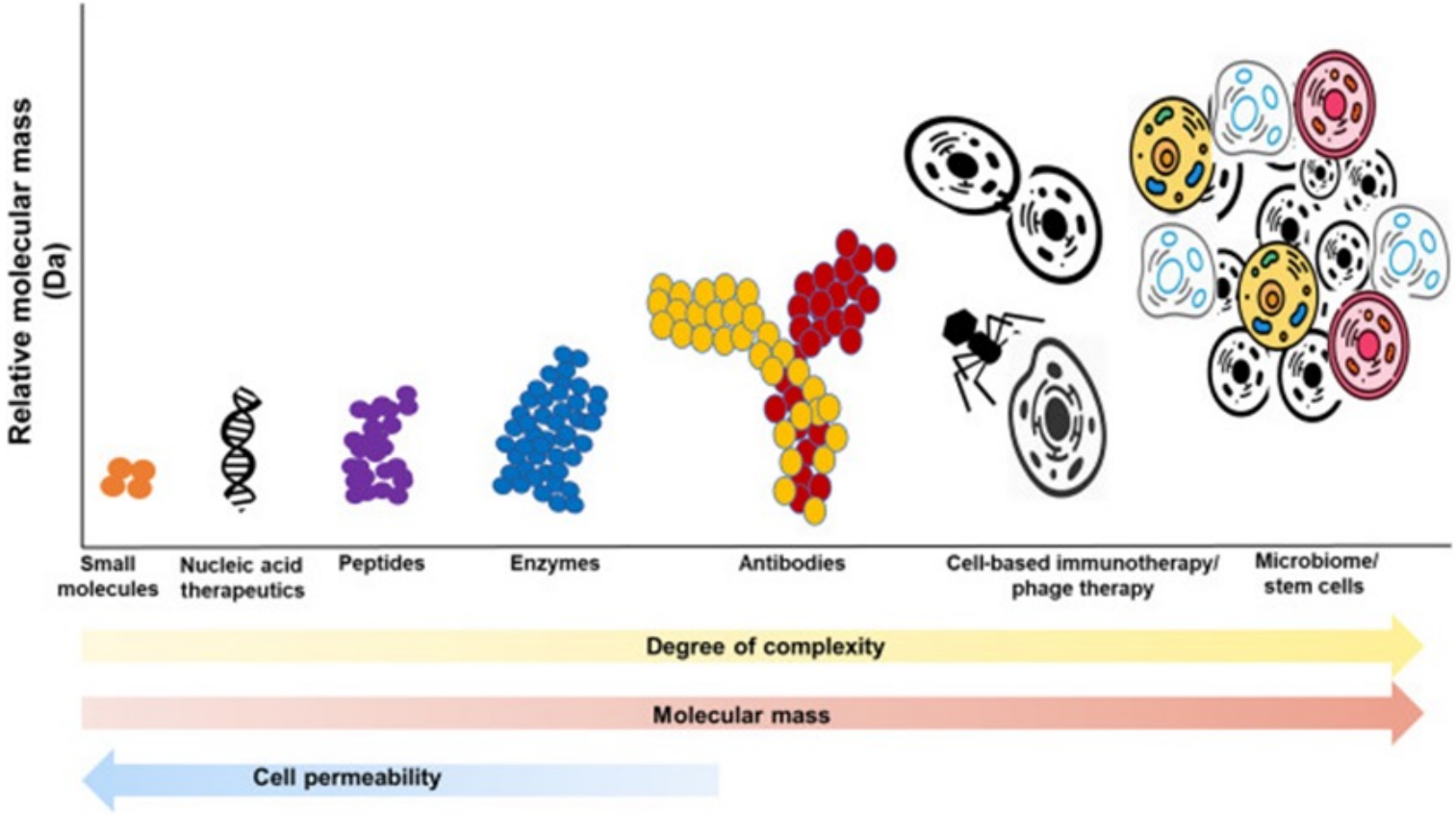
ADME

Lack of efficacy

Toxicity

[1] "Navigating chemical space for biology and medicine", Lipinsky and Hopkins. *Nature*, 432:855-862, 2004. [2] "Lead optimization in the nondrug-like space", Zhao, *Drug Discovery Today*, Dec 2010.
[3] "Targeting BCL2 with BH3 mimetics…."Roberts and Huang, *Clinical Pharmacology & Therapeutics*, 101(1):89-98, 2017

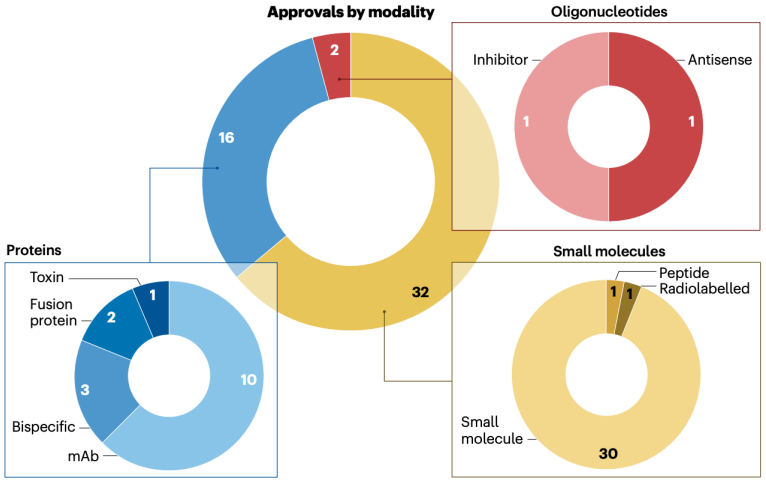
Which Drug modalities are out there?



https://www.biocompounding.com/exploring-drug-modalities-small-molecules-biologics-rna-cell-gene-therapy/

Which Drug modalities are out there?

In 2024 FDA approved 50 new drugs
64% small molecules



Seven of the top 10 selling biopharma products in 2024 are new modalities

2024 (actual)

Product	Modality	Worldwide sales ($B)
Keytruda	mAb	28
Ozempic	Recombinant	18
Dupixent	mAb	14
Biktarvy	Conventional	13
Eliquis	Conventional	13
Darzalex	mAb	12
Stelara	mAb	11
Opdivo	mAb	11
Skyrizi	mAb	11
Jardiance	Conventional	11

Conventional modalities New modalities

https://www.nature.com/articles/d41573-025-00001-5

https://www.bcg.com/publications/2024/new-drug-modalities-report

Key differences between Small Molecules and Biologics

Monoclonal antibody (IgG1)

Small Molecule

Features	Monoclonal Antibodies (mAbs)	Small Molecules
Size	**150 kilodaltons** (kDa)	**500 daltons**
Production	Produced using recombinant DNA technology in **living cells**, resulting in complex manufacturing processes.	**Chemically synthesized**, allowing for precise structural control.
Administration	Administered parenterally, usually via **intravenous** (IV) or **subcutaneous** (SC) injection	Can be taken **orally**, as they are typically absorbed through passive diffusion in the gastrointestinal tract.
Target specificity	**Highly specific** to a single epitope on their target antigen, minimizing off-target effects.	**May interact with multiple targets**, potentially leading to off-target effects
Mechanism of action	Bind to **extracellular targets**, such as cell surface receptors or soluble antigens, to modulate immune responses or block disease pathways	Can target both **intracellular and extracellular sites**, influencing various cellular processes.
Pharmacokinetics	**Exhibit long half-lives** (approximately 11–30 days in humans) and are cleared through linear and nonlinear processes. Low frequent dosing	Typically have **shorter half-lives**, necessitating more frequent dosing.

Small Molecules

Small-molecule drugs have been the pillars of traditional medicine and played an important role in shaping pharma research and improving global health

Aspirin approved by the FDA in 1965, is commonly used as a pain reliever (analgesic).

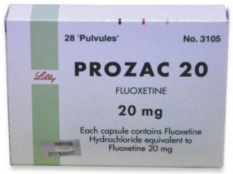
Imatinib (Gleevec) revolutionized the treatment of chronic myelogenous leukemia (CML) by targeting the specific genetic mutation responsible for the disease.

Fluoxetine (Prozac): Fluoxetine is a selective serotonin reuptake inhibitor (SSRI) antidepressant used to treat depression, anxiety disorders, and other mood conditions.

Warfarin: Warfarin is an anticoagulant (blood thinner) used to prevent and treat blood clots. It works by inhibiting vitamin K-dependent clotting factors, reducing the risk of stroke and heart attack

Amoxicillin is a widely used antibiotic belonging to the aminopenicillin class, effective against various bacterial infections. It works by inhibiting the growth of bacteria

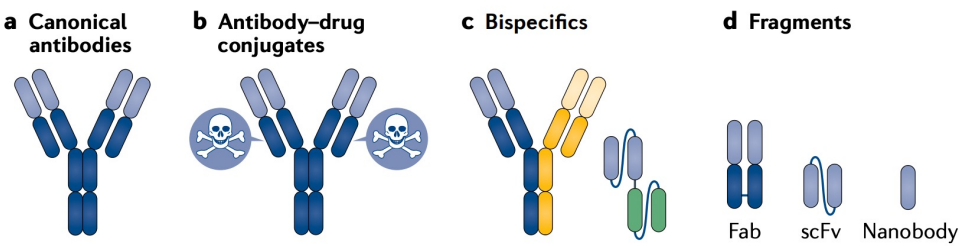
Statins are a class of drugs that lower cholesterol levels by inhibiting the enzyme HMG-CoA reductase.



Monoclonal antibodies (mAbs)

Monoclonal antibody (mAb) drugs have emerged as one of the cornerstone therapeutic modalities:

- They offer **exquisite specificity** and **affinity** for both secreted and cell-surface targets
- **Different formats** of antibody can be used to mop up circulating proteins, to block signalling pathways outright, to drive the internalization an degradation of cell-surface receptors, to deliver small molecule payloads to specific cell types, to recruit immune cells to cancer cells
- With a 22% overall success rate from phase I to approval, **antibodies are twice as likely to succeed in trials as small molecules**



the 100th mAb Dostarlimab, GlaxoSmithKline's anti-PD-1 drug, was approved by FDA in April 2021

Rituximab
Targets CD20 on B cells, effectively treating certain lymphomas and leukemias

Trastuzumab (Herceptin)
Binds to HER2 receptors, used in treating HER2-positive breast cancers

Adalimumab (Humira)
Targets TNF-alpha, reducing inflammation in conditions like rheumatoid arthritis and Crohn's disease.

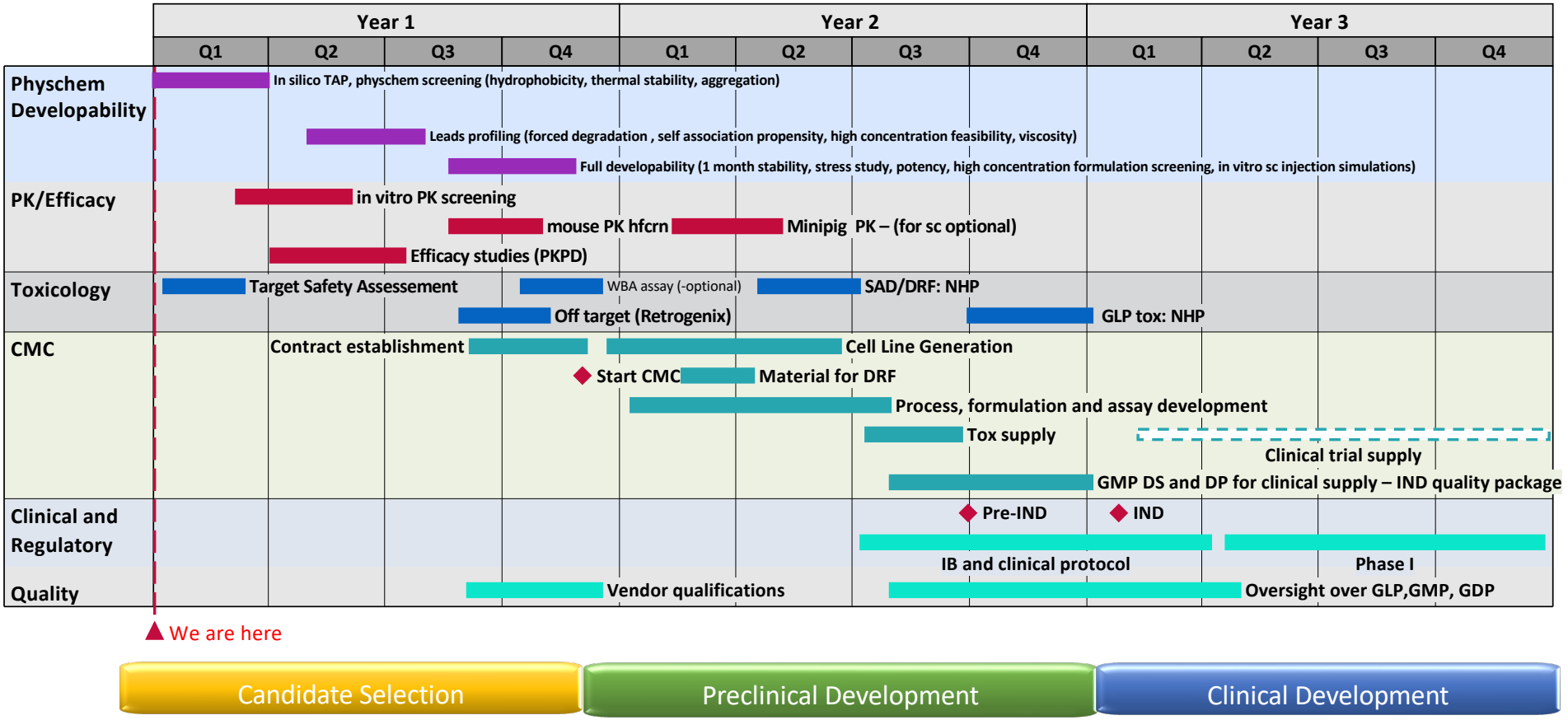
Pembrolizumab (Keytruda)
An immune checkpoint inhibitor that blocks PD-1, enhancing the immune response against tumors.

Preclinical and Early Clinical Development:
Key profiling to select Clinical Candidates

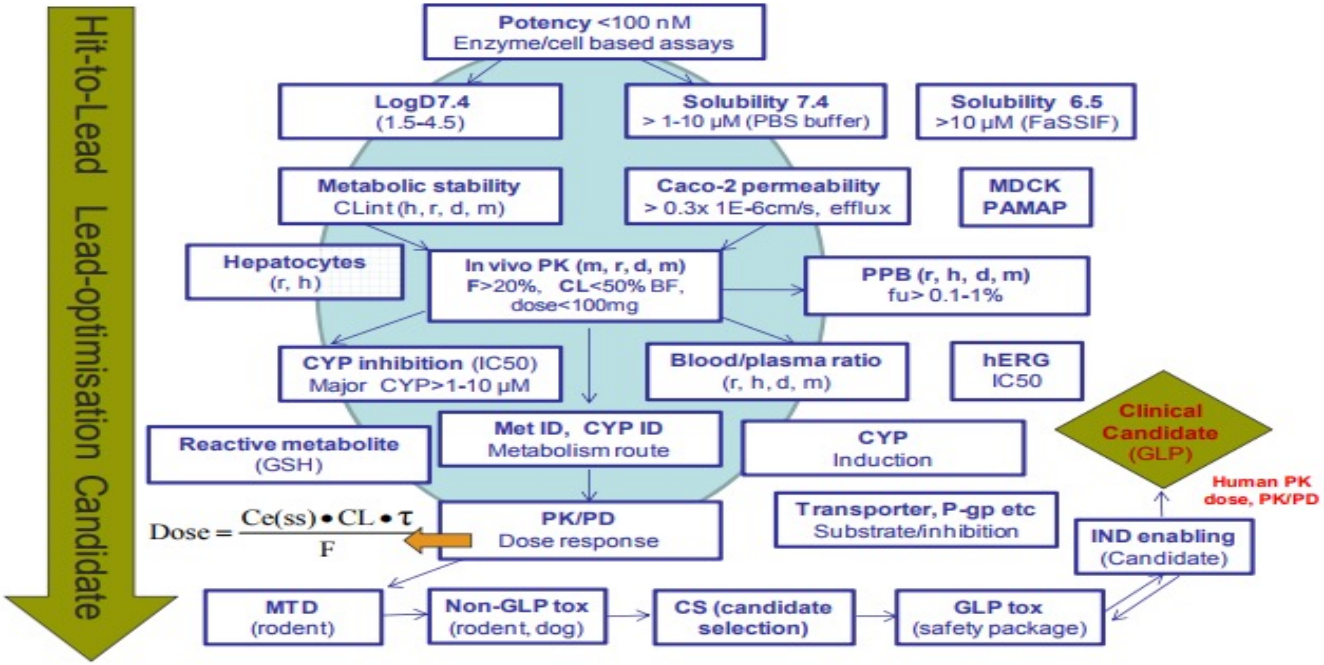
Candidate Selection	Preclinical Development	Clinical Development
Candidate nomination		**IND**
Phys/chem, developability and formulation	Bioanalytics and Biomarkers	Clinical protocols and subsequent updates
in vitro PK	PK/PD modeling & translation to human	Clinical Bioanalytics (PK, ADA and BM)
in vivo PK studies	Toxicology (DRF and GLP)	Clinical pharmacology: modeling and dose extrapolations (pharmacometrics and QSP)
In vivo pharmacology and PK/PD studies	Clinical dose selection / strategy (starting & efficacious dose projections)	Additional toxicology studies (e.g. chronic tox)
Early tox (Target safety assessment, off target, cross species reactivity, minitox)	CMC: Cell line, formulation and assay development, Process development & tox-manufacturing	CMC: clinical trial supply, process optimization, new formulations, continued manufacturing
	CMC: GMP manufacturing for clinical supply	Regulatory strategy/interactions for justification of clinical dosage/frequency and dose/route optimization studies
	Regulatory submission (IB, IMPD..)	

QA: Oversight of research, GLP/GMP/GDP activities, Preparation for GCP activities, Supplier Qualification and Oversight, QMS Maintenance and Training

Example Preclinical Dev. Ganntchart to IND for a mAb



Overview of DMPK Screening Assays for small molecules

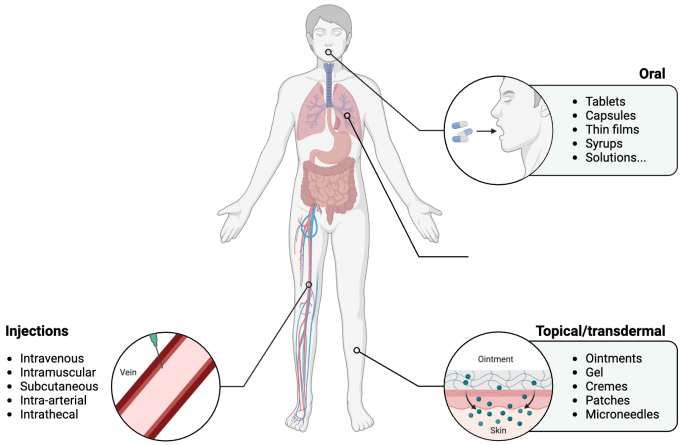


Small molecules are less specific than mAbs and can interact with many off-targets requiring a **more complex profiling**

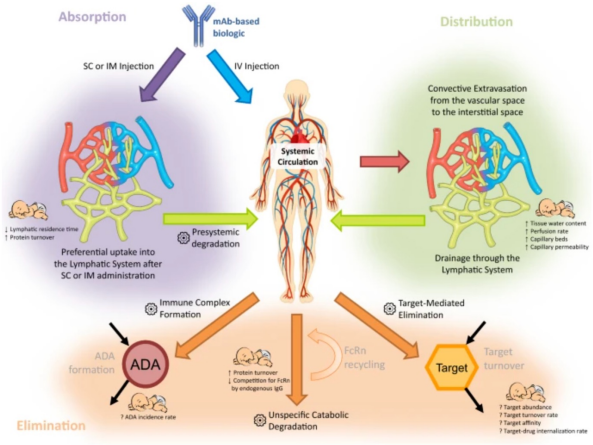
Principles of Pharmacokinetics

Pharmacokinetics

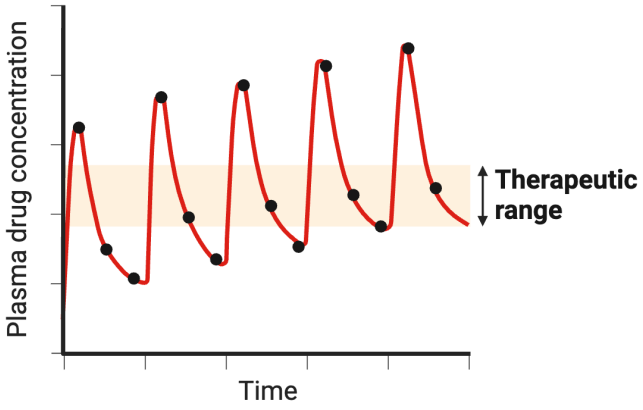
The description of a the drug journey from the administration to the elimination



Administration



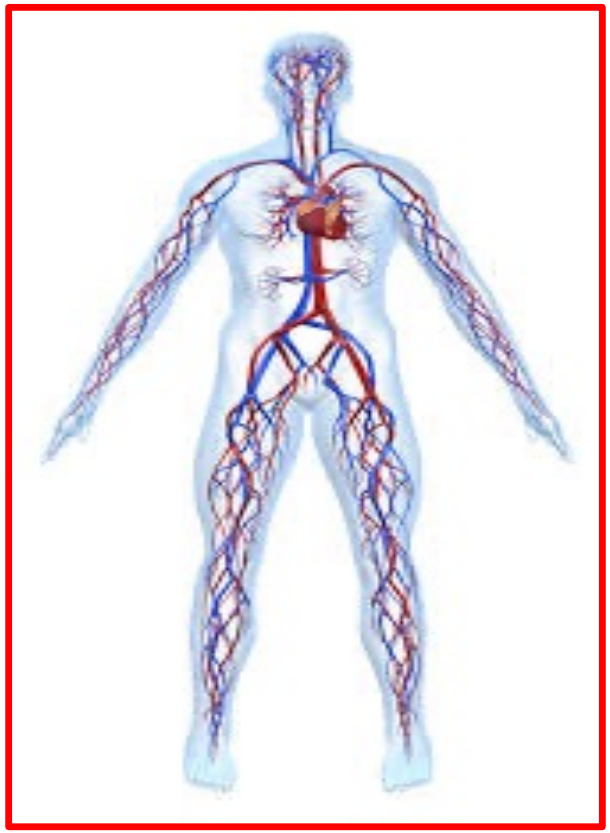
Disposition and elimination of the compound from the body

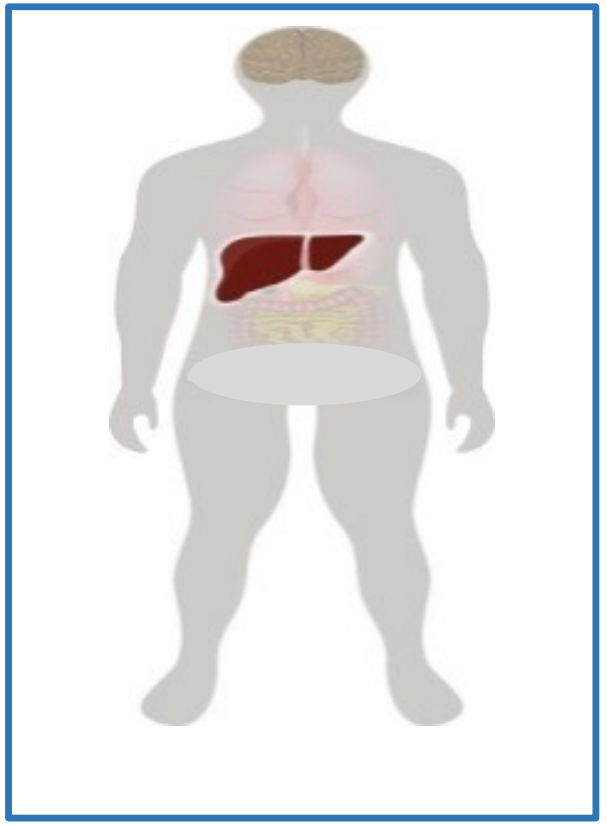


Plasma drug concentration allows to monitor and understand drug disposition

ADME evolution: from plasma PK to tissue PK

Assessment and optimization to reach optimal free concentrations in target organs

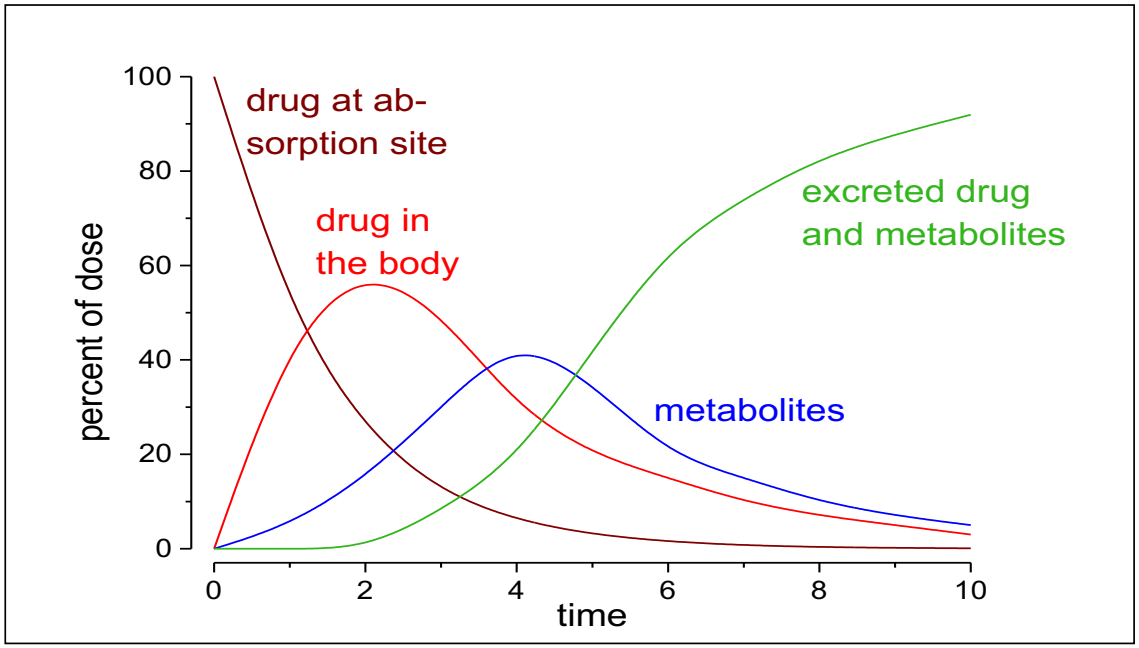




ADME (or DMPK..): an abbreviation in Pharmacokinetics that describes the disposition of a compound in the body

A	• The process by which a compound and its metabolites are transferred from the site of absorption to the systemic circulation
D	• The process by which absorbed compound and/or its metabolites partition between blood and various tissues/organs in the body
M	• High MW and lipophilic molecules get transformed to new compounds called metabolites. Metabolites are in general more polar, inactive or active
E	• Compounds and their metabolites need to be removed from the body via excretion, usually through the kidneys (urine) or in the feces

Drug exposure (PK) – Key Principles & Parameters



AUC = area under the concentration-time curve

C_{max} = maximum concentration

Clearance (CL) = Dose/AUC

Volume of distribution (Vd_{ss}) = MRT*CL

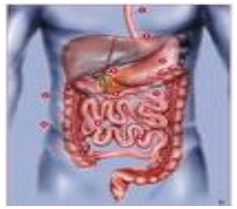
Half-life ($t_{1/2}$) = ln2 / lambda

Bioavailability (F) = (Dose IV * AUC_{po})/ (Dose PO * AUC_{iv})

Key PK differences between small molecules and monoclonal antibodies

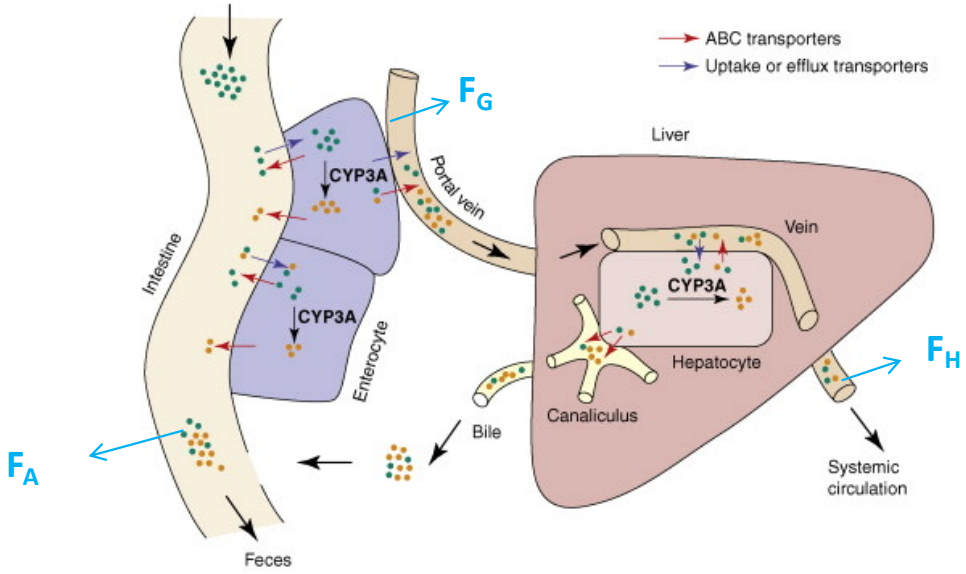
Pharmacokinetic Aspect	Small Molecules	Monoclonal Antibodies (mAbs)
Absorption and Bioavailability	Typically administered **orally**; absorbed through the gastrointestinal tract with varying bioavailability based on physicochemical properties.	Administered **parenterally** (e.g., intravenous or subcutaneous injection); poor absorption via the gastrointestinal tract; bioavailability depends on administration route and target antigens.
Distribution	Can diffuse across cell membranes to reach intracellular targets; small size facilitates widespread distribution, including the ability to **cross the blood-brain barrier**.	Predominantly remain in the extracellular space due to large size; **cannot cross cell membranes**; limited to vascular and interstitial compartments, restricting access to intracellular targets.
Metabolism	Primarily **metabolized in the liver** by cytochrome P450 enzymes, leading to active or inactive metabolites.	Undergo **catabolic processes** rather than traditional metabolic pathways; broken down into peptides and amino acids by proteolytic enzymes with minimal liver enzyme involvement.
Elimination	Eliminated mainly through **renal excretion** or **biliary secretion**, based on size, charge, and lipophilicity.	Eliminated via **target-mediated drug disposition** (TMDD); drug-target complex is internalized and degraded, leading to nonlinear pharmacokinetics with varying clearance rates.
Half-Life	Generally have **shorter half-lives**, often requiring multiple daily doses to maintain therapeutic levels.	Exhibit **longer half-lives** (days to weeks), due to size, Fc receptor interactions, and recycling mechanisms, allowing for less frequent dosing.

ADME bsorption



Major drug-specific factors affecting (oral) absorption (small molecules)

- Solubility/ Dissolution (pKa, lipophilicity, size)
- Stability in the GI tract
  (chemical/enzymatic stability)
- Permeability (pKa, lipophilicity, size)
- Active and facilitated transport mechanisms



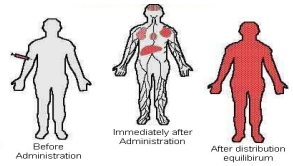
$$F = F_A * F_G * F_H$$

Absorption in vitro models

- Caco-2 cells
- MDCK cells
- PAMPA

System specific parameters affecting (oral) absorption:
pH, intestinal transit time, motility, transporter, enzyme expression

ADME istribution



Volume of distribution (Vd)

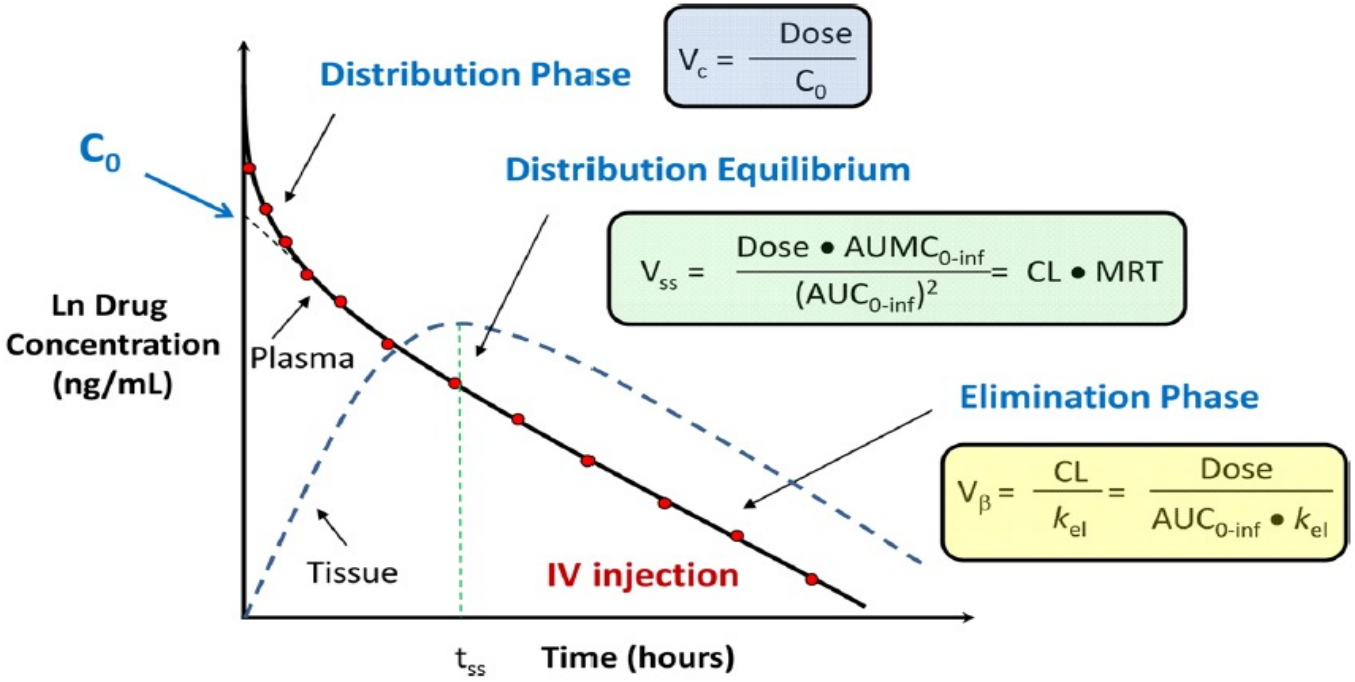
$$\textit{Definition: } Vd = \frac{\textit{Amount of drug in body at equilibrium}}{\textit{Plasma drug concentration}}$$

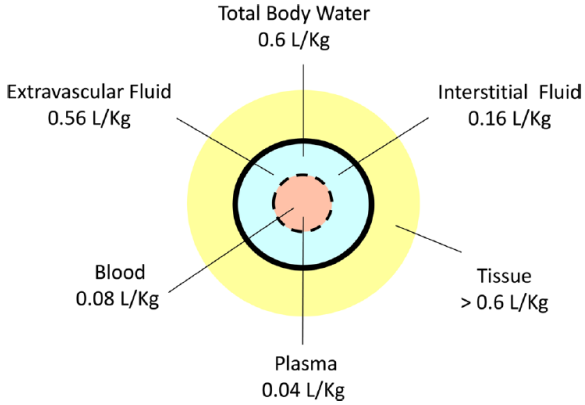
- Volumes of distribution of small molecules range from 3 to 40,000 L in humans
- Plasma water ~ 3 L, extracellular water ~ 12 L, total body water ~ 27 L
- **How can Vd be as high as 40,000 L when the physical aqueous volume of a human being is ~ 27 L?**
  - Vd does not refer to a physical but an apparent volume
  - Drugs have different affinities to tissue protein, lipids or other constituents and this may result in significant bindings or partitioning of drug into tissue
  - Acids (e.g., flubiprofen, warfarin) show strong affinity to plasma proteins (low fu_p) and have therefore typically much lower Vd compared to bases/ neutrals

Major drug-specific factors affecting the volume of distribution

- Lipophilicity
- pKa
- Plasma Protein Binding
- Transporters

ADME istribution





$$V_{ss} = V_p + \sum \left(\frac{fu_p}{fu_t} \right) V_t$$

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ADME Metabolism & Excretion

Metabolism (chemically converted to metabolite[s])

High MW and lipophilic molecules get transformed to metabolites that are generally smaller or more polar

Physiological factors: genetic related differences (polymorphisms), disease, environmental

Excretion (unchanged)

Route dependent on size & physicochemical properties of molecules:

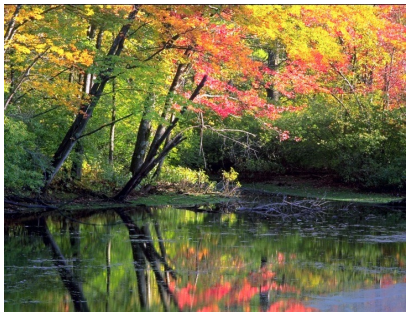
- Small polar molecules in urine (e.g. penicillin, atenolol, digoxin)
- Larger less polar molecules in bile (e.g. diazepam, indomethacin)
- Volatile/gaseous anaesthetics in expired air (e.g. alcohol)

What is Clearance?

$$CL = \frac{Dose}{AUC}$$

CL represents the proportionality constant between concentration and rate of elimination or upon integration dose and AUC

- **Apparent CL** refers to the observable clearance in blood or plasma (CL_b or CL_p)
- The apparent CL may be a composite of the contribution of various organs (**frequently liver and kidneys**)
  - Elimination of drug occurs by excretion and metabolism
- **Unbound intrinsic CL** (CLu_{int}) refers to the actual cellular clearance without any limitations caused by tissue perfusion
  - CLu_{int} is not directly observable *in vivo*
  - CLu_{int} is ≥ CL

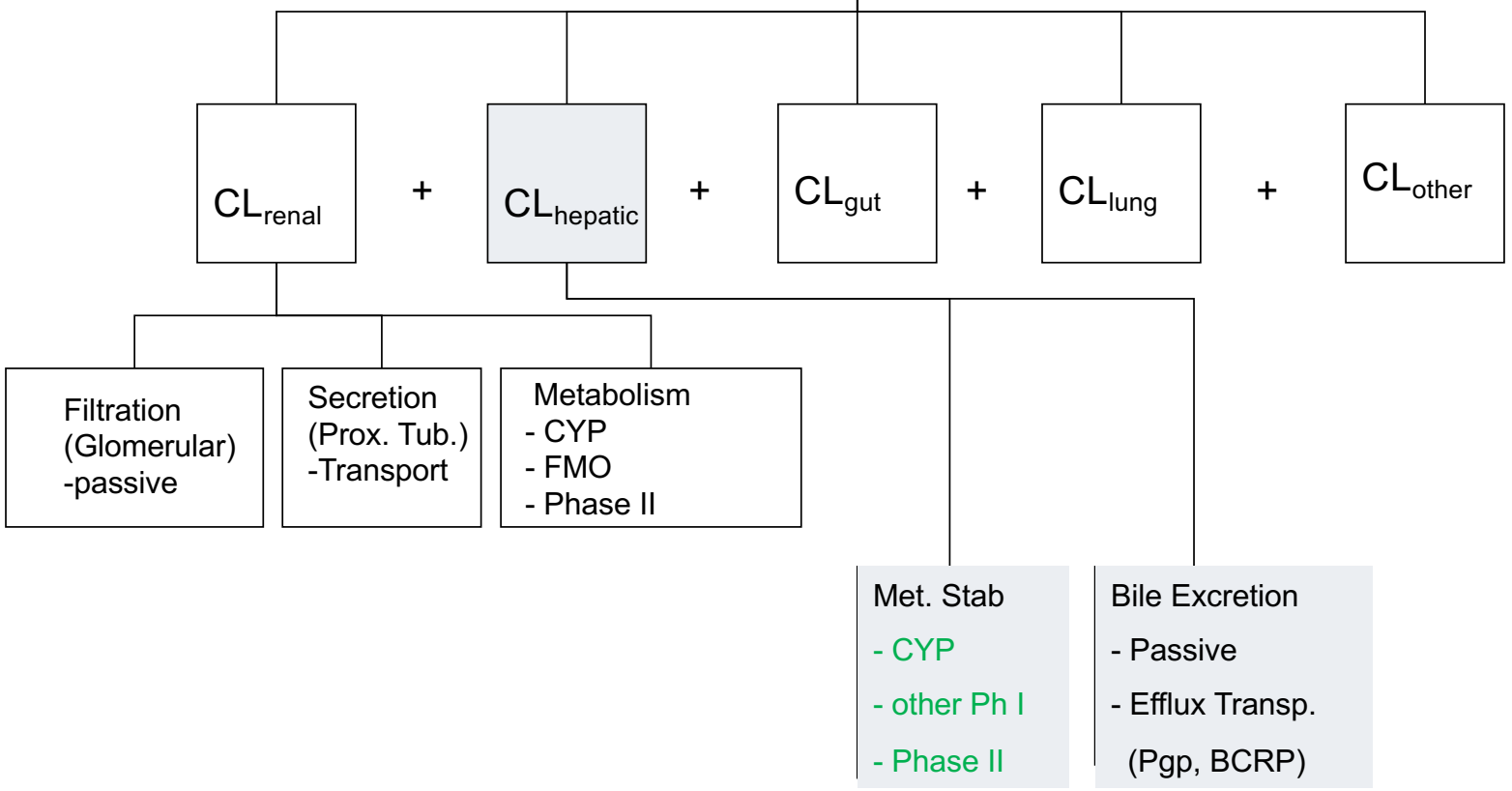


Small molecules

What is Clearance?

CL is bulk phenomenon
What takes drug out of plasma?

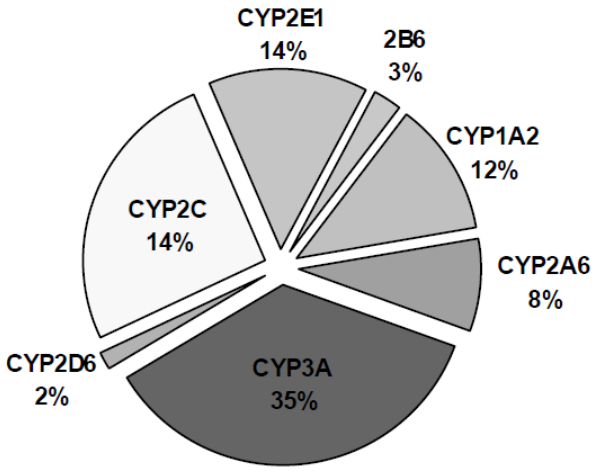
$$CL_{IV} = \frac{Dose}{AUC}$$



ADME Metabolism & Excretion

Hepatic metabolism

- **Oxidative metabolism**
  - Referred to as **Phase I** metabolism as frequently the first metabolic step
- **Cytochrome P450** enzymes are key contributors to small molecule metabolism
  - **CYP3A4** is the main enzyme



- **Conjugation**
  - Referred to as **Phase II** metabolism as frequently following oxidative metabolism
  - However, conjugation may also occur as first step
  - UGT (glucuronidation) or SULT (sulfation) are primary conjugating enzymes
- **Other metabolically active enzymes**
  - FMO (flavin mono-oxygenase), AD or XO (aldehyde or xantine oxidase), ADH (alcohol dehydrogenase), carboxylesterases

1, Paine et al. (2006) Drug Metab and Dispos 34: 880-6

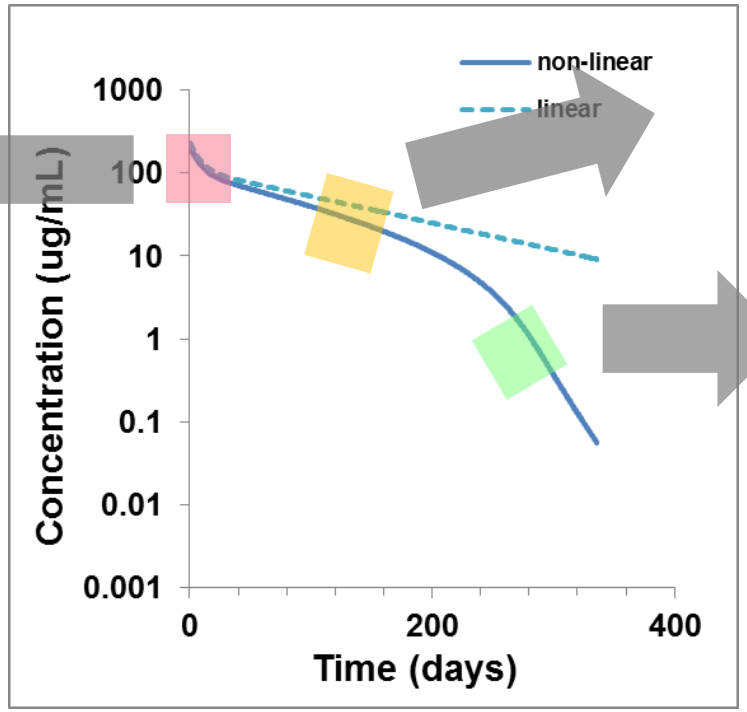
Small molecules

What are the major determinants of mAb PK?

Example conc. vs. time profile following single dose intravenous administration

Distribution

- Size
- Target binding
- Unspecific binding



Non-specific elimination

- Pinocytosis, FcRn recycling
- endocytosis
- proteolysis

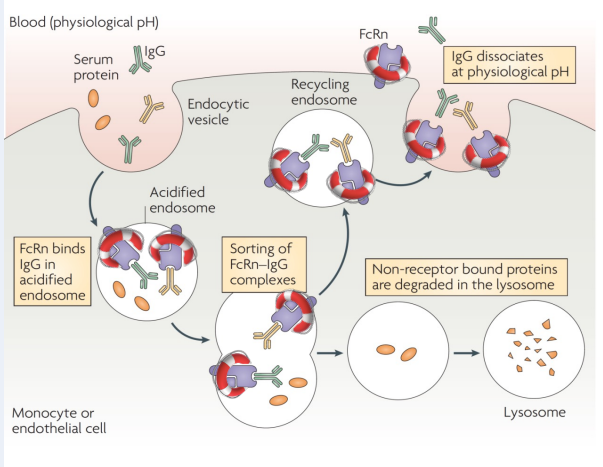
Specific (Target) related elimination

- Target expression, abundance
- Turnover
- Synthesis rate

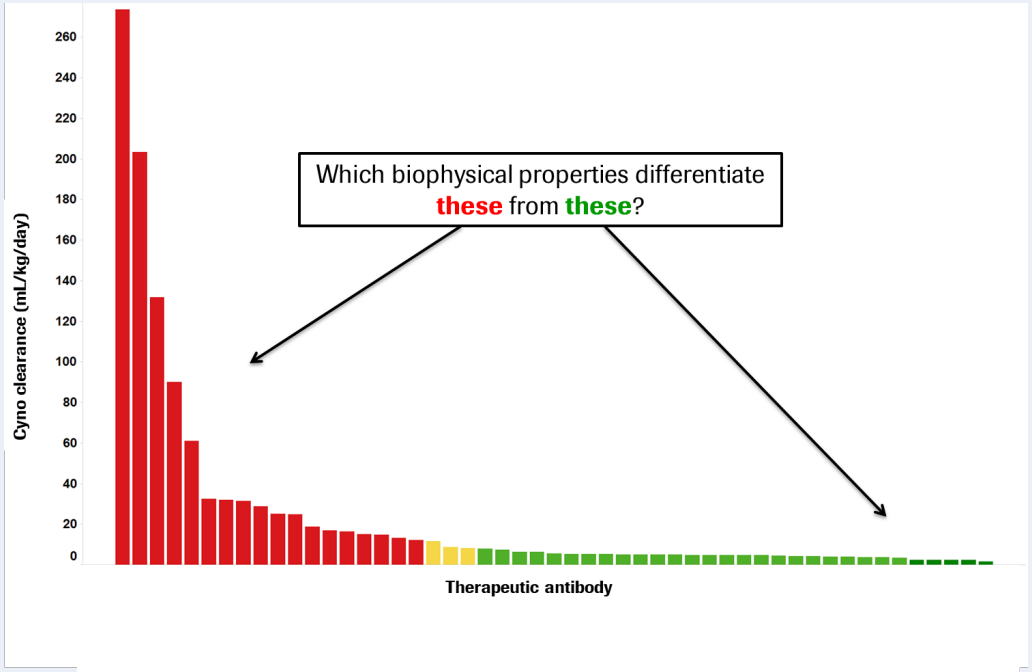
ADA mediated elimination

PK profiling of mAbs: relevance of FcRn recycling

Classical understanding: FcRn recycling drives mAb PK and half life

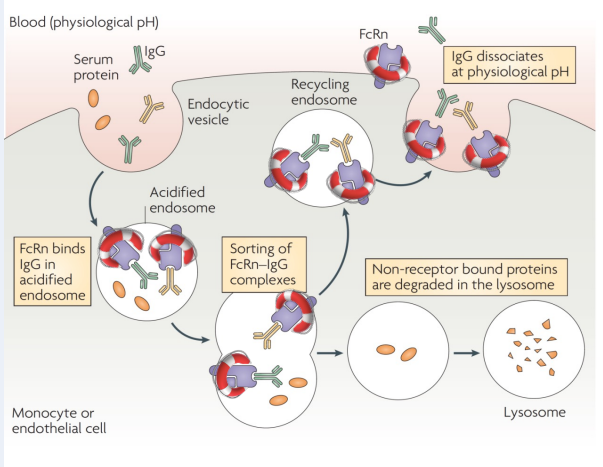


BUT, same Fc, different PK….

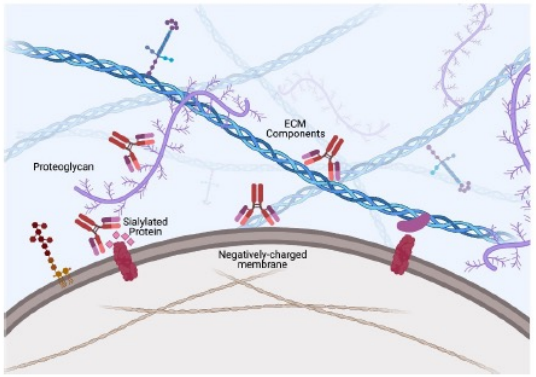


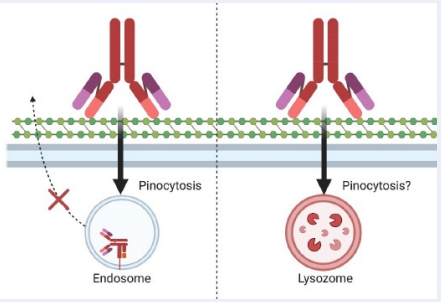
PK profiling of mAbs: properties beyond FcRn

Classical understanding: FcRn recycling drives mAb PK and half life



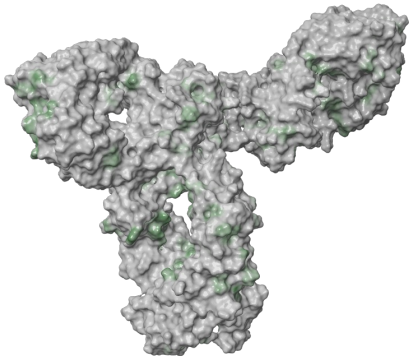
Excessive positive charge or hydrophobicity can lead to non-specific interactions with negatively charged or hydrophobic cell membranes and ECM components across tissues



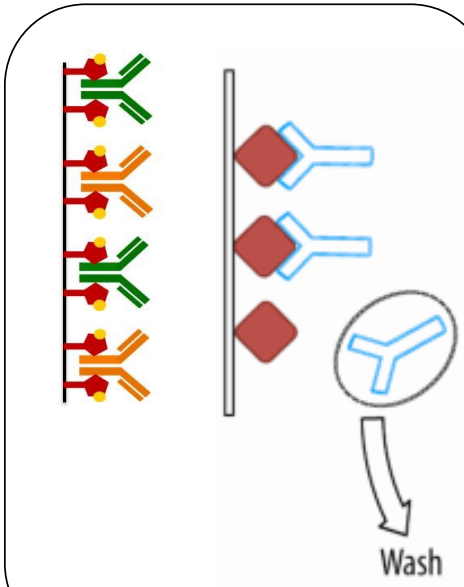


=> Charge imbalance and excess hydrophobicity can increase pinocytosis rates, affecting PK and biodistribution

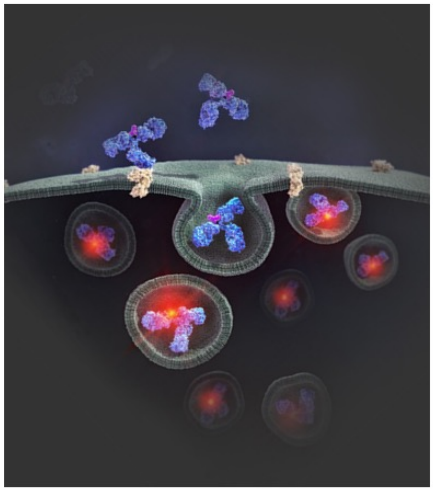
Tools for the assessment of key PK-relevant properties



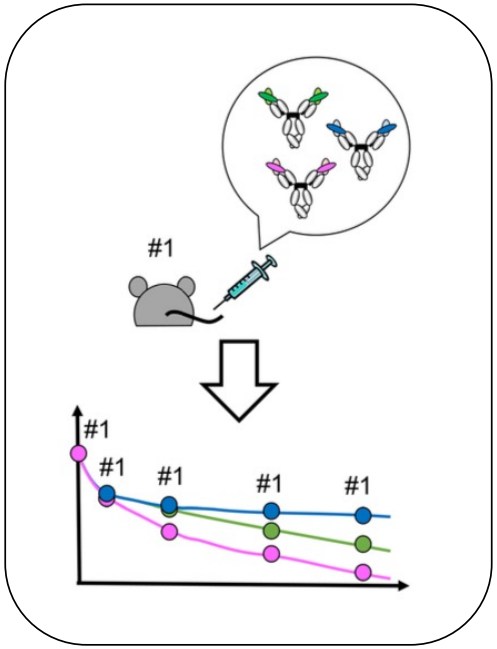
TAP
(in silico
charge patch analysis)



in-vitro
(heparin and FcRn
chromatography)



in-vitro
(cellular uptake / clearance/
recycling)



in-vivo
(PK in mouse / cyno)

Principles of Pharmacokinetics & Pharmacodynamic

(PKPD)

Why PK/PD in Drug Discovery?

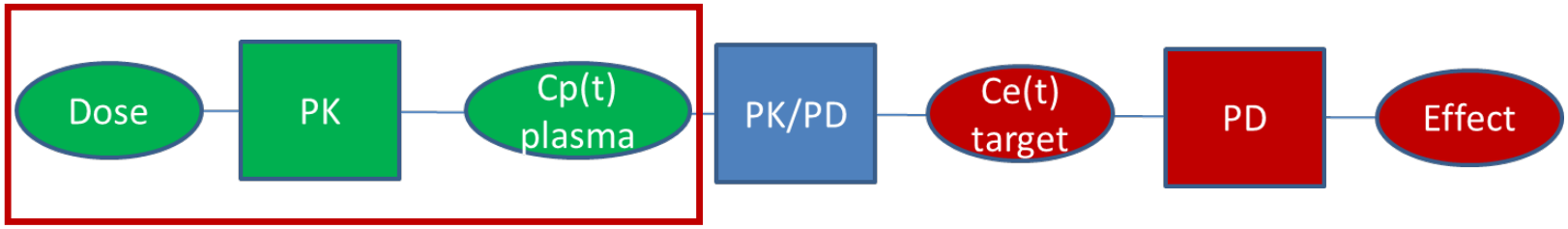
- **Examine drug concentration-effect relationships** in vivo, establish confidence for a novel target and correlation with in vitro assays
- Establish in vivo potency over an effective concentration or dose range (EC50) or at least a “minimal effective exposure/dose” from animal models (also useful for safety margins)
- Quantitatively describe the **time course** of **drug effects** (if possible) in relation to plasma concentrations or doses
- Recognize the **presence of active metabolites**
- Assess the **PK driver for efficacy** (C_{max}, C_{min}, AUC?)
- **Translation into men** for human efficacious dose and dosing regiment projection to help design “FIM” and “POC” studies
- Ultimately to **identify** the overall most promising **clinical candidate**

Pharmacodynamics and Pharmacokinetics Relationships

- Drugs produce a therapeutic effect when there is an adequate exposure profile at the target site (Ce).
- Measurement of systemic drug exposure (Cp) most often offers a useful surrogate for exposure at the active site.
- Delays may exist between plasma concentrations and the response. Such delays can obscure the concentration/response.

PKPD deals with the relationship between **drug conc**. at the **effect site** or drug conc. in **plasma** in equilibrium with effect site and the magnitude of the **observed PD effect**.

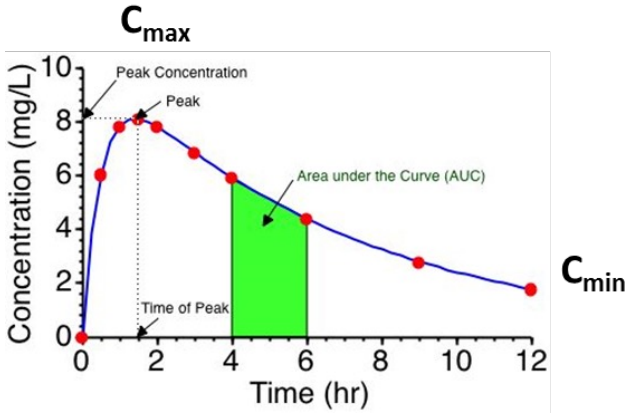
Pharmacodynamics and Pharmacokinetics Relationships



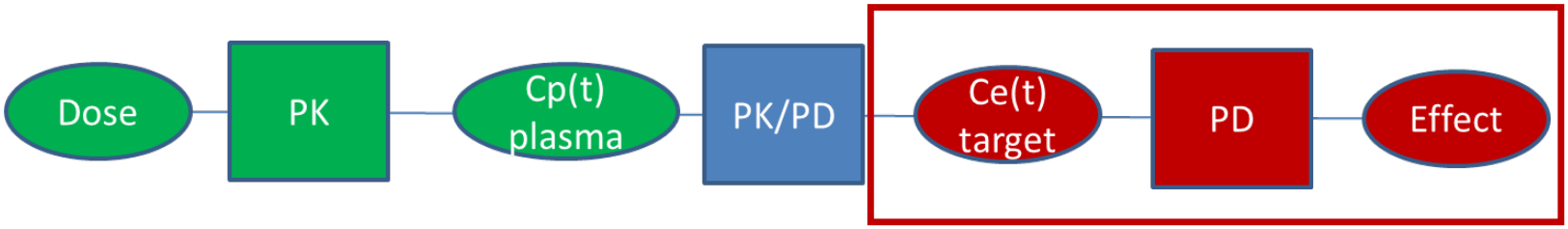
PK describes:

- How a xenobiotic compound gets into the body
- How it distributes in the body to the different organs
- How it gets eliminated from the body by metabolism or excretion

PK helps to identify which **exposure profile** is **most important** for the desired **effect**



Pharmacodynamics and Pharmacokinetics Relationships

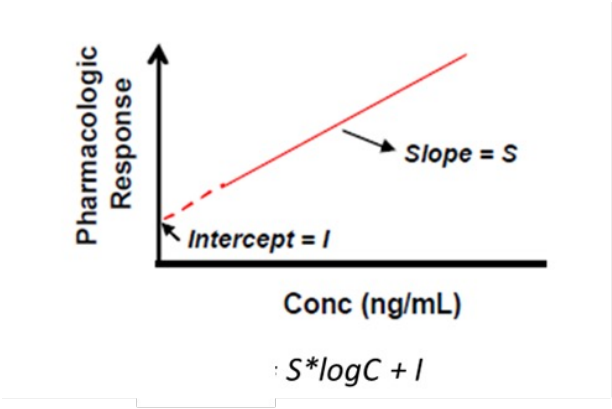


PD models:

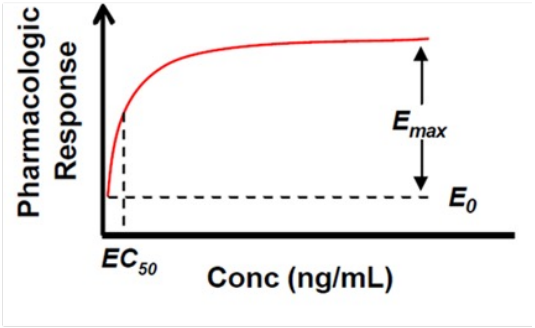
- Useful to describe the PD profile and to gain insights into the biological processes
- Assumptions:

  (a) drug response is reversible

  (b) there is only one type or receptor with one binding site

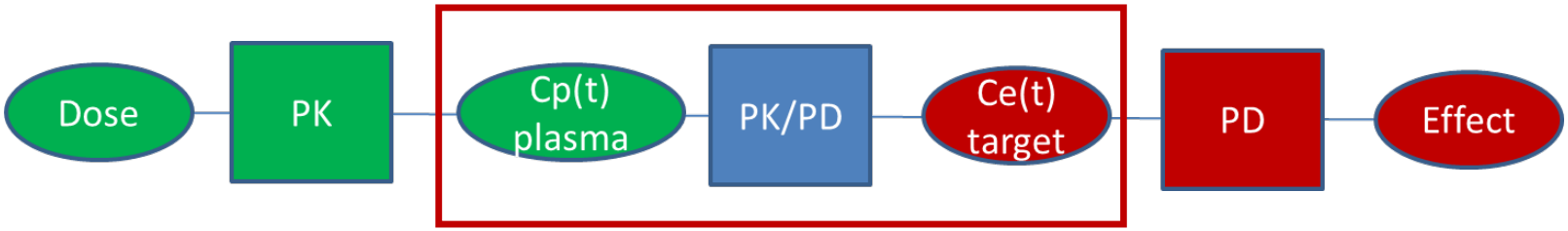


$S*\log C + I$



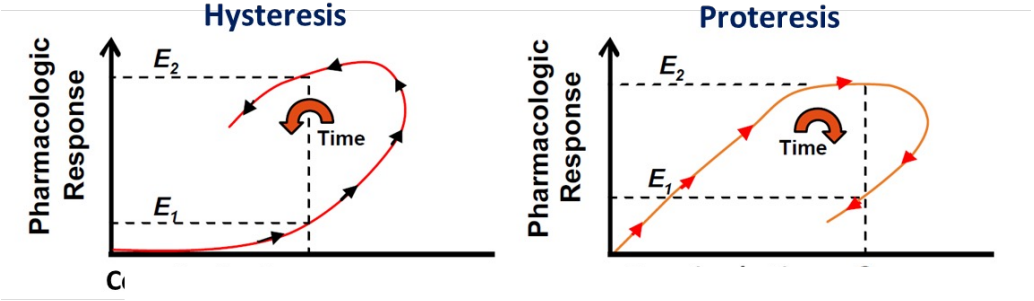
$E = E0 + Emax*C/(EC50 + C)$

Pharmacodynamics and Pharmacokinetics Relationships



PKPD models:

- If there is a delay between Cp and effect, Cp(t) might not be in equilibrium with Ce(t)
- Such delays can obscure the concentration/response
- PKPD model can reveal the true PD of a drug predicting the "effect site" concentrations



A given drug concentration elicits **a different pharmacological response**, **depending on the time** at which the concentration is measured.

Preclinical PK-PD approaches

Select a relevant (translatable) model

Is it translatable in man?
Do we have competitor/reference info?

Target Information

Where is the target expressed?
Which target occupancy is required?
Does unbound plasma unbound conc. ≈ unbound conc. at the target?

in vitro potency data

Biochemical and/or cellular assays

PK assessment

Same species/strain and administration route as in PKPD model. MetID info

PKPD Study Design

Efficacy is Cmax/AUC driven?
Selection of dose/dose regimen with modeling support

PKPD Study

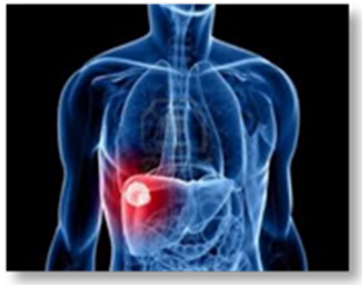
With PK sparse sampling and/or PK satellite arm

PKPD data analysis

Quantify link between concentration and effect (direct/indirect?)
Assess potency and efficacy

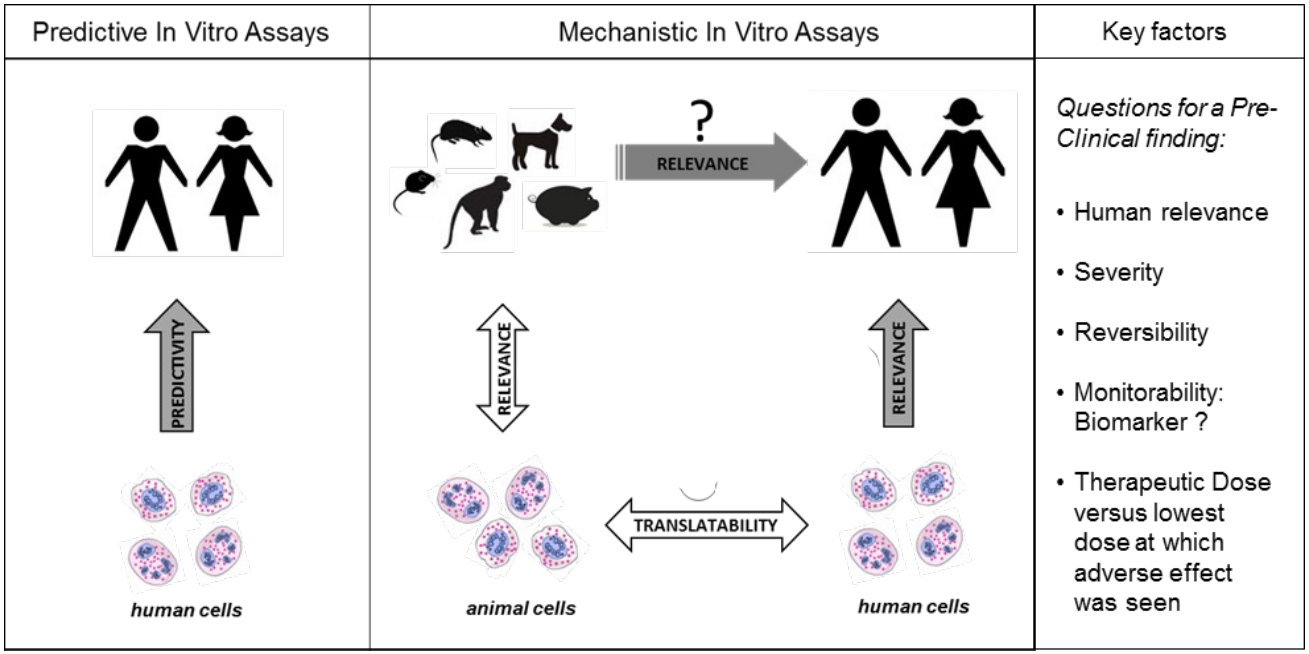
PKPD translation in men

Understand the concentration-effect relationship



in vitro and animal models

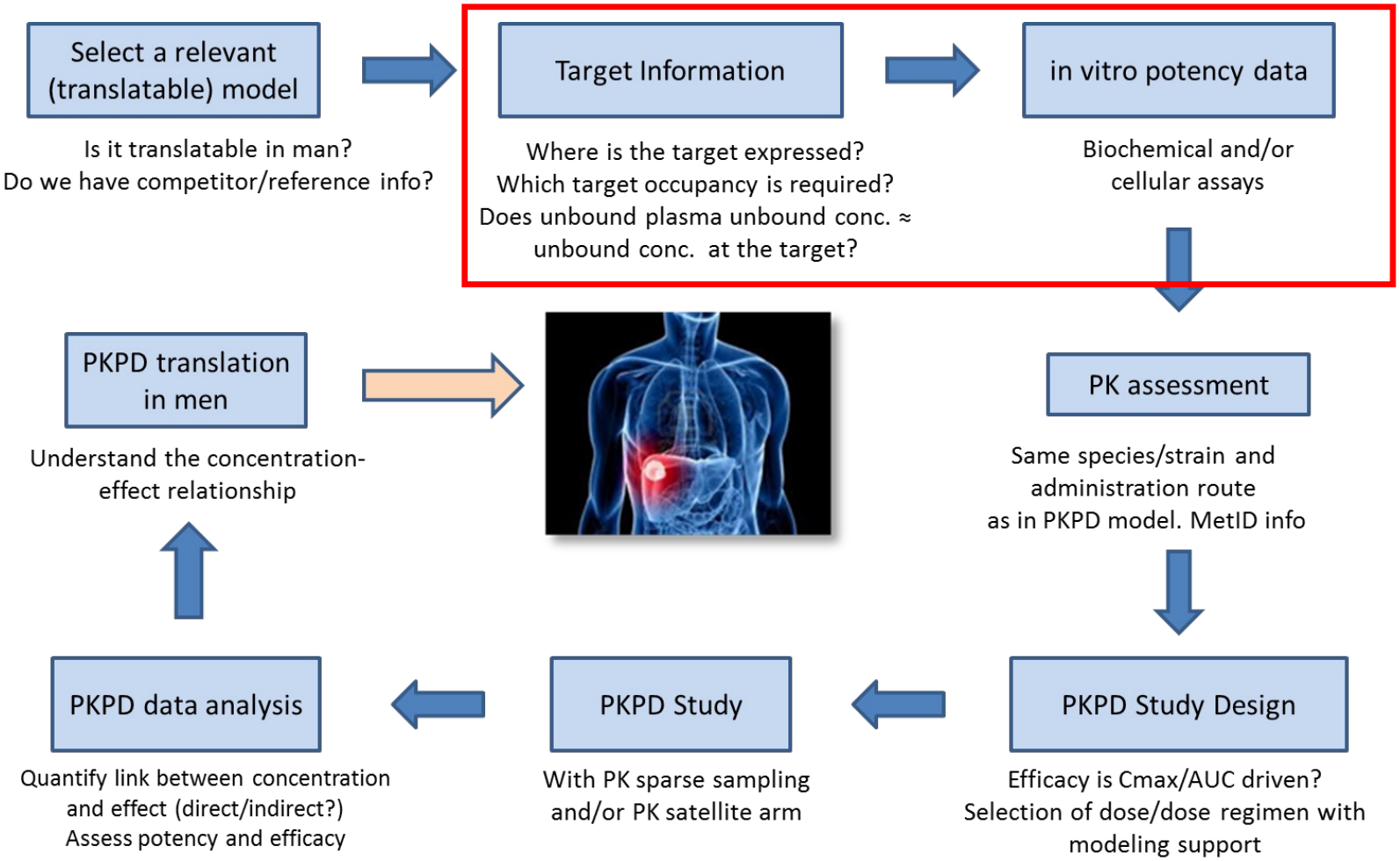
Used pre-clinically to holistically predict human relevant underlying mechanism for PKPD



Predictive Assays – direct quantitative extrapolation to humans using established scaling factors

Mechanistic Assays – validation in animals, then prediction from human systems to humans

Preclinical PK-PD approaches



In vitro Receptor Occupancy (RO) /Enzyme Inhibition (EI)

- The ultimate targets for most compounds are binding sites on receptors, ion channels, transporters and enzymes
- Degree of in vivo occupancy/inhibition needed for efficacy in animals and humans is of outmost value in guiding drug discovery and development efforts.
- In vivo RO/EI can be predicted based on unbound plasma exposure and in vitro binding under the default assumptions:
  - the animal used is predictive of human situation
  - unbound plasma conc. ≈ unbound tissue conc. (free drug hypothesis)
  - no active transport occurs
  - in vitro binding data are predictive of in vivo binding (often needs to be tested)

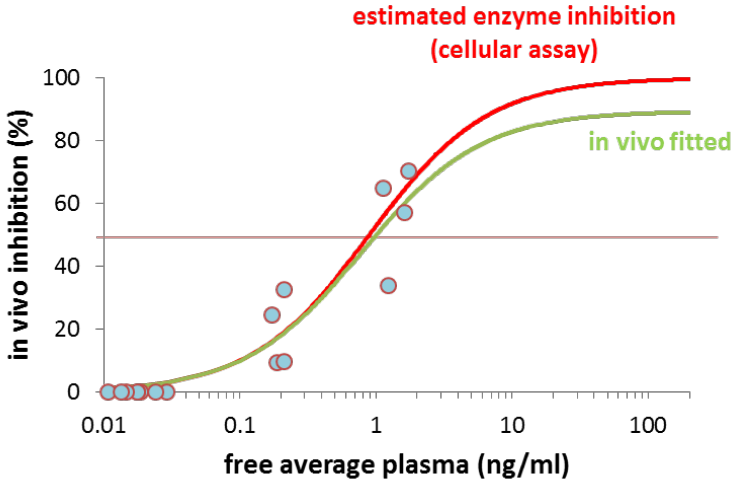
$$\mathrm{RO} = B\max \times \frac{\frac{Cp \times fup}{Ki}}{1 + \frac{Cp \times fup}{Ki}}$$

Bmax = maximum binding, generally assumed 100%
Cp = plasma concentration measured in the PD experiment
fup = free fraction in plasma
K_i = in vitro binding to the receptor

in vitro and in vivo Enzyme Inhibition

example from a cyno PKPD model

- Target is known and expressed in cyno and human
- unbound plasma conc. ≈ unbound tissue conc.
- Full PK-PD time course profile



In vivo IC50	ng/ml	0.8 (estimated)
In vitro IC50	ng/ml	0.9 (measured)

- The direct E_{max} model adequately fits the in vivo experimental data
- The estimated in vivo IC50 matches nicelv with in vitro one

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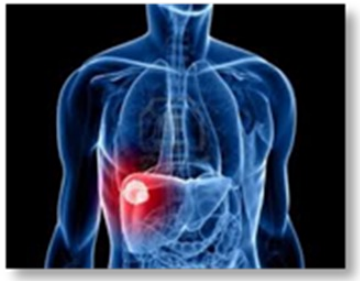

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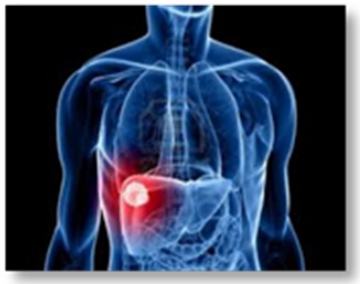


PK Driver for Efficacy

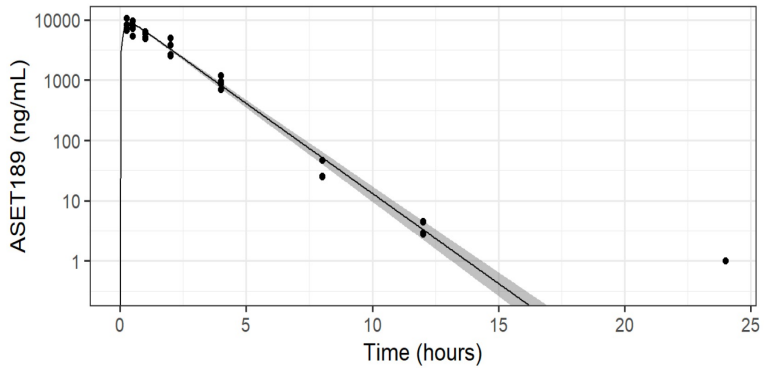
- By evaluating the overall exposure expressed as **AUC**, **C_{max}** and **C_{min}** we can identify which **exposure profile** is the most important for the desired effect

- For example for some drugs given chronically it is only important to maintain the plasma conc. above a defined minimum, than a drug with a slower decline (longer t1/2) is an advantage as the duration of the clinical effect will be longer

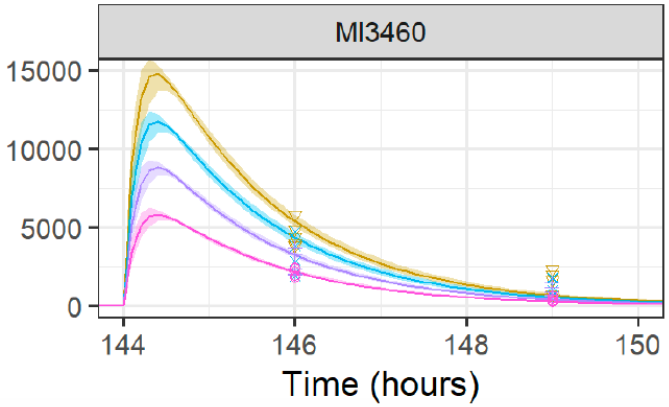
- For other drugs instead, relief of headache, the critical factor is the rapid achievement of an adequate concentration after which maintenance is less important (in this case C_{max})

PK Driver for Efficacy

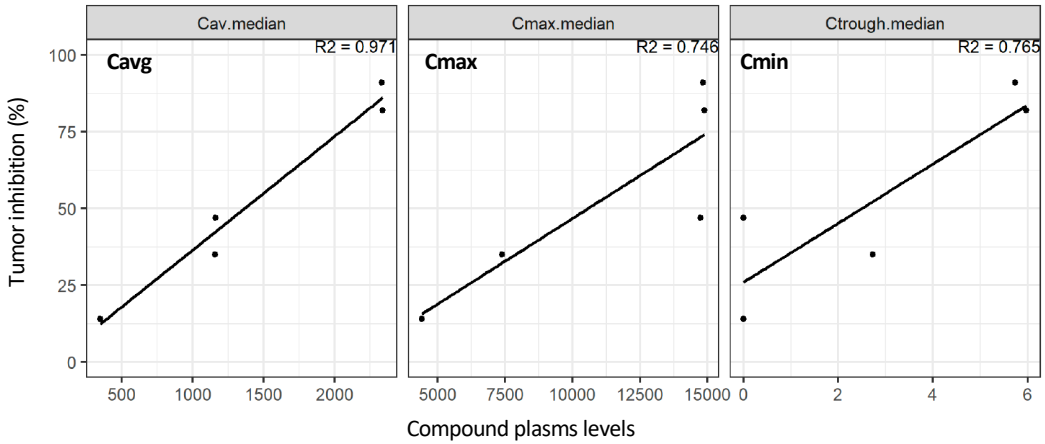
Measure PK in the Efficacy Model in a satellite group



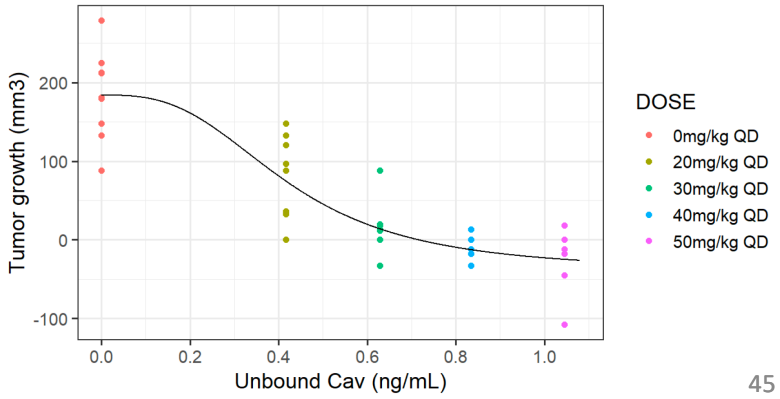
Spot check PK in the Efficacy Model in the main group



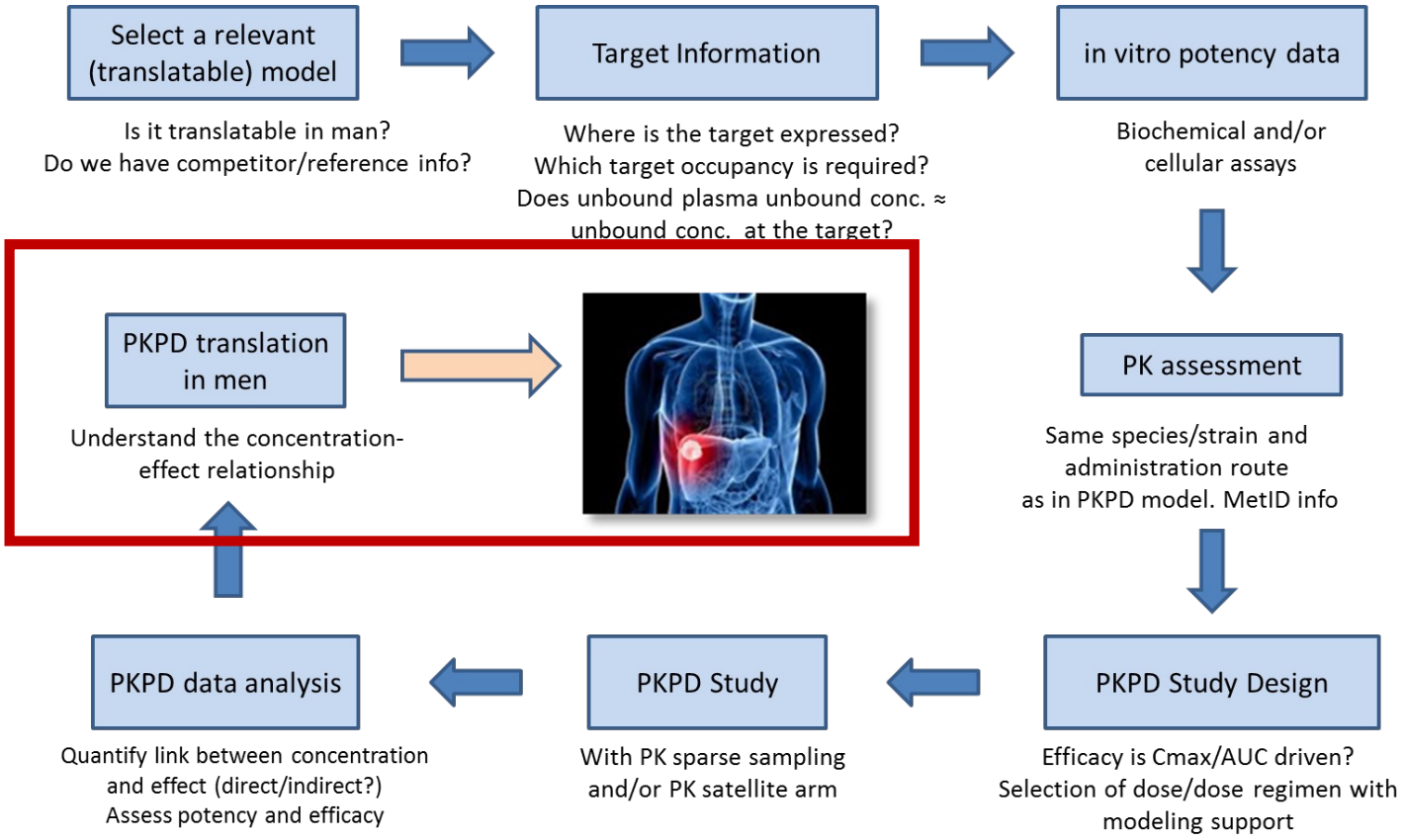
Correlate PD with PK



Build PKPD model



Preclinical PK-PD approaches



PK/PD modeling & translation to human

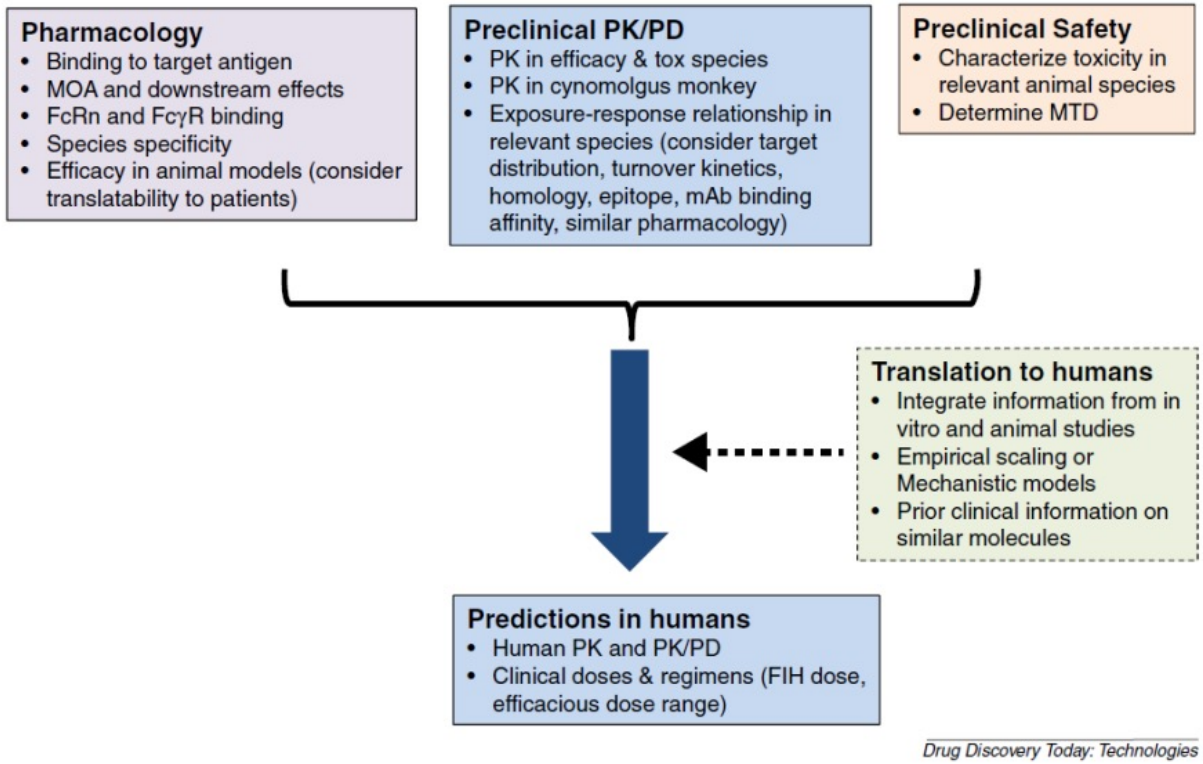


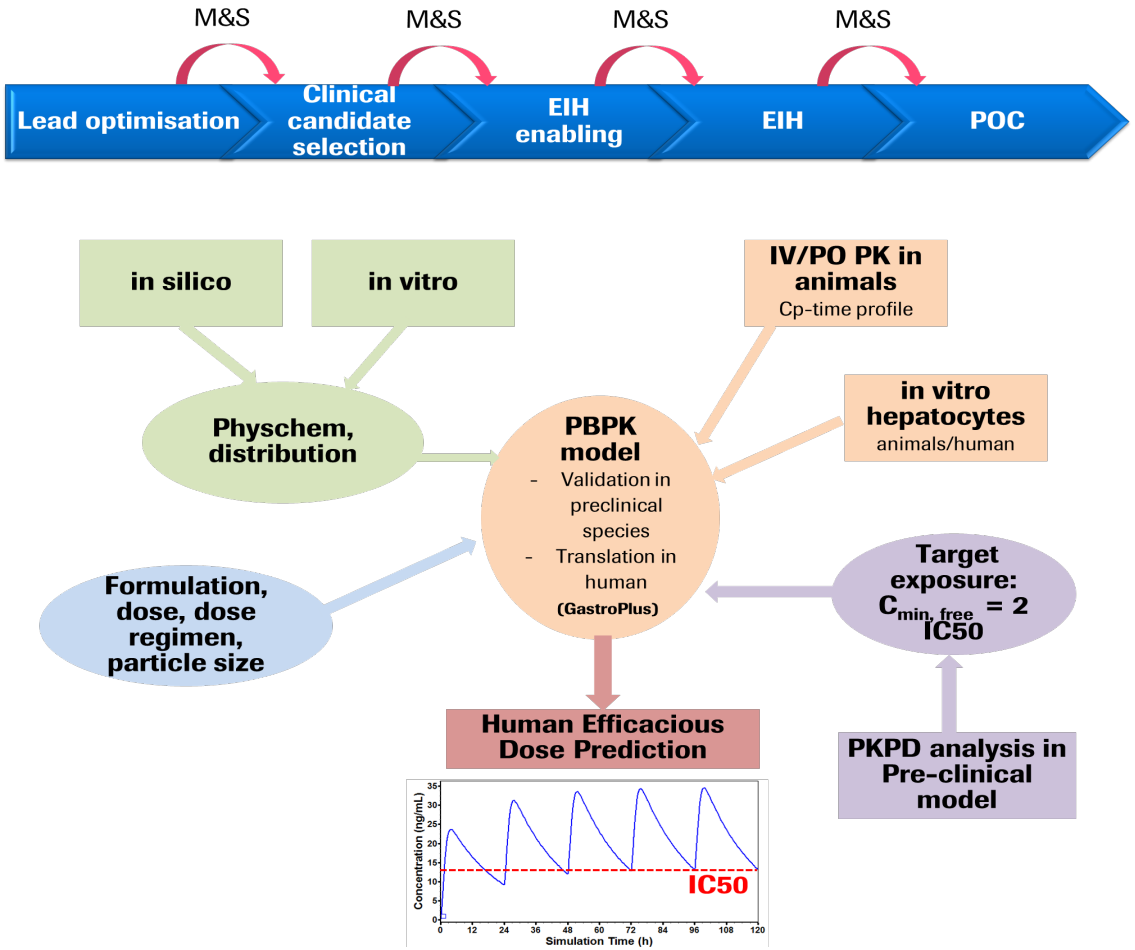
Figure 2. Framework for translation of PK/PD of mAbs from *in vitro* and animal data to humans.

1. Getting appropriate efficacy, safety, PK and PD data from in vitro and in vivo studies
2. Understanding exposure-response (PK/PD) relationships
3. Integrating in mathematical models and translating the PK, efficacy and safety data to predict PK/PD and PK/safety profiles in humans, select optimal first in human (FIH) dose, identify dose escalation steps and potential efficacious dose ranges in human, etc

Drug Discovery Today, Vo. 21-22, 2016, page 75

How to Translate From Animal to Human?

Model based approach to predict safety efficacy in human from preclinical data



Conclusions

- Preclinical PKPD models are crucial to **screen** and **select** a **clinical candidate**
- Focus on selected compounds to **run properly designed PK/PD trials** at the **beginning of the projects**
- Proper design needs to be conducted to extract the maximum amount of information such as : *“What level of effect and what duration of effect is required for clinical efficacy?”*
- Useful to **translate** the preclinical PKPD **into humans** for human efficacious dose projection and facilitates the **design of dosing regimens** in **first-in-man** and **POC studies**

Selected PKPD References

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