

# Setting the starting dose in FIH studies - key considerations

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# XenoGesis - quick introduction

- The services offered:
  - Experimental *in vitro* and *in vivo* DMPK/ADME studies, bioanalysis and *in vitro* pharmacology
  - Pre-clinical PK/PD modelling, interpretation and dose to man
  - Consultancy delivering expert drug research & development advice
- The Client base:
  - UK, Europe, US, Singapore, Australia
  - 180 companies in 7.5 years - SMEs, mid-sized Pharma. 13 academic institutions
  - High % repeat business (72% of all quotes issued)
- The Company:
  - Founded in November 2011 at BioCity, Nottingham, with 3 staff
  - 95% privately owned
  - High year on year growth (30% growth and 50% overseas revenue 2018/19 YE)
- The Team:
  - Richard Weaver Ph.D., FRSC – CEO and Founder
  - 31 further members of staff
  - Highly experienced scientific staff, with Pharmaceutical Industry or CRO backgrounds

# Contents

- Scene setting. What considerations are required?
- Pharmacology considerations. *in vitro* efficacy data (both preclinical species and human), and preclinical *in vivo* efficacy PK/PD studies
- DMPK and TK. Exposure considerations. C<sub>max</sub>, AUC, T<sub>1/2</sub> and "free exposure". Exposure is the glue that brings it all together. Wanted exposure versus unwanted exposure.
- Pre-clinical regulatory safety studies
- Healthy volunteers or patients?
- Maximum Recommended Starting Dose (MRSD), NOEL, MABEL, HED and additional safety factor
- Learning points

# Dose and exposure

- “All things are poison and nothing is without poison, only the dose makes a thing be poison”

Paracelsus (1493–1541)

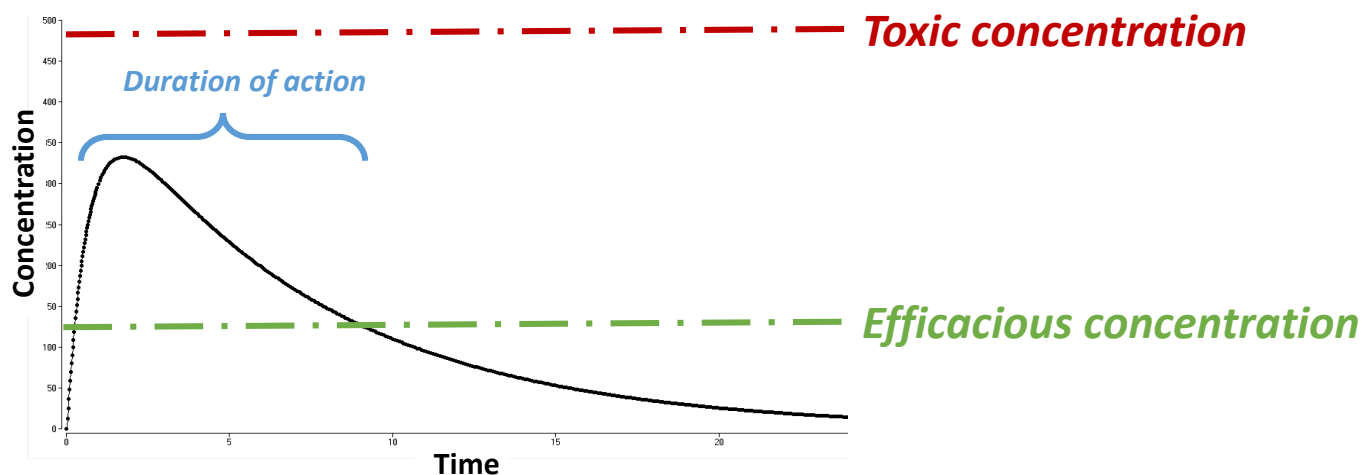
- 500 years later and it's very valid
- But dose does not necessarily equal exposure...



# Dose, exposure and efficacy

Biologic effects (desired and toxic) are a result of drug exposure

- Too little exposure – not enough desired effect. Too high exposure – toxicity

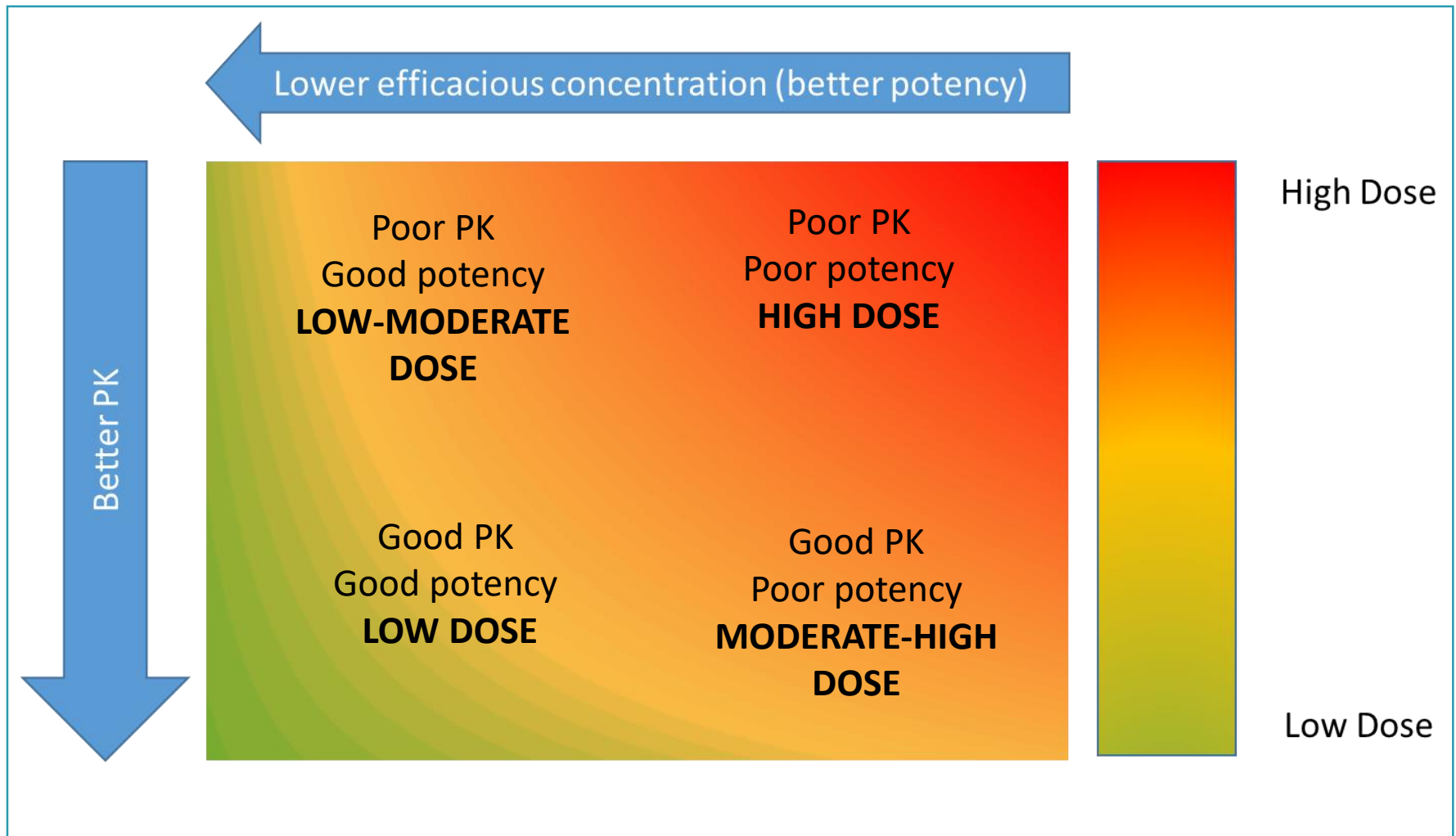


- Drug exposure (concentration-time profile) is a function of dose and pharmacokinetics

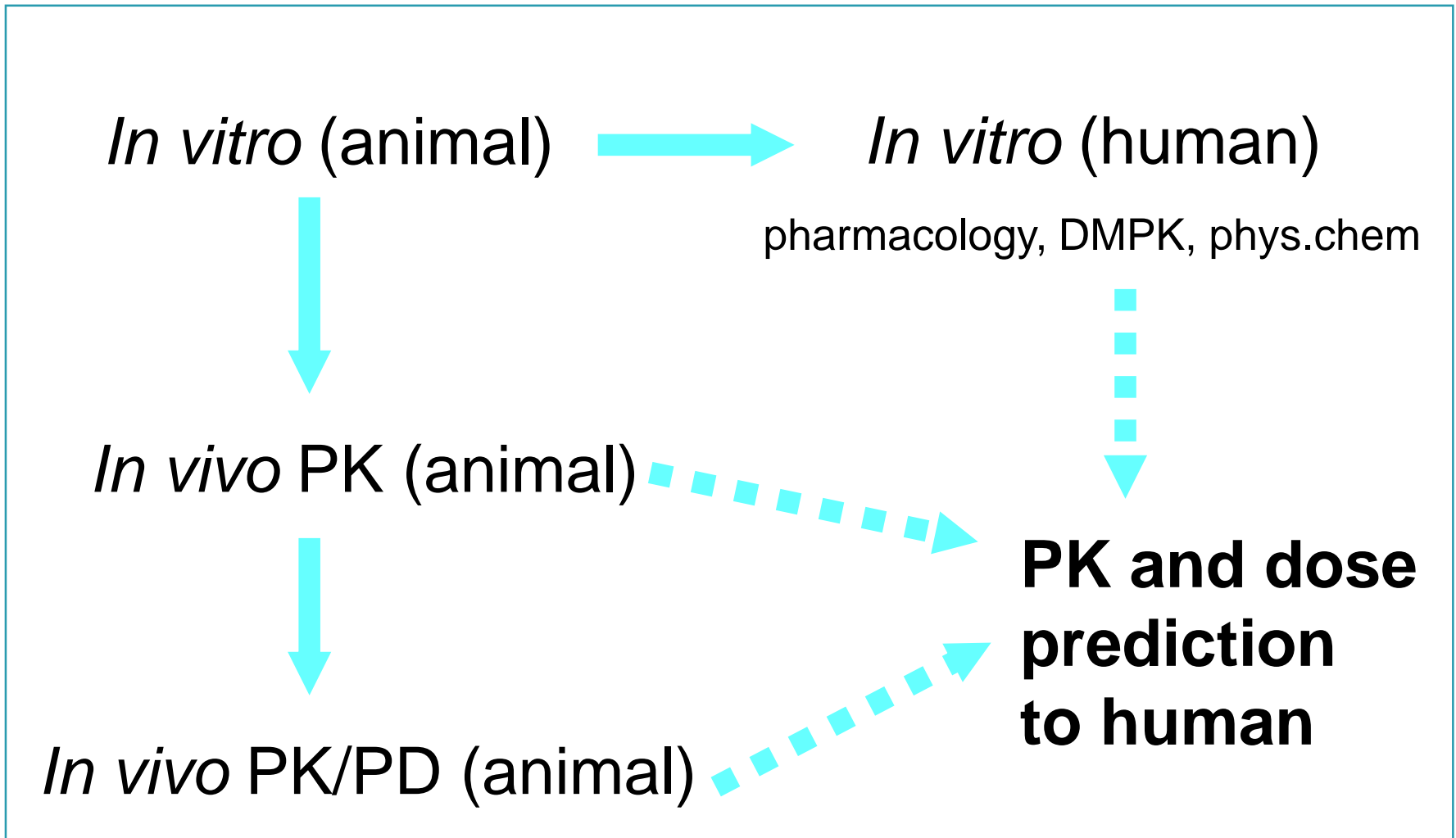
DOSE is the means of achieving the required EXPOSURE for sufficient EFFICACY

*“Whenever dose is mentioned in this guideline, the expected exposure at that dose should always be taken into consideration”* [EMA draft guideline 2017](#)

# Target absorbed dose: PK and efficacious concentration

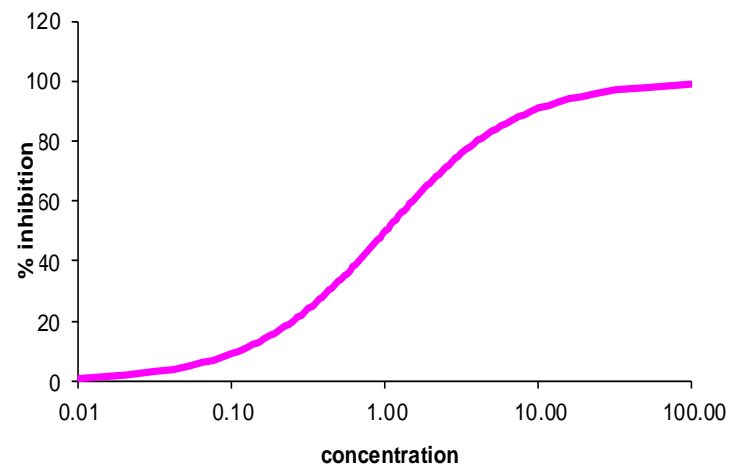
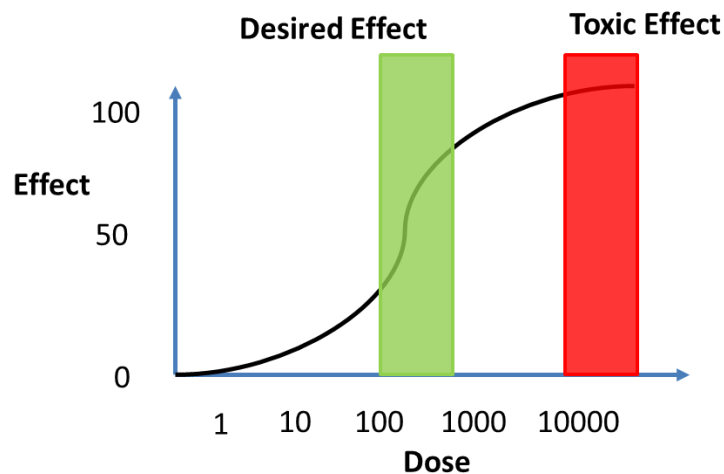


# How do we predict efficacious exposure?



# What dose?

- What dose should we give, whether *in vitro* or *in vivo*, to cause the desired effect without inducing a negative (toxic) response?



$$E = \frac{E_{\max} * C}{C + C_{50}}$$

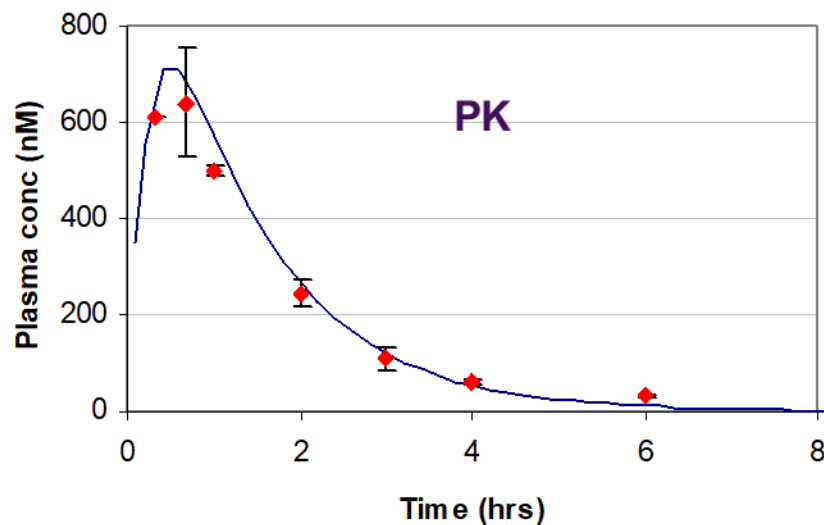


# PK/PD

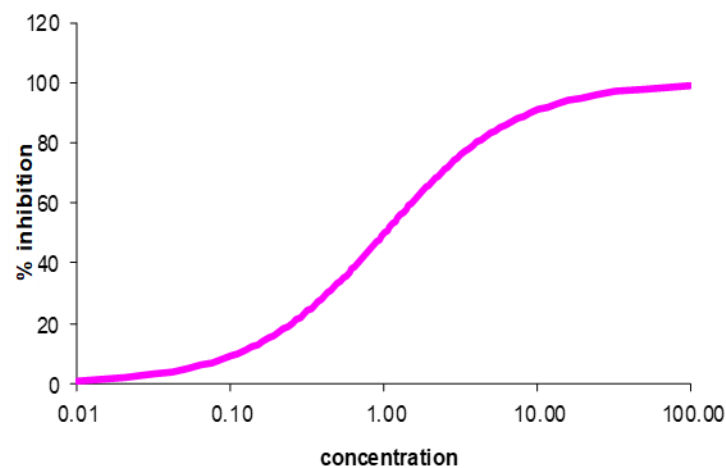
- Pharmacokinetics
  - What the body does to the drug
  - Relationship between drug concentration and time
  
- Pharmacodynamics
  - What the drug does to the body
  - Relationship between drug concentration and effect

# PK/PD

- PK and PD are mathematically related by concentration
- Therefore human dose prediction is dependent on **both** pharmacology AND PK



$$C_t = C_0 * e^{-kt}$$



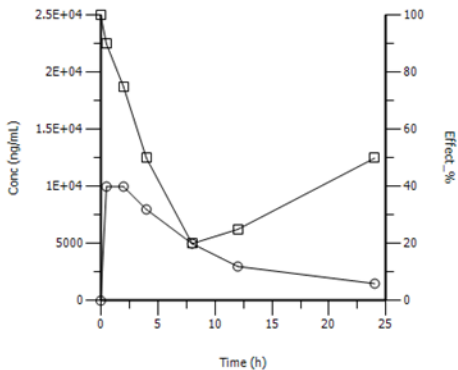
$$E = \frac{E_{\max} * C}{C + C_{50}}$$

# PK-PD modelling and prediction

Single dose PK-PD  
(target engagement)

## Measure PK-PD

- Effect v time
- Concentration v time

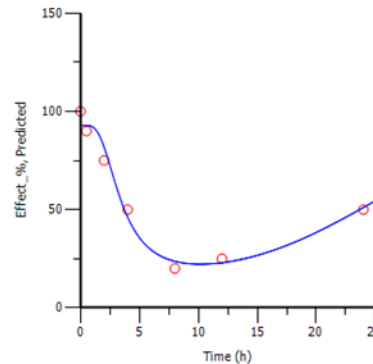


- Generation of single dose PK-PD models
- Design of repeat dose studies (optimal dose/schedule)

PK-PD modelling  
(single dose)

## Model single dose PK-PD

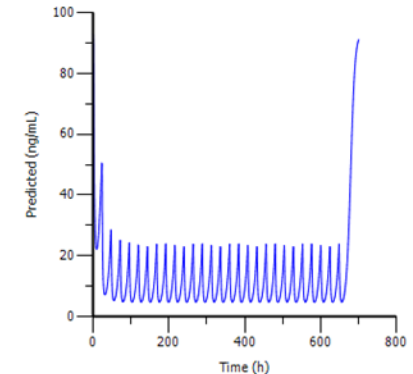
- Biomarker response over time from single dose



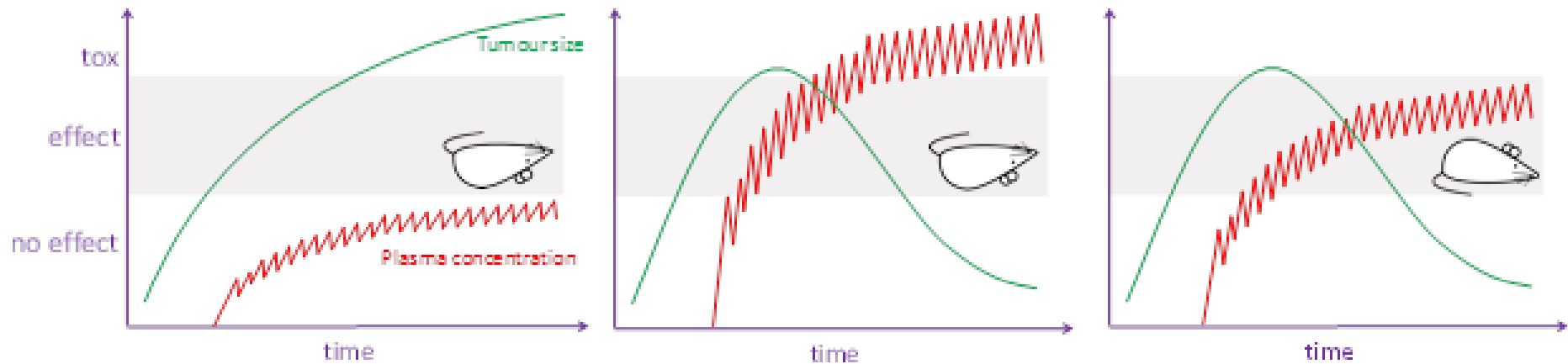
PK-PD modelling  
(repeat dose selection)

## Predict repeat dose PK-PD

- Select dose and schedule to test hypothesis



# PK/PD – the correct exposure



- A) Dose too low: Animals die from uncontrolled tumour growth despite dosing a potent drug
- B) Dose too high: Animals suffer/die from administered drug, despite reduction in tumour growth
- C) Correct dose: Only when potency and exposure are within the right range tumour growth can be inhibited without serious side effects

# But how do we predict PK with confidence?

- We don't really know what the human PK will be until we dose volunteers in FTIH, but we can estimate it based on the wealth of pre-clinical data
- How do we build the evidence?:
  - Can we predict rat clearance from rat *in vitro* data?
  - Can we predict rat volume of distribution from *in vitro* data?
  - Can we predict absorption, and with the clearance prediction, predict bioavailability?
  - Can we predict the actual concentration/time profile from an oral dose?
- If the answer is “yes” to some, or all of these, then we can probably predict mouse, dog, cynomolgous monkey, mini-pig and human PK
- How do we do this?

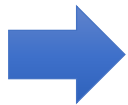
# GastroPlus™: *in vitro* – *in vivo* predictions and correlations

## *In vitro* data

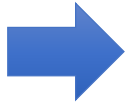
- Hepatocyte CL<sub>int</sub>, PPB, LogD<sub>7.4</sub> and pKa



- PPB, B:P, LogD<sub>7.4</sub> and pKa



- Solubility, pKa, Caco-2 permeability, particle size



## *In silico* model

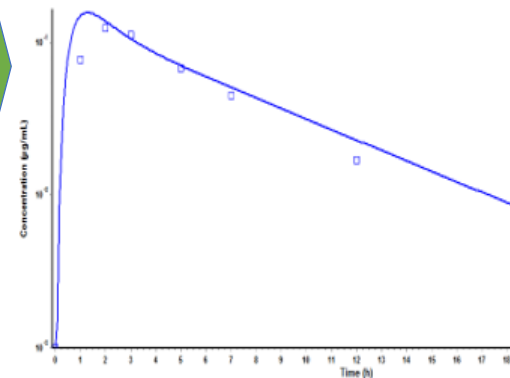
- Hepatic Clearance

- Tissue partitioning
- Volume of distribution

- Absorption (rate and fraction over time)
  - Dose dependence



## Predicted oral exposure



- Integration of PK with PK-PD (efficacy) data allows us to predict efficacious dose

# Key Pharmacokinetics parameters

- **Clearance (Cl):** A measure of how efficiently the body removes drug from the body. Measured in ml/min/kg (or l/h) and is measured from blood or plasma. Clearance is the volume of blood from which ALL drug is removed in unit time
- **Volume of distribution (V<sub>ss</sub>):** Tells us information about whether the drug is mostly restricted to the volume of blood or distributes freely into tissues. This is not a physiological volume. Acids tend to have a V<sub>ss</sub> of 0.1-0.3 l/kg, neutral compounds 1-2 l/kg and bases >4l/kg. Distribution is the *reversible* transfer of drug between the site of measurement (usually plasma) and other sites within the body (tissues etc.)
- **Half-life (T<sub>1/2</sub>):** The time taken for the blood concentration to drop by half. Half-life is a function of BOTH Cl and V<sub>ss</sub>
- **AUC:** Is a measure of total blood (and body) exposure and is the area under the curve from the conc./time profile
- **C<sub>max</sub> and C<sub>min</sub>:** C<sub>max</sub> is the maximum blood concentration received after a dose (IV, PO, SC, IM, Inhaled). C<sub>min</sub> is usually defined as the minimum concentration achieved before the next dose
- **Bioavailability (F):** Bioavailability is the fraction of administered dose that reaches the systemic circulation as intact drug. F is 1 for an IV dose.

# Total versus free exposure

- A very important concept that many people still don't understand or appreciate
- Only unbound (or "free" drug) can exert a pharmacological or toxicological effect or be able to be cleared/metabolised by the body
- In the absence of active transport, the "free" plasma concentration = "free" tissue concentration at steady-state
  - Steady-state is reached after approximately 5 half-lives from repeat dosing (when 97% of steady-state conc. is reached)
  - Therefore, a compound with a short half-life reaches steady state faster than one with a long half-life
- But we measure total blood or plasma concentration in PK and TK. Therefore, it's important to measure plasma protein binding (PPB) in all relevant species and convert to "free" exposure when considering PK/PD and safety margins
  - Differences in plasma protein binding between species are absolutely possible. If human PPB is 10-fold more "free" than rat for example, then using total plasma concentrations for safety margins could be dangerous



# Total versus free exposure

- “When protein binding is low in both humans and rodents, or when protein binding is high and the unbound fraction of drug is greater in rodents than in humans, the comparison of total plasma concentration of drug is appropriate. When protein binding is high and the unbound fraction is greater in humans than in rodents, the ratio of the unbound concentrations should be used”. [ICH5\(R2\)](#)
- “The choice for the use of total vs. fraction unbound pharmaceutical exposures should be justified. The total exposure can be used as the default, unless the fraction unbound results in a lower exposure margin than that of the total; in this case the lower exposure multiple should be used for the comparison of animal vs. human exposures. Alternatively, the fraction unbound pharmaceutical exposure can be used regardless of whether it generates a lower or greater exposure multiple than that of the total exposure provided the following applies: The fractions unbound can be calculated accurately from the total pharmaceutical exposure, is reproducible at the effective concentrations in humans and at the toxicological concentrations in animals, and the fractions unbound are statistically significantly different.
- Two examples of how this calculation might impact the exposure multiples are provided below.
  - 25 fold exposure multiple not met: If the total exposure is 25  $\mu\text{M}\cdot\text{hr}$  in animals and 1  $\mu\text{M}\cdot\text{hr}$  in humans and unbound protein fraction is 5% and the unbound fraction in animals is 1%, then the margin would be 5.
  - 25 fold exposure multiple exceeded: If the exposure is 10  $\mu\text{M}\cdot\text{hr}$  in animals and 5  $\mu\text{M}\cdot\text{hr}$  in humans and unbound protein fraction is 1% in human and 20% in animals, then the unbound ratio would be 40 rather than the apparent ratio of 2 based on total. [ICH5\(R3\)](#)

# PK versus TK

- PK = pharmacokinetics
- TK = toxicokinetics
- There are no fundamental differences between the two mathematically
- PK is determining all the pharmacokinetic parameters at generally a low (non-toxic) dose
- TK is determining  $C_{max}$ ,  $T_{max}$ ,  $C_{min}$ , AUC,  $T_{1/2}$ , dose linearity etc. from regulatory toxicology studies and thus generally at substantially higher doses

# What dose in FTIH (volunteers)?

- Let's say we're reasonably confident we can predict the human PK well
- We understand the dose : exposure, with increasing dose in preclinical species
- We understand the PK/PD relationship in pre-clinical species, target engagement and any differences in potency, receptor expression etc. between pre-clinical species and human
- Would we dose our FTIH at a therapeutically relevant concentration?
- What have we not talked about yet?

# What are the primary objectives of Phase 1?

- Assess safety and tolerability
- Characterize dose-limiting adverse reactions
- Determine maximum dose associated with acceptable safety profile
- Characterize pharmacokinetic parameters
- Explore drug metabolism and drug interactions (if dosed on background of other drug(s))
- Secondary: some measure of target engagement or biomarker

# Why do we choose healthy volunteers?

- Less confounding issues
  - no disease(s), significant co-medication introducing the possibility of DDI's, less likely to withdraw from trail, no COPD, long and historic alcohol abuse etc.
  
- Patients are used when agent is likely to be cytotoxic e.g. oncology patients, special populations are needed e.g. renal or hepatic impairment, potential breakthrough therapy for terminally ill patients

# What dictates the starting dose in man?

- Need to have ‘one-eye’ on likely efficacious dose
- Need to understand toxicity profile in pre-clinical species plus human *in vitro* data etc.
- “The development and evaluation of a new IMP is a stepwise process involving animal and human efficacy and safety information. The non-clinical data in PD, PK and toxicology and their translation to human are important basis for planning and conduct of a FIH/early CT” [EMA draft guideline 2017](#)
- Is the projected human started dose commensurate with the compound properties?
  - Solubility etc.
  - Starting dose 50mg with a dose concentration of 1mg/mL but drug solubility only 10 µg/mL...isn't going very far
- Introducing the concept of Maximum Recommended Starting Dose (MRSD)
  - Principles in selecting an MRSD:
    - avoid toxicity at the initial clinical dose
    - allow reasonably rapid attainment of the trial objectives (tolerability and PK)
- <https://www.fda.gov/media/72309/download>

# Pre-clinical species requirements

- “For small molecule entities, in line with ICH M3(R2), at least one species used for toxicity testing (rodent or non-rodent) should be “pharmacologically” relevant, where both the presence of the target and the relative potency of the molecule against the target in the selected animal species and the intended patient population should be considered. The species should also be chosen based on their similarity to humans with regard to *in vitro* metabolic profile. Need to understand toxicity profile in pre-clinical species plus human *in vitro* data etc.
- “Qualitative and quantitative differences may exist in biological responses to an IMP in animals compared to humans, e.g. differences in affinity of the new candidate for molecular targets, or physiological differences in tissue distribution of the molecular target, or cellular consequences of target binding, cellular regulatory mechanisms, metabolic pathways, or compensatory responses to an initial physiological perturbation”.
- “In this context, the use of *in vitro* human cell systems or human-derived material could provide relevant information about these translational differences and improve the understanding of the relevance of the animal models”. [EMA draft guideline 2017](#)

# MRSD, NOAEL, MABEL and PAD (acronym overload!)

- Determine NOAEL
  - No Observable Adverse Effect Level in at least 2 species. One rodent and one non-rodent
  - “The highest dose level that does not produce a significant increase in adverse effects in comparison to the control group. In this context, adverse effects that are biologically significant (even if they are not statistically significant) should be considered in the determination of the NOAEL”
  - “As a general rule, an adverse effect observed in nonclinical toxicology studies used to define a NOAEL for the purpose of dose-setting should be based on an effect that would be unacceptable if produced by the initial dose of a therapeutic in a phase 1 clinical trial conducted in adult healthy volunteers”.
- <https://www.fda.gov/media/72309/download>
- “Exposure showing PD effects in the non-clinical pharmacology studies, including *ex vivo* and *in vitro* studies in human tissues if feasible, should also be determined and these data should be used to determine the minimal anticipated biological effect level (MABEL) in humans and an estimation of the pharmacologically active dose (PAD) and/or anticipated therapeutic dose range (ATD) in humans” [EMA draft guideline 2017](#)



# Starting dose for volunteers and patients

- “In addition, the calculation of the MABEL, PAD and/or ATD should consider target binding and receptor occupancy studies *in vitro* in target cells from human and the relevant animal species and exposures at pharmacological doses in the relevant animal species”.
- “The starting dose for **healthy volunteers** should be a dose expected to result in an exposure lower than the PAD, unless a robust scientific rationale can be provided for a higher dose”.
- “The goal of selecting the starting dose for FIH/early CTs in **patients**, i.e. where there are no previous data in healthy volunteers, is to identify a dose that is expected to have a **minimal pharmacological effect** and is safe to use.
- [EMA draft guideline 2017](#)

# NOAEL to HED

- The NOAEL is converted to Human Equivalent Dose (HED) normalised to body surface
- Human Equivalent Dose (HED): Conversion factor applied that converts mg/kg dose for each animal species to a mg/kg dose in humans
- Selection of animal species:
- The most sensitive species is chosen (i.e. the species in which the lowest HED can be identified)
- Some instances, especially with biologics, appropriate animal species used based on *in vitro* binding and functional studies
- <https://www.fda.gov/media/72309/download>

# NOAEL to HED

| <b>Table 1: Conversion of Animal Doses to Human Equivalent Doses Based on Body Surface Area</b> |   |   |                         |
|---|---|---|-------------------------|
| Species   | To Convert Animal Dose in mg/kg to Dose in mg/m <sup>2</sup> , Multiply by k <sub>m</sub> | To Convert Animal Dose in mg/kg to HED <sup>a</sup> in mg/kg, Either: |                         |
|   |   | Divide Animal Dose By   | Multiply Animal Dose By |
| Human   | 37  | ---   | ---                     |
| Child (20 kg) <sup>b</sup>  | 25  | ---   | ---                     |
| Mouse   | 3   | 12.3  | 0.08                    |
| Hamster   | 5   | 7.4   | 0.13                    |
| Rat   | 6   | 6.2   | 0.16                    |
| Ferret  | 7   | 5.3   | 0.19                    |
| Guinea pig  | 8   | 4.6   | 0.22                    |
| Rabbit  | 12  | 3.1   | 0.32                    |
| Dog   | 20  | 1.8   | 0.54                    |
| Primates:   |   |   |                         |
| Monkeys <sup>c</sup>  | 12  | 3.1   | 0.32                    |
| Marmoset  | 6   | 6.2   | 0.16                    |
| Squirrel monkey   | 7   | 5.3   | 0.19                    |
| Baboon  | 20  | 1.8   | 0.54                    |
| Micro-pig   | 27  | 1.4   | 0.73                    |
| Mini-pig  | 35  | 1.1   | 0.95                    |

# The process

1

- Determine NOAEL

2

- Convert each animal NOAEL to HED

3

- Select HED from most appropriate species

4

- Choose safety factor and divide HED by that factor

5

- Maximum recommended starting dose (MRSD)

# The safety factor

- The safety factor provides a margin of safety for protection of human subjects receiving the initial clinical dose
  - The default safety factor is usually 10
  - Allows for variability in extrapolating from animal toxicity studies to studies in humans, uncertainties due to enhanced sensitivity in humans vs. animals
- Difficulty in detecting certain toxicities in animals (Headache, myalgia)
- Differences in receptor densities or affinities
- Unexpected toxicities
- Interspecies difference in absorption, distribution, metabolism, excretion (ADME)
  - (this is where we come in!)

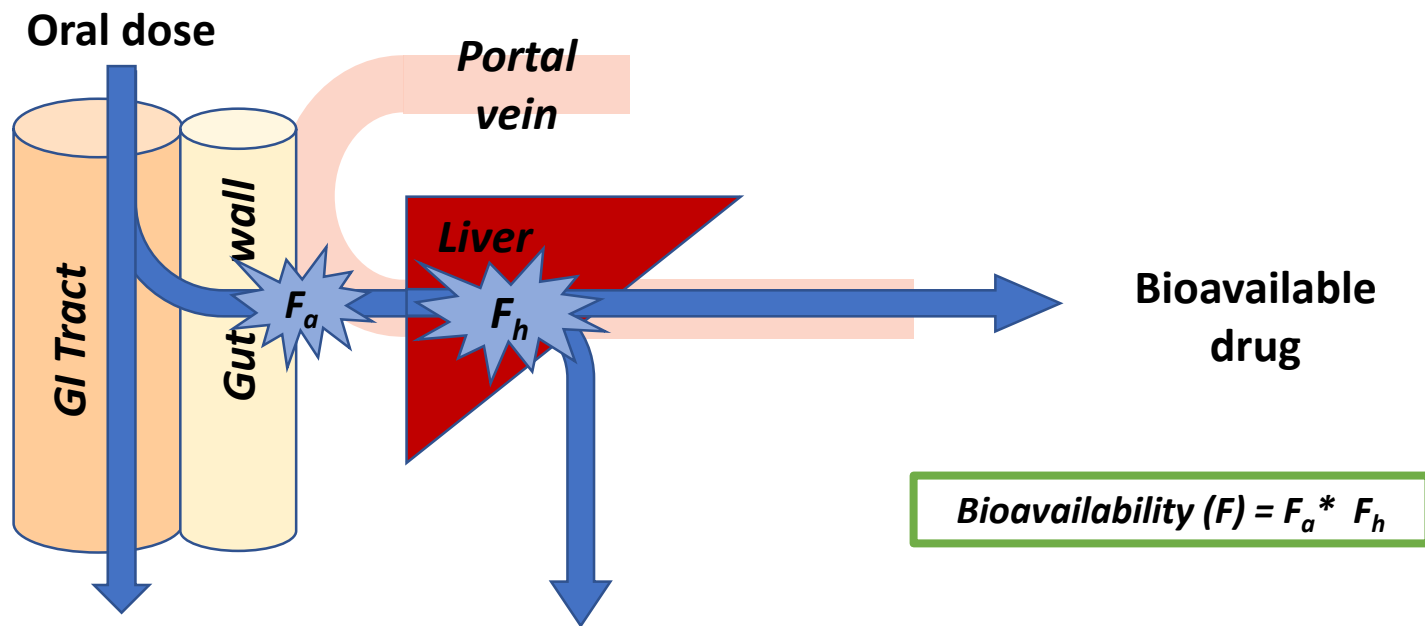
# Why we might want to raise the safety factor?

- Novel therapeutic class
- Toxicities:
  - Severe or irreversible
  - Non-monitorable toxicity- histopathologic changes in animals, not readily monitored clinically/markers
- Steep dose response curve
  - May indicate a greater risk in humans
- Non-linear pharmacokinetics
  - Limits the ability to predict dose-related toxicity
- Variable bioavailability
  - Poor bioavailability in test species may underestimate toxicity in humans

# Why we might want to decrease the safety factor?

- Members of a well-characterized class
- Toxicities produced by the therapeutic agent are easily monitored, reversible, predictable
  - If the NOAEL was determined based on toxicity studies of longer duration
  - assuming toxicities are cumulative
  - are not associated with acute peaks in therapeutic concentration, and
  - did not occur early in the repeat dose study

# What happens if the PK studies give exposure that is too low to progress to efficacy studies?



*Loss of drug:*

Unabsorbed drug in  
faeces  
Metabolism in GI  
tract/gut wall

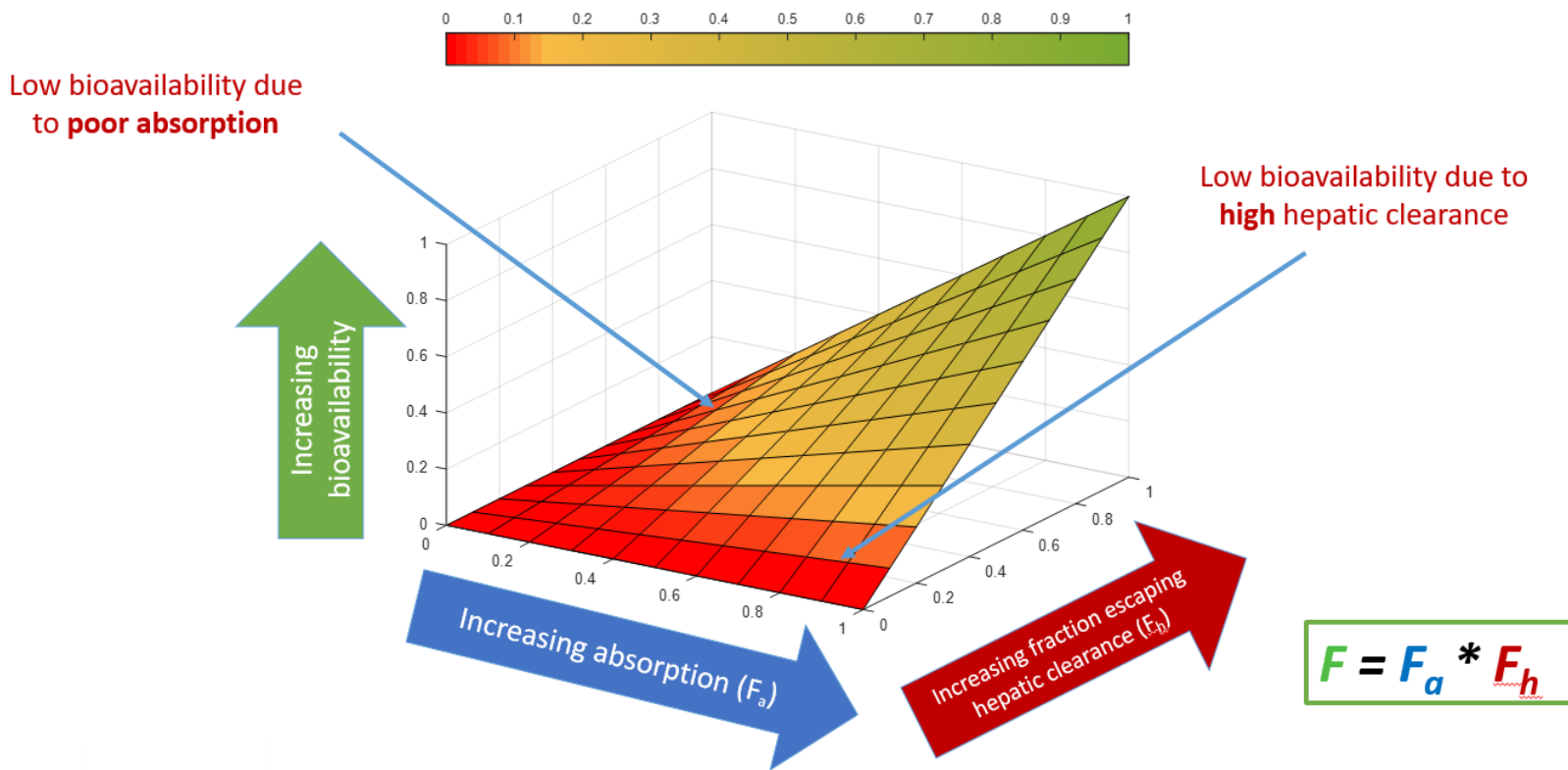
Hepatic  
clearance

$F_a$  = Fraction of drug absorbed and  
escaping GI metabolism  
 $F_h$  = Fraction of drug escaping  
hepatic clearance

**Bioavailability can be limited by absorption or  
metabolism/clearance**



# Metabolism and absorption both contribute to bioavailability



**Formulation strategies will generally only improve bioavailability if this is absorption limited**

# Case study – Enhancement of oral bioavailability for NCE

- Background:
  - Requirement: To provide a formulation to enhance exposure for use in toxicology studies
  - Very poor aqueous solubility ( $< 1 \mu\text{g/mL}$  at pH 7.4), moderate permeability (Caco-2 A-B:  $6 \times 10^{-6} \text{ cm/s}$ )
  - Limited exposure observed in animal studies (plateau at 20 mg/kg dosing).
  - Clearance was  $< 5\%$  liver blood flow – if all compound is absorbed, bioavailability  $> 95\%$  should be possible
  - Fraction absorbed and escaping GI metabolism ( $F_a$ ) is limiting bioavailability:
- 
- Data suggests poor solubility is limiting exposure due to poor absorption

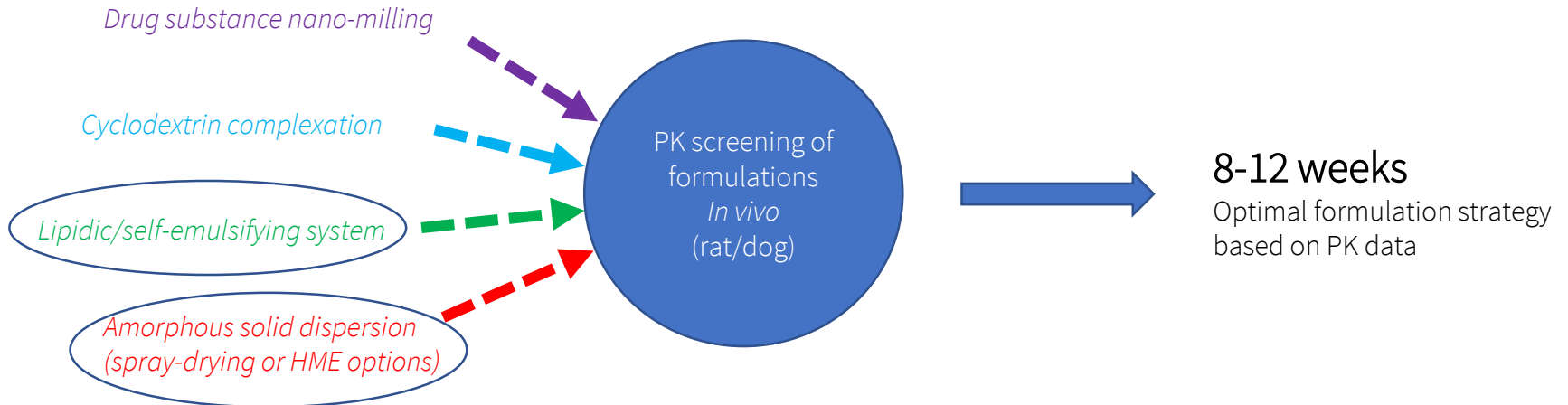
# Formulation strategy

## Stage 1: Drug substance review

Physicochemical and biological (DMPK) characterisation, anticipated dose in humans (or dose range in animals)  
- gap analysis and gap filling

## Stage 2: Enabling technologies rapid screen

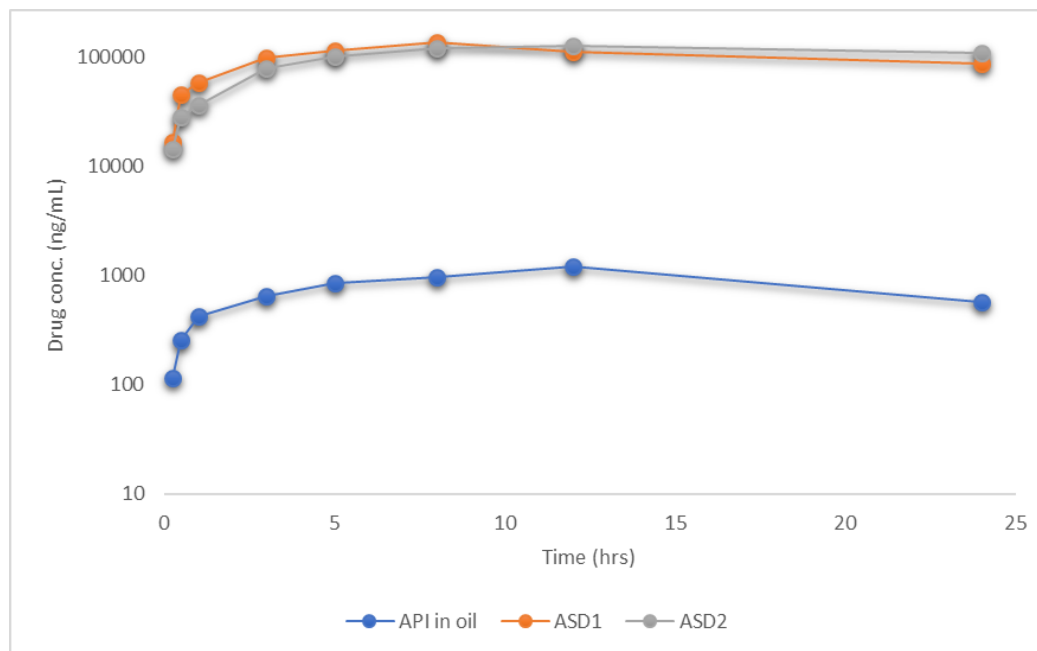
Rapid assessment of solubility-enhancing formulation technologies  
5 g drug substance for full screen



Test compound very poorly soluble in water, moderate permeability

# Success !

- Significantly improved exposure for both spray-dried formations at 500mg/kg
- Bioavailability > 80% for both formulations & dose levels versus < 2% for simple API suspension
- AUC and Cmax generally increased with dose from 50 to 500mg/kg



Outcome consistent with bioavailability being limited by solubility & poor absorption rather than clearance

# Take home messages

- Pre-clinical PK, pharmacology, toxicokinetics, *in vitro* human potency assessments are **all important and should be used** in helping design FTIH dose
- PBPK modelling, PK/PD modelling, target engagement/exposure data should have been used well in advance of FTIH
- Don't forget plasma protein binding in safety margin calculations, if appropriate
- The key point is to understand (unbound) exposure relative to effect, whether wanted effect or unwanted effect
- Dose does not always equate to exposure!
- PBPK modelling, PK/PD modelling will be continuingly be used throughout Phase 1, 2 and 3 to refine the dose prediction
- Don't forget how formulation strategies can help or even rescue a compound

# Thank you



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