EDDC Experimental Drug Development Centre

Great Science for Great Medicines

EDDC Satellite Seminar Series

Preclinical *in vivo* studies for drug discovery & development

4th September 2023

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Outline

1. Introduction: Why and when do we need *in vivo* models in drug discovery & development, how does EDDC manage it?

Dr Hannes Hentze
Assoc. Director,
Translational Sciences

2. Animal models & efficacy capabilities @EDDC: Example of humanised mouse models in oncology

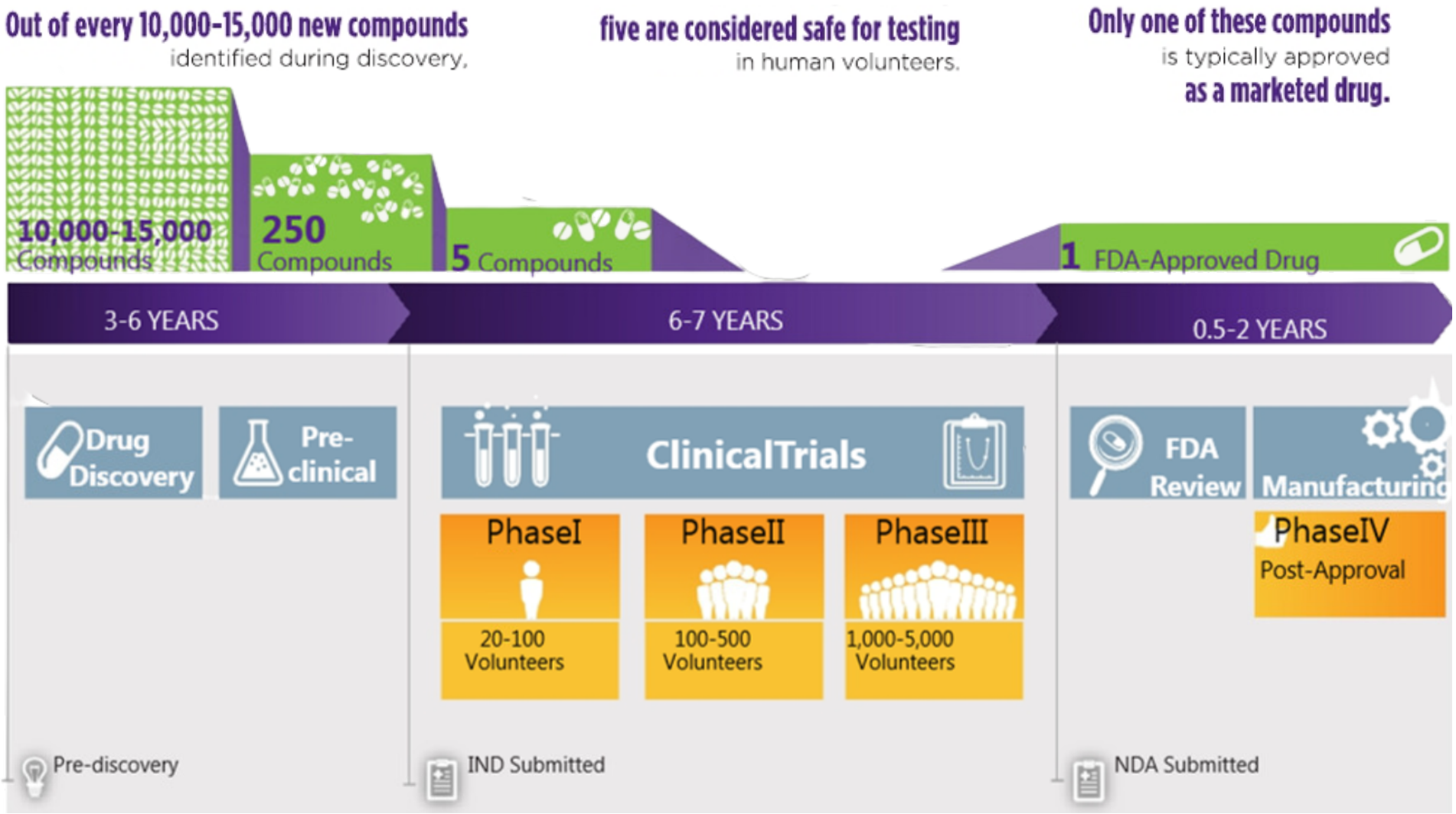
Dr Vikas Madan
Head, *In vivo*
Pharmacology

3. PBPK & DMPK experience & capabilities @EDDC

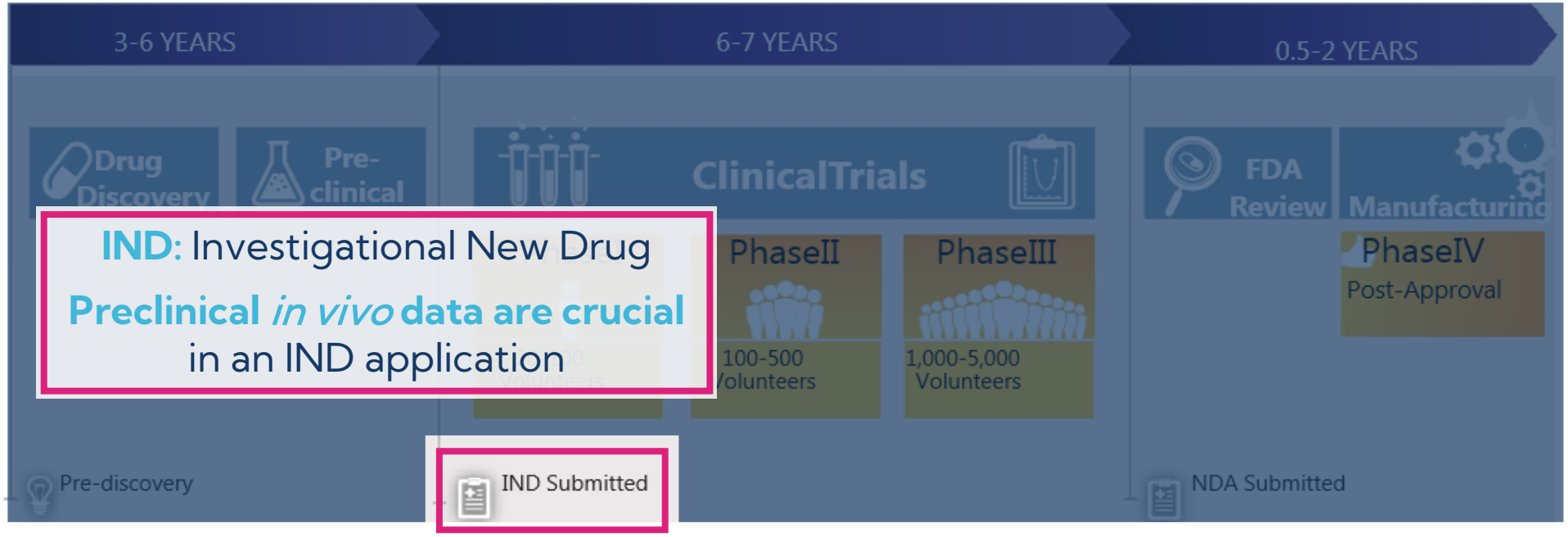
Vishal Pendharkar
Senior Manager,
In vivo Pharmacology

The drug discovery and development process is a long and costly journey

It typically takes 10-15 years and costs over $2 billion on average



Why do we need *in vivo* models in drug discovery & development?



Preclinical animal studies are required for demonstrating:

A. **Efficacy** (Pharmacology) → Animal-based disease models = "Animal Models"

B. **PK** (Pharmacokinetics) → PK animal studies

C. **PD** (Pharmacodynamic) → PD animal studies

D. **Safety** (Toxicology) → GLP tox animal studies

When do we need *in vivo* models in drug discovery & development?

Glossary:

PK	Pharmacokinetics
PD	Pharmacodynamics
MTD	Maximum Tolerated Dose (TI?)
GLP	Good Lab Practice
IND	Investigative New Drug *(application)*

	Drug Discovery	Pre-clinical	IND submission
Pharma / Industry	**PK/PD** **MTD** **Efficacy**	**PK/PD** **MTD** **Efficacy** **PK** **GLP Tox**	• Discovery: A few efficacy studies at appropriate time • Project handed over to development (includes regulatory, quality assurance, clinical input…) • **High quality studies in appropriate order with clinical-grade material, FDA-compliant reports**
Academic Lab	Efficacy? Efficacy?	→ **?**	**Common pitfalls:** • Study design/execution/quality? • Documentation & reports? • Efficacy only and too early, **exposure/MTD unknown** • **Focus on publishing, not FDA-compliant reports** • **Pivotal studies to be repeated…**

When do we need *in vivo* models in drug discovery & development?

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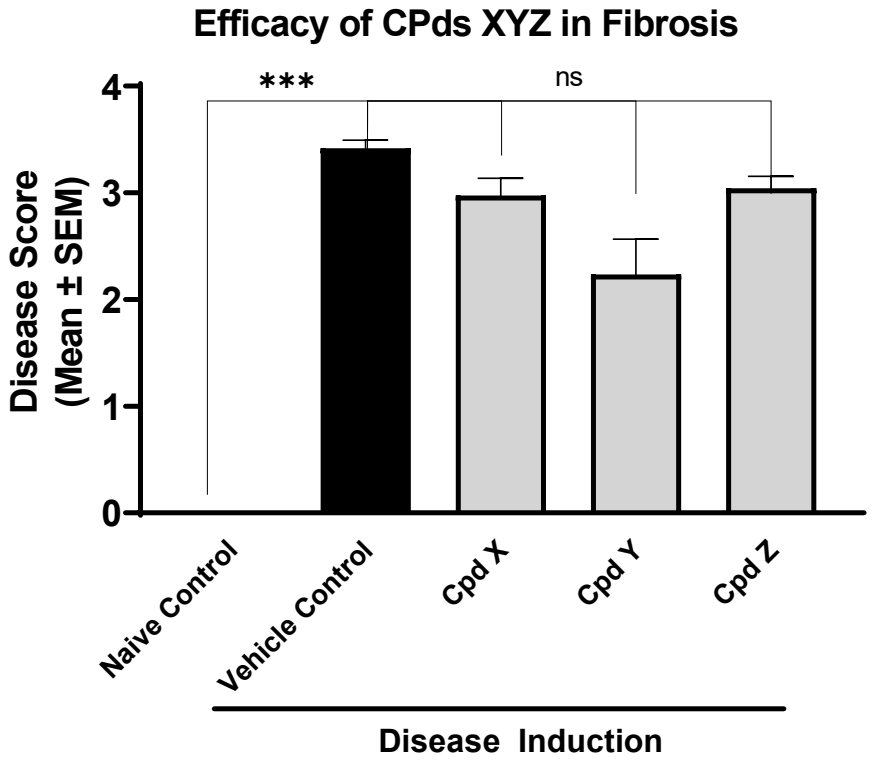
Academic Lab⁺	**PK/PD** **MTD** **Efficacy**	**A comprehensive, high-quality preclinical package enables development & deals!**	• Set of high-quality studies with most promising candidate • **Study design, execution, and reporting at high quality standards (FDA-compliant?)** • **In-house, or at CRO** • Publishing is easier too (**ARRIVE** guideline)!

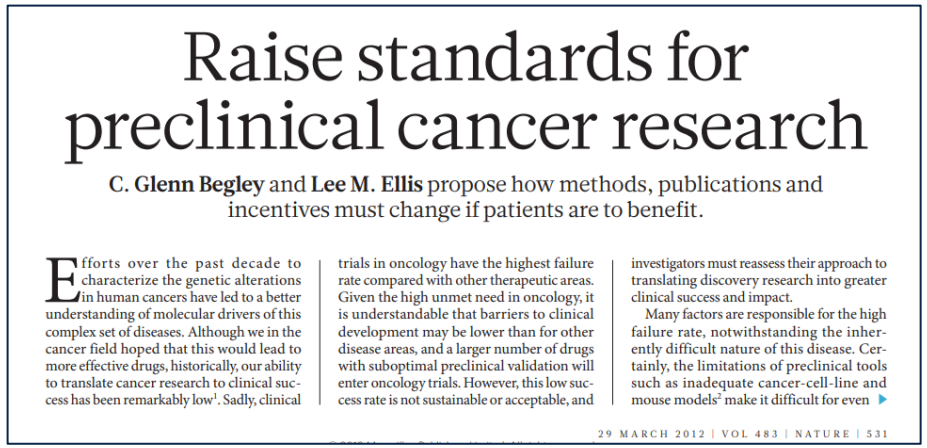
A typical example for Efficacy – what can we conclude from this study result?



We learned nothing – absence of evidence is not evidence of absence!

1. Is this model working, validated? No positive control or SOC (standard-of-care)
2. Is the effect for compound Y real, was the study statistically powered, for which effect size – 10% or 50%? What kind of statistics was done?

"Irreproducibility crisis" – why preclinical research translates so poorly?



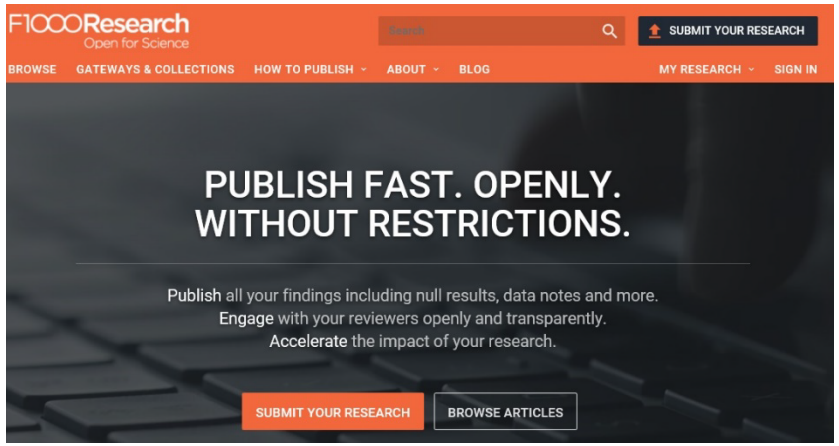
Raise standards for preclinical cancer research

C. Glenn Begley and Lee M. Ellis propose how methods, publications and incentives must change if patients are to benefit.

Efforts over the past decade to characterize the genetic alterations in human cancers have led to a better understanding of molecular drivers of this complex set of diseases. Although we in the cancer field hoped that this would lead to more effective drugs, historically, our ability to translate cancer research to clinical success has been remarkably low[1]. Sadly, clinical trials in oncology have the highest failure rate compared with other therapeutic areas. Given the high unmet need in oncology, it is understandable that barriers to clinical development may be lower than for other disease areas, and a larger number of drugs with suboptimal preclinical validation will enter oncology trials. However, this low success rate is not sustainable or acceptable, and investigators must reassess their approach to translating discovery research into greater clinical success and impact.

Many factors are responsible for the high failure rate, notwithstanding the inherently difficult nature of this disease. Certainly, the limitations of preclinical tools such as inadequate cancer-cell-line and mouse models[2] make it difficult for even

29 MARCH 2012 | VOL 483 | NATURE | 531



Back in 2012 Begley and Ellis shocked the academic community by reporting that scientists at Amgen, a major biotech company, could **not replicate** the findings of **nearly 90% of 53 high-profile oncology publications**.

Key drivers why pre-clinical data do not translate well to human clinical trials:

- Random error & fraud
- Disease mechanisms differ between human and animal model = **i) un-validated animal models**
- Bias & lack of rigour - due to **ii) poor experimental design,** iii) lack of statistics**, iv) incomplete documentation** & reporting

i) un-validated animal models – what is validity and validation (for animal models!)?

FACE VALIDITY = Similarity between clinical disease presentation in humans & signs/symptoms in animal model

TARGET VALIDITY = Target of interest should have the **same/similar role** in disease model as compared to clinical situation

PREDICTIVE VALIDITY = Model can correctly predict the results of a particular intervention when applied in a clinical setting.

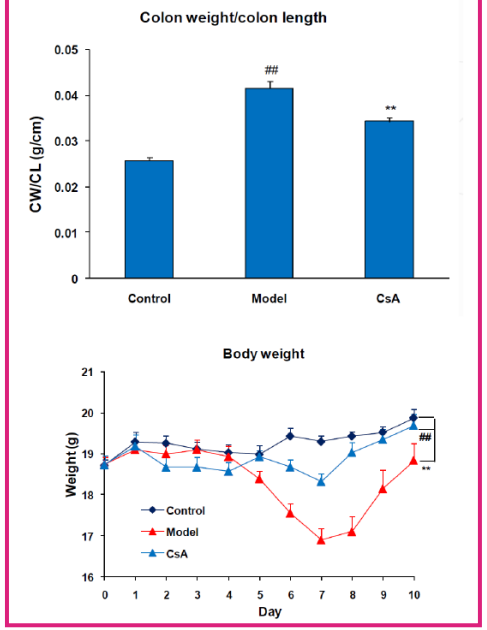
*This is most important and can be tested by **validation studies**…*

Conn PM. Sourcebook of models for biomedical research. Humana Press, 2008, **CHAPTER 24**
Denayer T, New Horizons in Translational Medicine 2 (2014), 5-11

Small molecule drugs approved for Ulcerative Colitis in UC mouse models

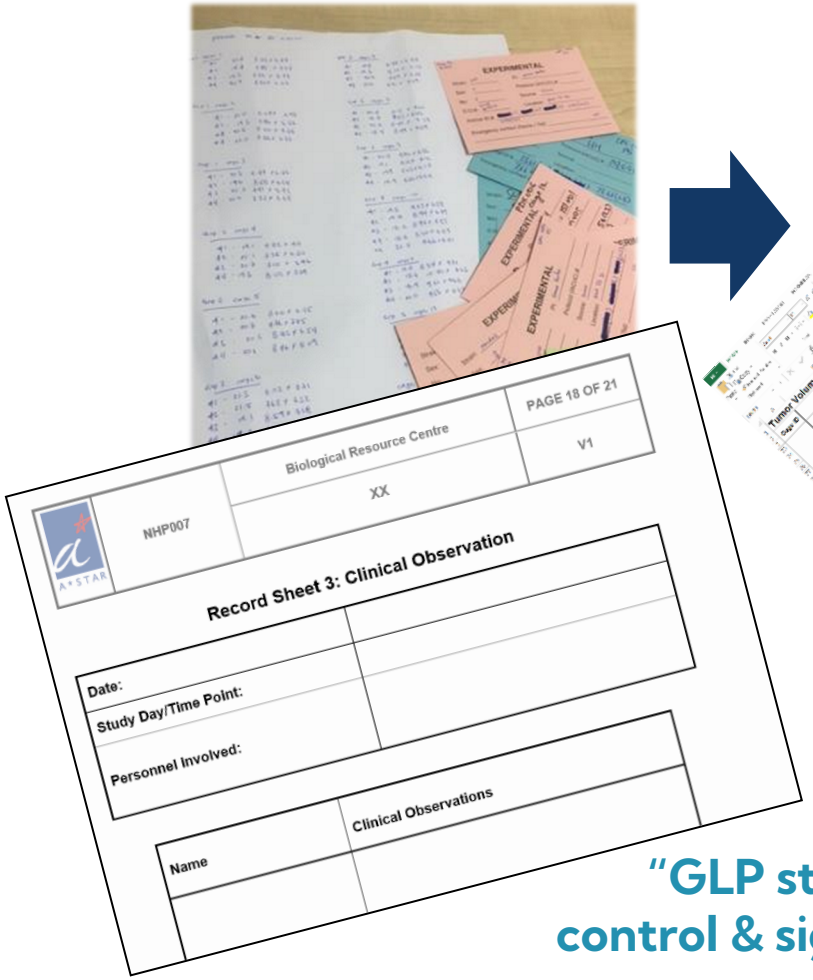
Approved Compound Classes	DSS References/CROs (Acute/Chronic Models)	TNBS References/CROs
Corticosteroids (Prednisolone, Budesonide)	Adgyl (Acute) Creative Biolabs (Acute & Chronic)	Creative Biolabs (TNBS), Inotiv
5-Aminosalicylates (Sulfasalazine/Osalazine/Azulfidine, Mesalamine/Delzicol, etc.)	Creative Biolabs (Chronic Model)	Creative Biolabs (DNBS)
Calcineurin Inhibitors (Cyclosporin A)	Adgyl and Inotiv (Acute), Crown Bioscience (Acute & Chronic)	Crown Bioscience
PDE4 Inhibitors (Roflumilast)	Adgyl (Acute)	Brazilian Journal of Medical and Biological Research (2022) 55: e11877
JNK Inhibitors (Upadacitinib/RINVOQ, tofacitinib/Xeljanz)	Crown (Acute and Chronic)	Crown
Sphingosine-1-phosphate Receptor Inhibitor (Ozanimod/Fingolimod/Zeposia)	Crown (Acute)	Crown

Cyclosporin A in acute DSS

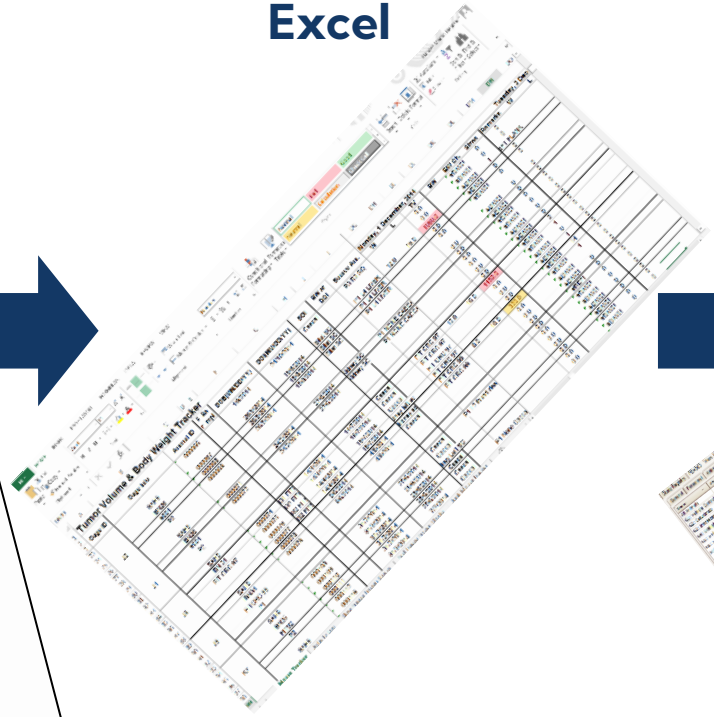


iv) incomplete documentation & reporting – the best method to record raw data?

Paper



Excel



Database

"GLP style":
control & sign

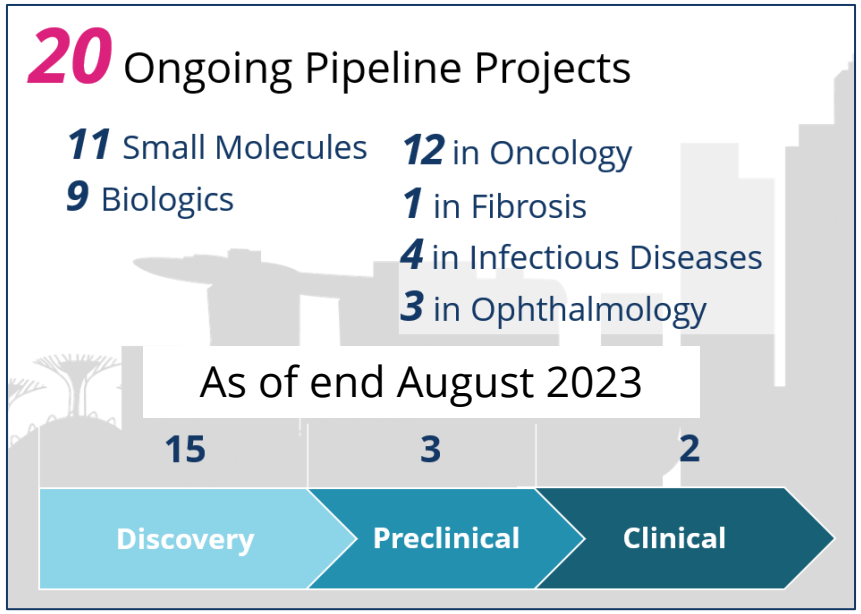
Required expertise & international network to support all projects preclinically work @EDDC

In-house in vivo lab @BRC

- CDX
- Hu-mice
- MTD
- PK/PD
- Ulcerative colitis

Long-term partnerships with academia

- External academic centres with unique *in vivo* disease models



The most 4 important aspects for outsourcing & collaborations:

1) **Quality**
2) **Speed**
3) **Cost**
4) **Location** …

Specialised CROs

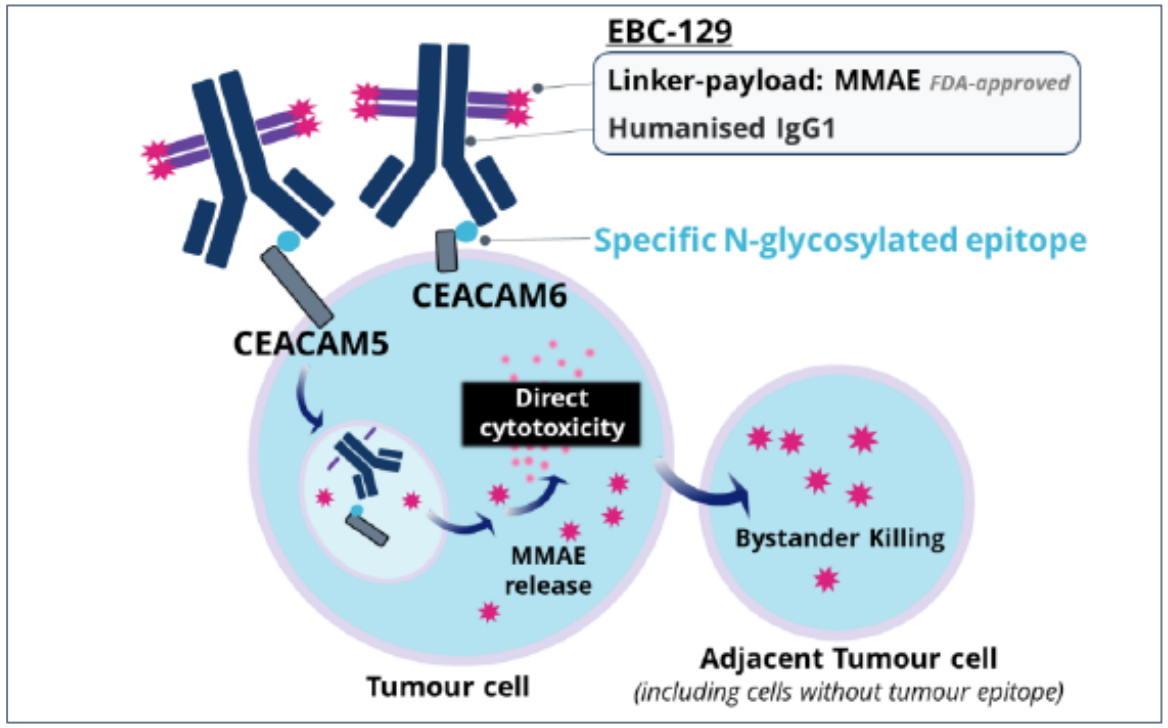
- Hu-mice
- Fibrosis models
- PDX
- Infection models
- ….

GLP Toxicology CROs

- A
- B
- C
- …

First-in-class ADC EBC-129 targeting a glycosylated tumour antigen approved to enter clinical trials

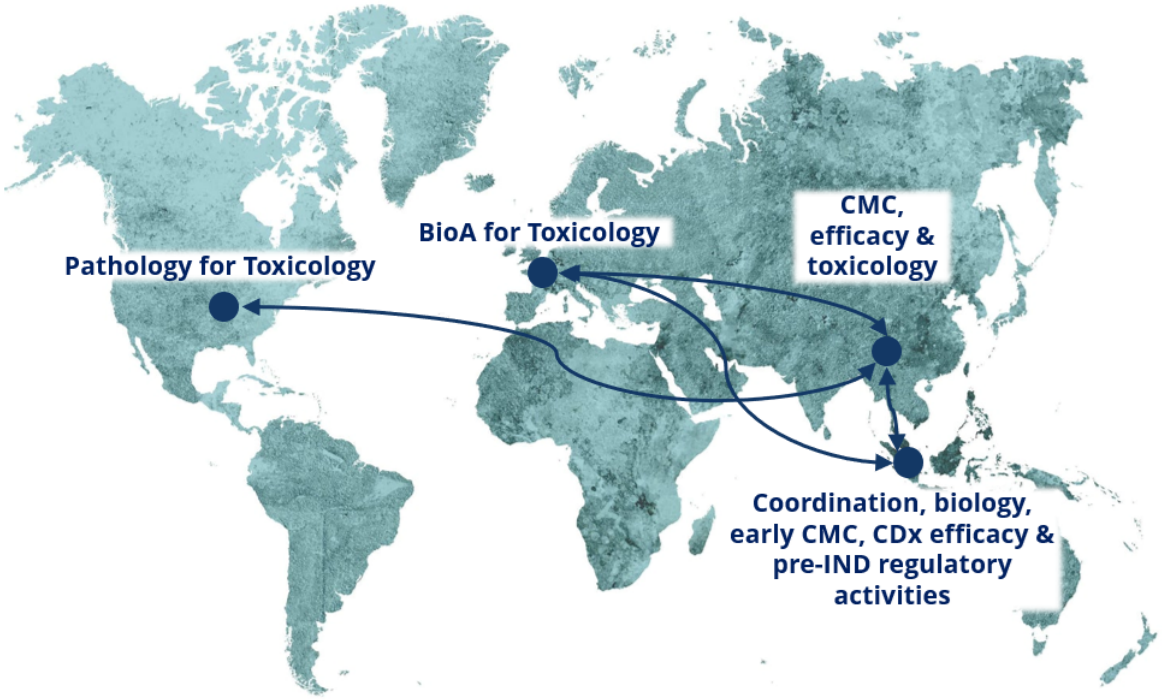
- **ADC** = a combination of an **antibody** directed against a specific tumour (cell surface antigen), a **linker** and a **payload**, (toxin, "warhead", in our case we use MMAE which already validated clinically, allowing fast track development
- MOA demonstrated, confirming **first-in-class**/differentiation from competitors
- DEC 2022: IND application was cleared by US FDA for progress into first in human studies (Singapore & USA)
- ADC & patient selection test developed in **collaborative efforts of A*STAR's BTI and IMCB, EDDC, and NCCS**



https://www.a-star.edu.sg/docs/librariesprovider35/annual-report/eddc-annual-report-2022.pdf

World-wide preclinical activities EBC-129 in 2022 – lessons learned!

- Shipment logistics between Singapore - China – France - USA (↓ speed)
- CITES applications for sample transfer (↓ speed)
- Animal supply for GLP toxicology study (⇊ speed)
- Collaboration between partners in Singapore ecosystem (quality & speed)
- Long-standing good relationship with reputed CRO for BioA (quality)
- In-house CDx (xenograft) and PK studies (quality & speed)



Mouse models in preclinical oncology

Mouse models of cancers

Spontaneous	Carcinogen-induced	Genetically modified	Implanted
• Mouse strain specific • Age-dependent	• Chemical or radiation-induced	• Overexpression of oncogenes • Targeted deletion of tumour suppressor genes	• Implantation of cancer cells at site of origin (orthotopic) or elsewhere (ectopic)

Tumour cell implantation–based mouse models

Implanted

- Implantation of cancer cells at site of origin (orthotopic) or elsewhere (ectopic)

Syngeneic

- Tumour tissue from same genetic background
- Functional immune system of recipient mice
- Cell line-derived or mouse-derived
- Valuable for testing immune checkpoint inhibitors e.g., anti PD-1 and anti-CTLA4 therapy

Xenogeneic

- Tumour cells and host of different species/strain

How can human tumour cells engraft and proliferate in mice?

Immunocompromised mouse strains facilitate xenograft studies

- ❑ Severely immune deficient strains developed
- ❑ Ideal hosts for implanting xenogeneic tumours

Strain	Key feature	Immune cell status
Nude (1962)	Foxn1 mutation	Lack T cells; normal macrophages, NK cells and APCs; normal complement activity
SCID (1983)	Deletion of Prkdc gene	No functional T and B cells
SCID/beige	Crossbreeding beige and SCID mice	No mature T and B cells; impaired macrophage and NK cell function
NOD-SCID	Crossbreeding NOD (1980; diabetic) and SCID mice	Multiple defects in innate (macrophages and dendritic cells) and adaptive (T and B cells) immunity; residual NK cell activity
NSG	Crossbreeding NOD-SCID mice with IL2Ry-deficient mice	Complete loss of NK cells

Fragile Superheroes

Tumour cell implantation–based mouse models

Implanted

- Implantation of cancer cells at site of origin (orthotopic) or elsewhere (ectopic)

Syngeneic

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- Cell line-derived or mouse-derived
- Valuable for testing immune checkpoint inhibitors e.g., anti PD-1 and anti-CTLA4 therapy

Xenogeneic

- Tumour cells and host of different species/strain
- Require immuno-deficient recipients
- Human cell line- (CDX) or patient-derived (PDX)
- Extensively used model

Considerations for an *in vivo* study design (xenograft tumour in immunocompromised mice)

- Objective(s), endpoint and analyses
- Mouse strain, sex, age
- Group size (statistical power required?)
- Cell line(s)/PDX selection
- Negative and positive controls

Tumour inoculation
- Site of inoculation
- Number of cells

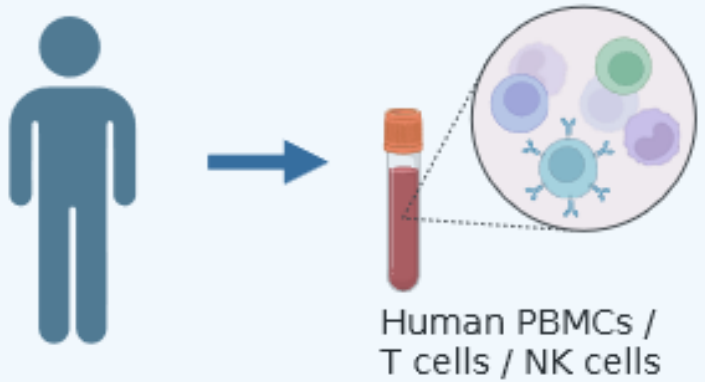
Therapeutic candidate
- Formulation
- Route of administration
- Dose level
- Dosing regimen

Xenograft tumour models using immunocompromised mice are widely used in preclinical research

...but lack cancer cell-immune system interactions

Development of humanised mouse models (human immune system)

PBMC/PBL model
(transplantation of human leukocytes)



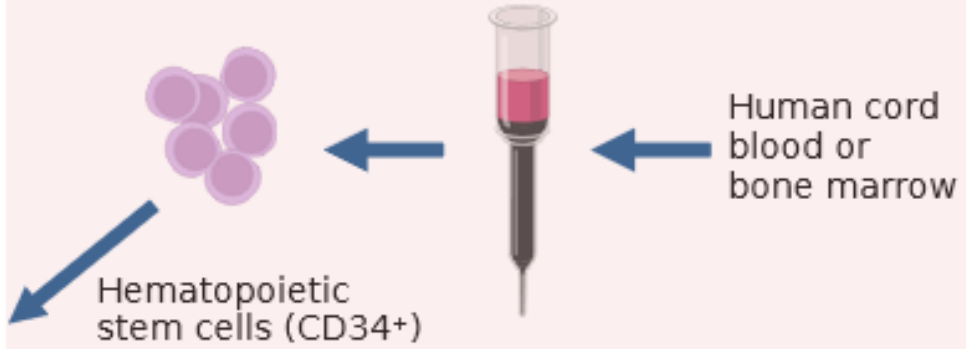
- ✓ Quicker to establish (~2 weeks)
- ✓ Robust T cell engraftment
- ✓ Donor recall possible

- ○ Poor engraftment of other lineages
- ○ Rapid GvHD (short-term studies)

- ○ **Donor variability**

Humanized mouse

HSC model
(transplantation of human hematopoietic stem cells)



- ○ Longer to establish (12-16 weeks)
- ○ Human T cell restricted to murine MHC
- ○ Donor recall not possible

- ✓ Multilineage reconstitution
- ✓ Suitable for long-term studies

- ○ **Donor variability**

GvHD: Graft *versus* Host Disease; HIS: Human Immune System; HSC: hematopoietic stem cell; NK: Natural Killer; PBL: Peripheral Blood Lymphocyte; PBMC: Peripheral Blood Mononuclear Cell

Considerations for an *in vivo* study design (xenograft tumour in immunocompromised mice)

- Objective(s), endpoint and analyses
- Mouse strain, sex, age
- Group size (statistical power required?)
- Cell line(s)/PDX selection
- Negative and positive controls

Tumour inoculation
- Site of inoculation
- Number of cells

Therapeutic candidate
- Formulation
- Route of administration
- Dose level
- Dosing regimen

Considerations for an *in vivo* study design (xenograft tumour in humanised mice)

Objective(s), endpoint and analyses

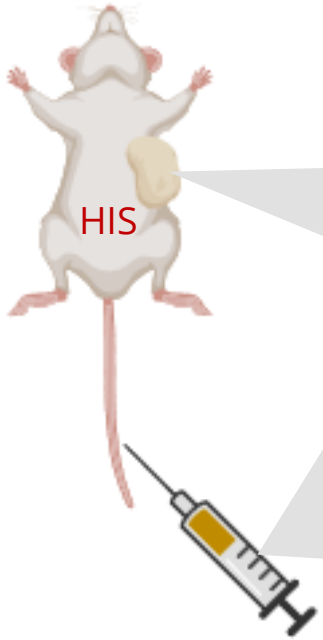
Mouse strain, sex, age

Group size (statistical power required?)

Cell line(s)/PDX selection

Negative and positive controls

Humanization method:
- Route of human PBMC/PBL transplantation
- Number of human PBMC/PBL
- Human cell purification/QC
- Irradiation of mice

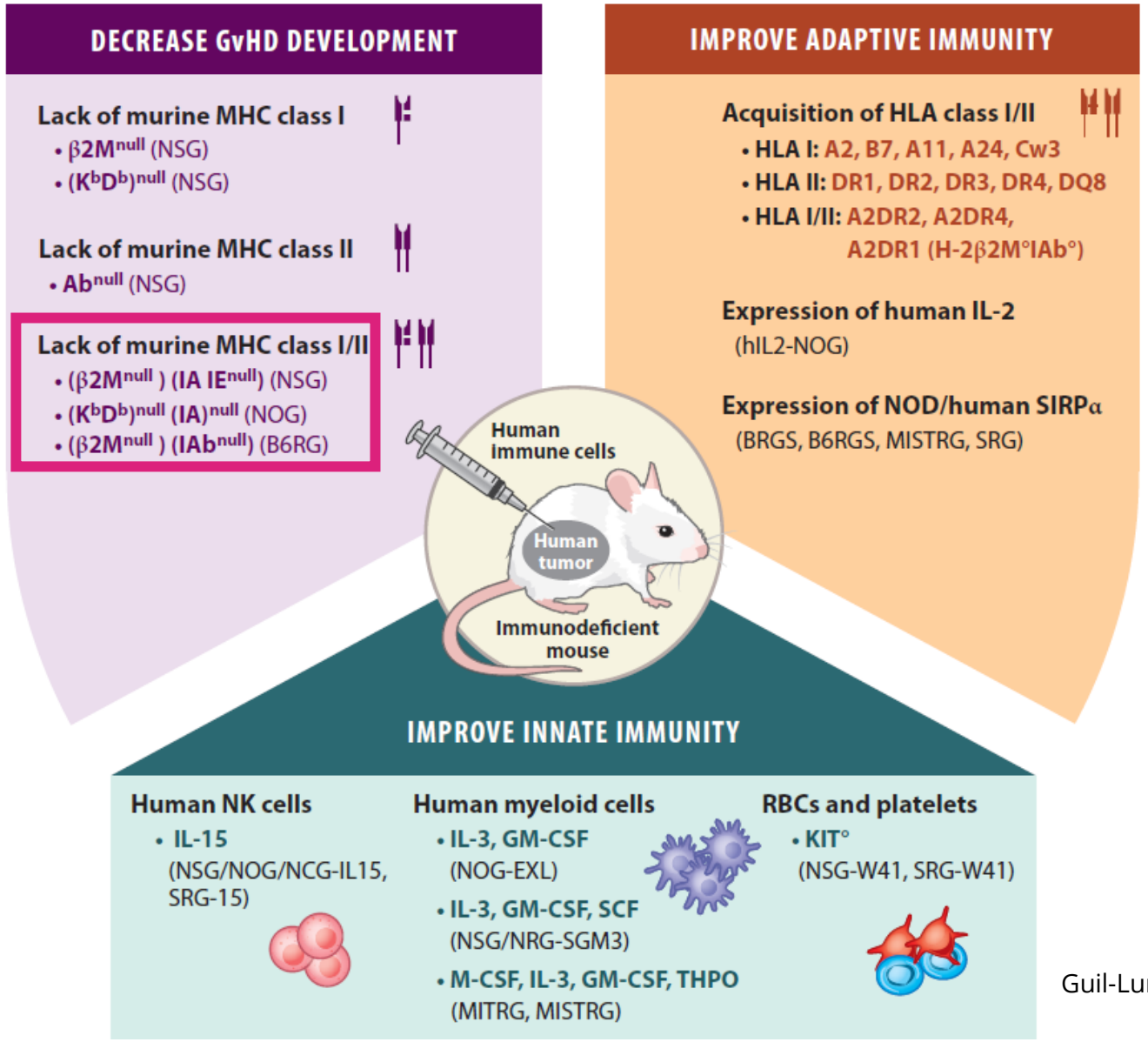


Tumour inoculation
- Site of inoculation
- Number of cells
- Timing of inoculation wrt humanisation

Therapeutic candidate
- Formulation
- Route of administration
- Dose level
- Dosing regimen
- Treatment initiation wrt humanisation
- Exposure in “humanised” setting

PBMC or HSC donor variability

Approaches to overcome current limitations of humanised models



Guil-Luna *et al*, Ann Rev Can Biol, 2021

Dose selection

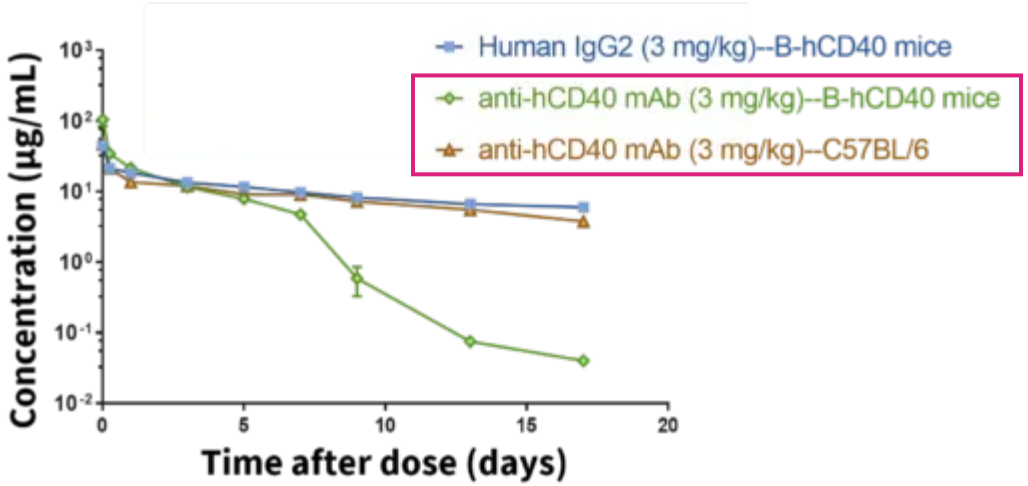
Alle Dinge sind Gift, und nichts ist ohne Gift; allein die Dosis macht, dass ein Ding kein Gift ist.

All things are poison, and nothing is without poison; the dosage alone makes it so a thing is not a poison. — Paracelsus, 1538

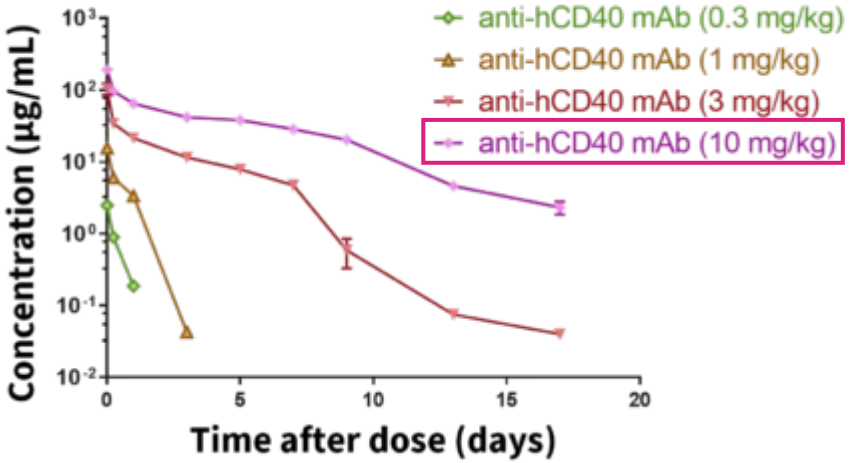
"*The dose makes the poison/drug*"

Better: "*The exposure makes the poison/drug*"

Target Mediated Drug Disposition (TMDD) in **Tg mice Vs WT mice**

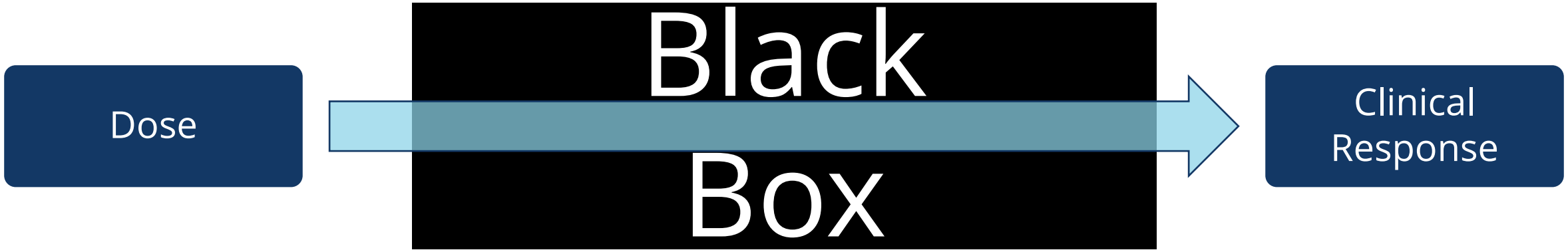


Dosing 10 mg/kg of antibody saturates CD40 receptors and overcomes TMDD. **Best dose** to test efficacy in a mouse model



Pharmacokinetic profile and exposure determines the efficacious dose

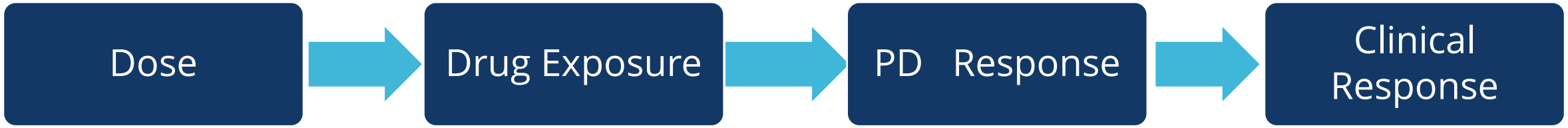
Pharmacokinetic–pharmacodynamic (PK–PD) relationship



In traditional drug discovery, clinical response corresponding to a dose is measured.

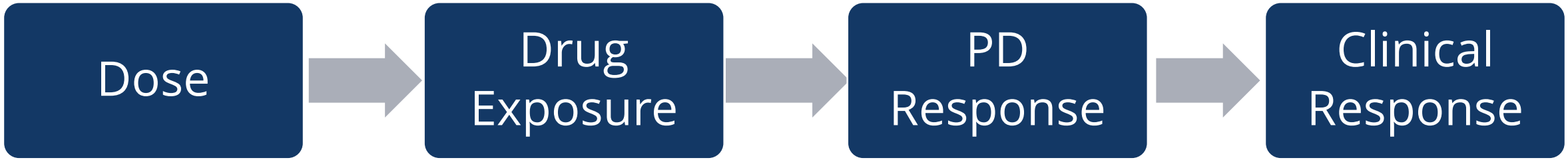
But the clinical response is not always objective and warrants mechanistic understanding.

In recent years, drug discovery projects increasingly seeks to understand how the dose and the resulting clinical response are related to and affect each other.



Oncogene **35**, 2197–2207 (2016)

Pharmacokinetic–pharmacodynamic (PK–PD) relationship



Pharmacokinetics

- Exposure at the site of action
- Systemic circulation
- Route
- Major processes:
  - Absorption
  - Distribution
  - Metabolism
  - Excretion
  - Translation from preclinical to clinical

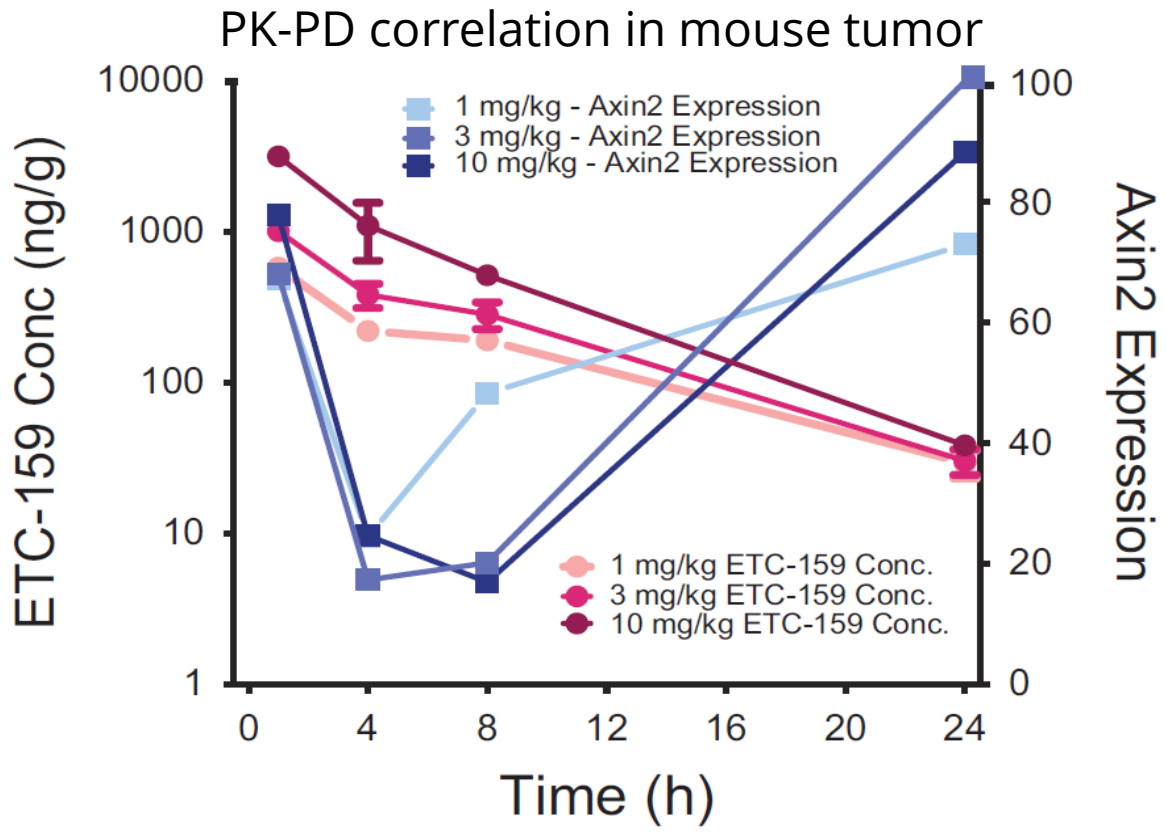
Pharmacodynamics

- Measurable/ quantifiable
- Time course
- Invasive/non-invasive
- Target occupancy
- Proximal to target
- Imaging
- Binding kinetics: equally or sometimes more important than binding affinity
- Translation from preclinical to clinical

Oncogene **35**, 2197–2207 (2016)

Pharmacokinetic–pharmacodynamic (PK–PD) relationship

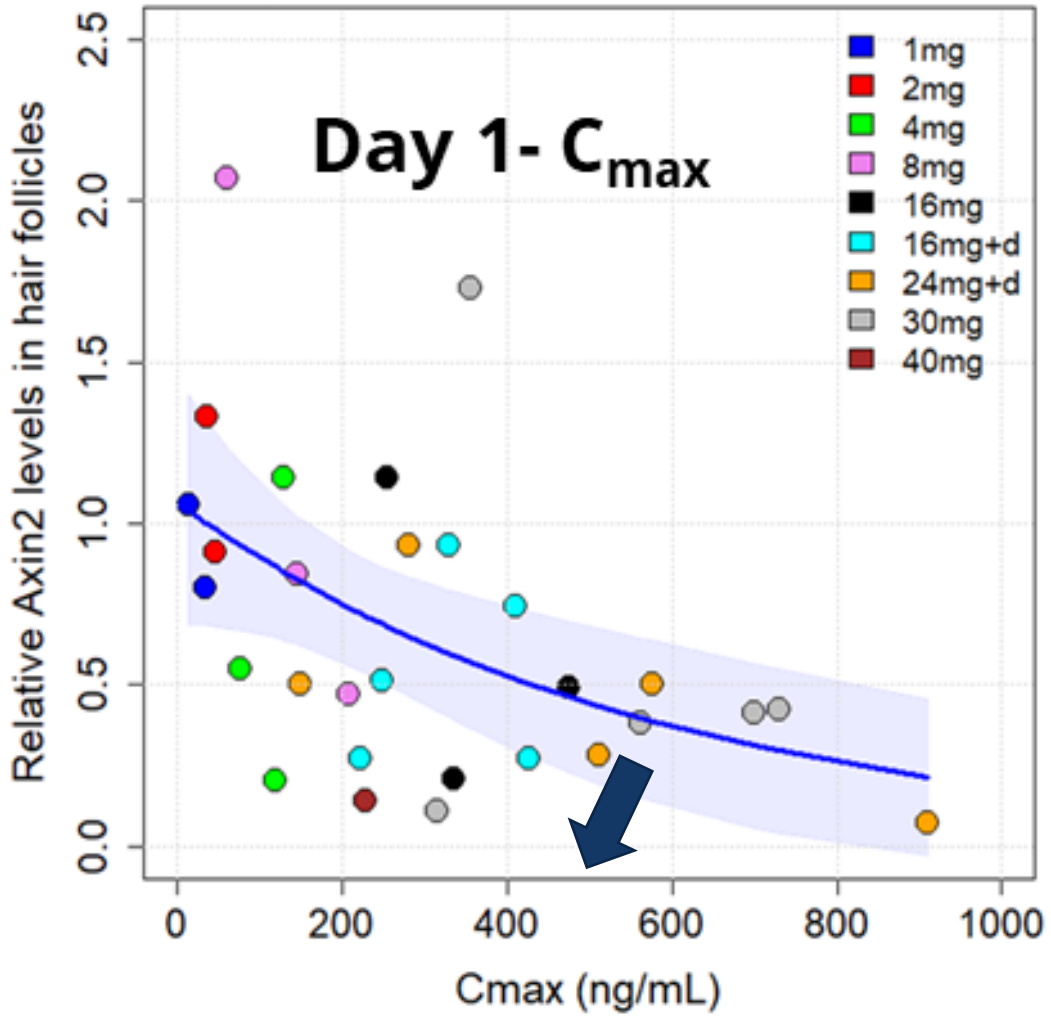
Dose → Drug Exposure → PD Response → Clinical Response

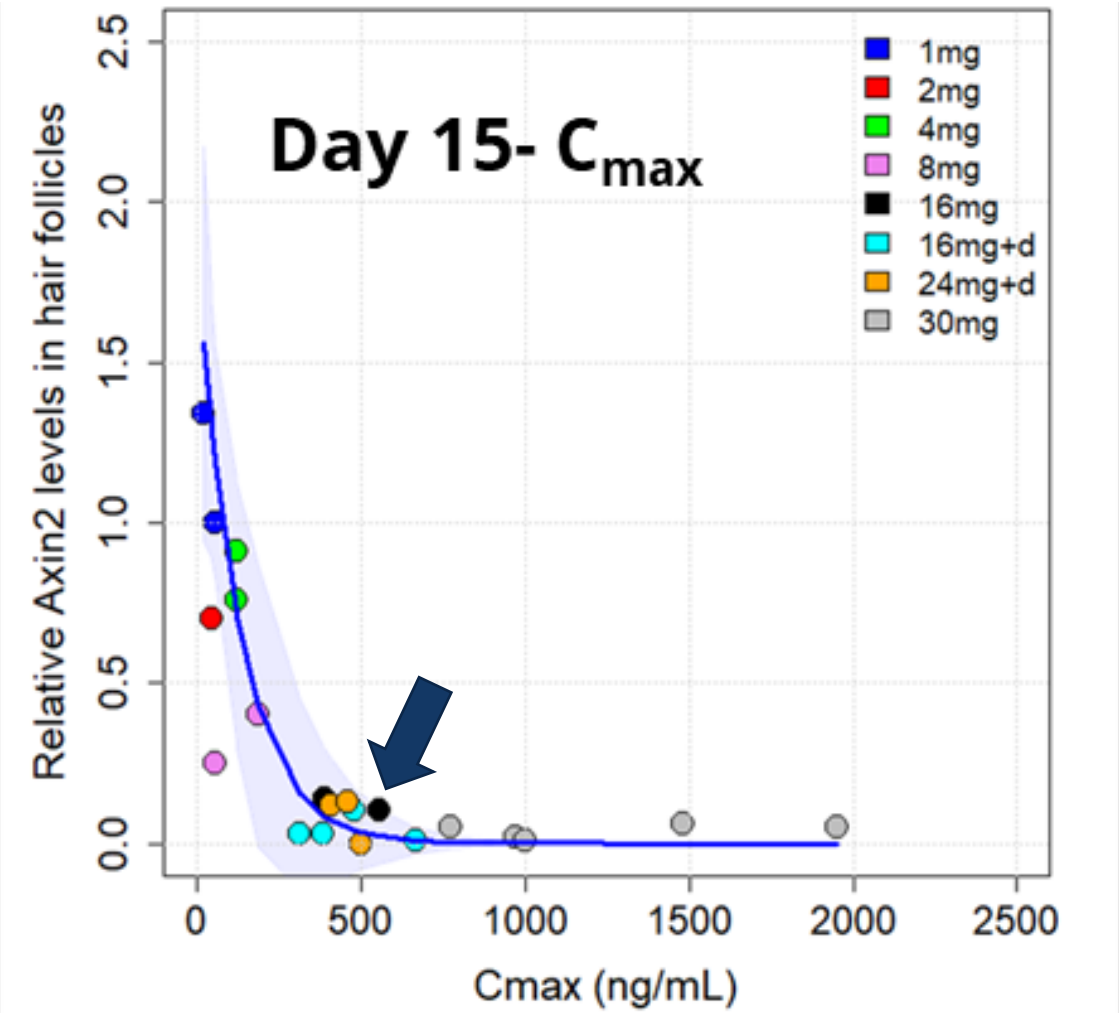


Oncogene **35**, 2197–2207 (2016)

Pre-clinical to Clinical PK-PD translation

Phase 1a PK-PD data: Changes in the hair follicle Axin2 levels as a function of C_{max} values of ETC-159





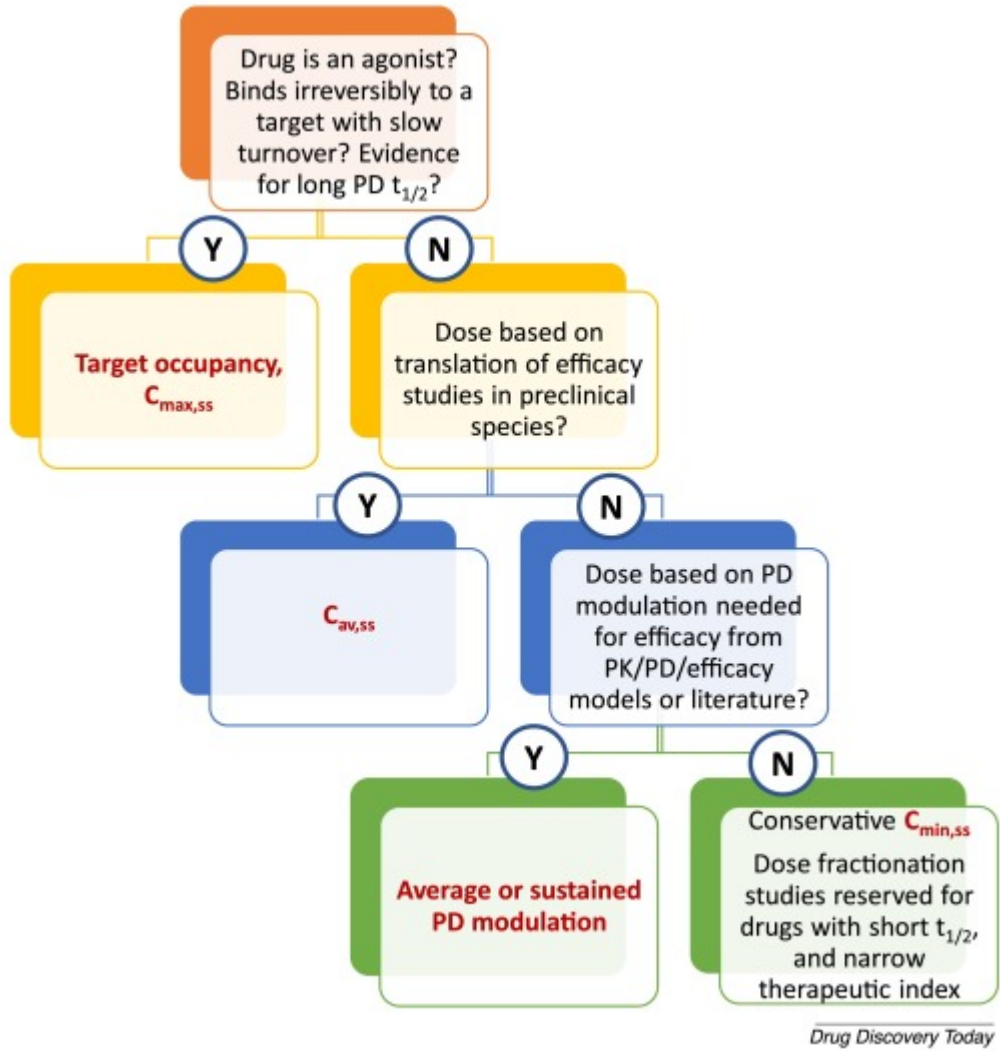
ISSX/MDO 2022 Annual meeting Poster P44

Pharmacokinetic–pharmacodynamic (PK–PD) driver

When a drug is an agonist or binds irreversibly to a **slow-turnover target**: **C_{max} or target occupancy** serves as a good PK metric for efficacy

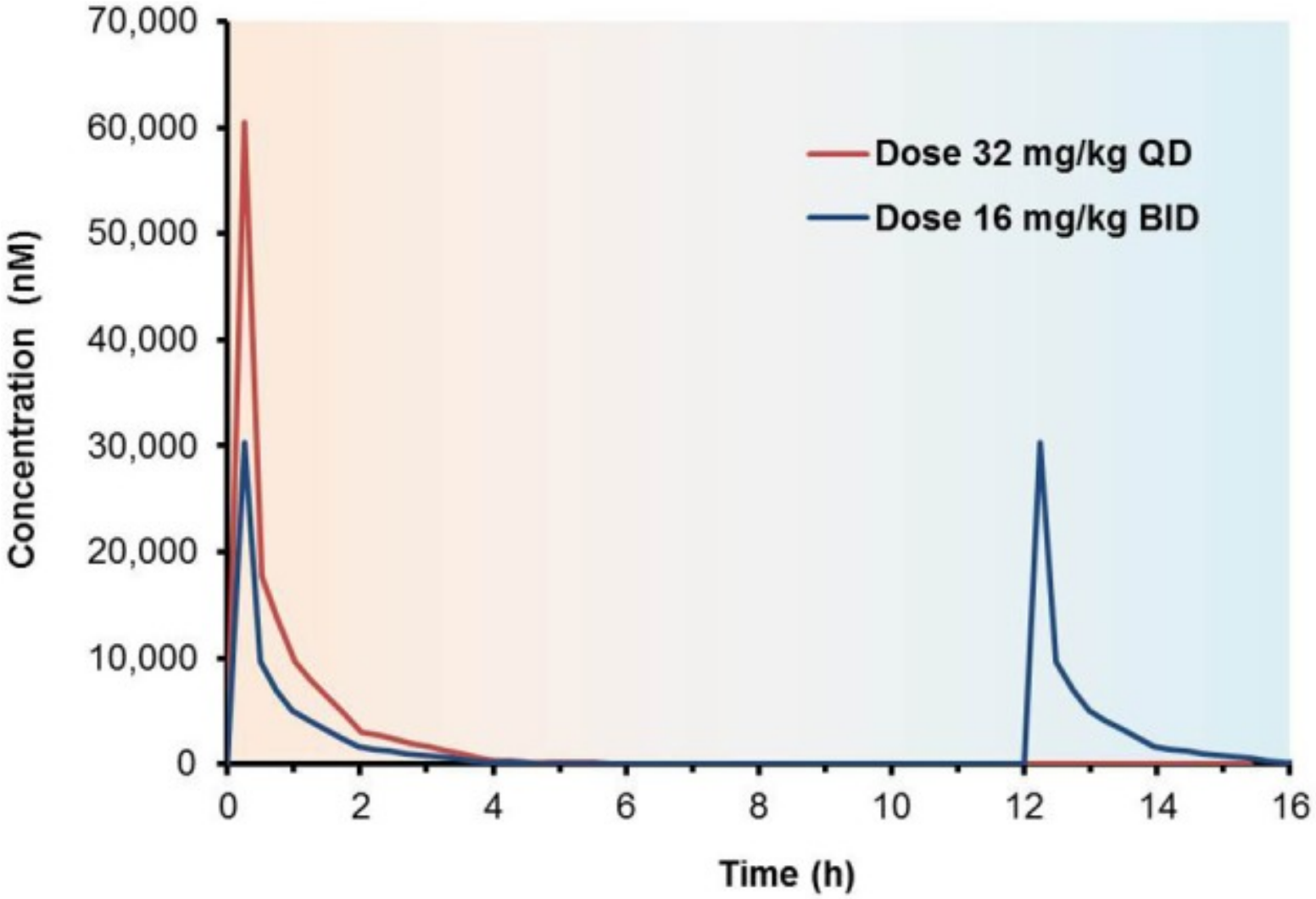
The **more distal the biomarker** is to the target occupation: greater likelihood for **C_{avg}** to be the PK metric for efficacy

$C_{min,}$ is a conservative PK metric. Especially for fast turnover targets. **Dose fractionation studies** are important to compounds with a short half-life or narrow therapeutic index



Drug Discovery Today Volume 25, Number 5 May 2020. P909

Dose fractionation for compounds with short half life or narrow therapeutic index



32 mg/kg QD is **more** efficacious than 16 mg/kg BID: **C_{max} –PK driver**
Ex. Ciprofloxacin, All fluoro-quinolones

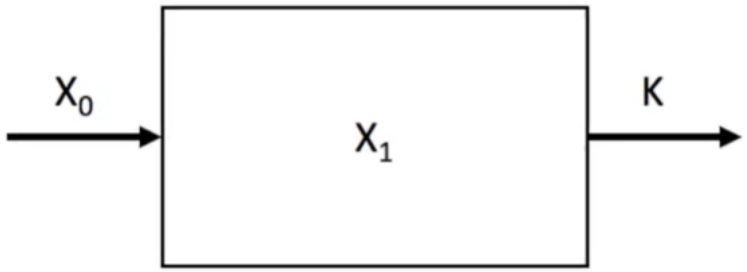
32 mg/kg QD is **equally** efficacious than 16 mg/kg BID: **AUC –PK driver**
Ex. Macrolides- Azithromycin and Tetracyclines
Toxicity can be reduced by dividing the dose (chemotherapy)

Why do preclinical PK modelling?

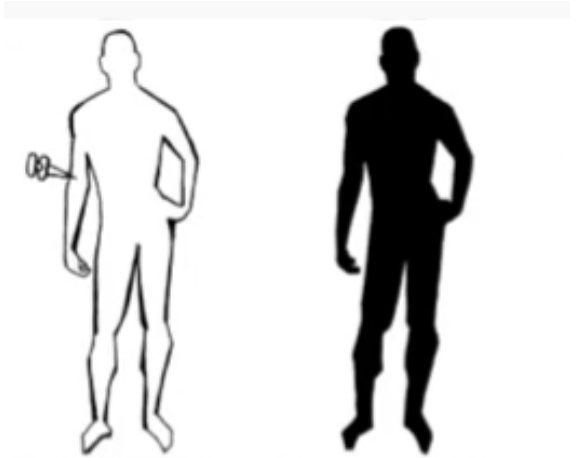
- Regulators and investors need confidence in the outcome of first-in-human (FIH) and phase 2 trial-
- FIH: Right **starting Dose and exposure**
- Ph2: Right **patient selection**
- Ph2RD: **Right dose and efficacious Exposure**
- Major **COST SAVING**
- Modeling has diverse applications from preclinical to post-market
- In translational sciences we can predict exposure across species - Mouse, rat, dog, monkey > human
- Prediction is based on 'assumptions' and these assumptions need to be validated and refined periodically with new preclinical or clinical data.

Compartment models of describing PK profile

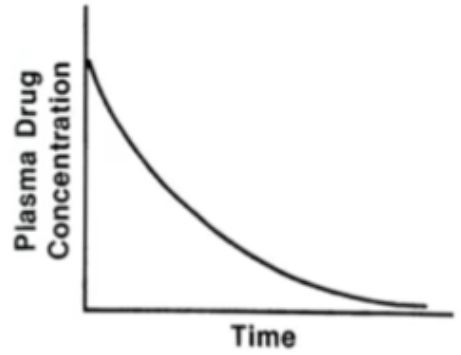
One compartment model



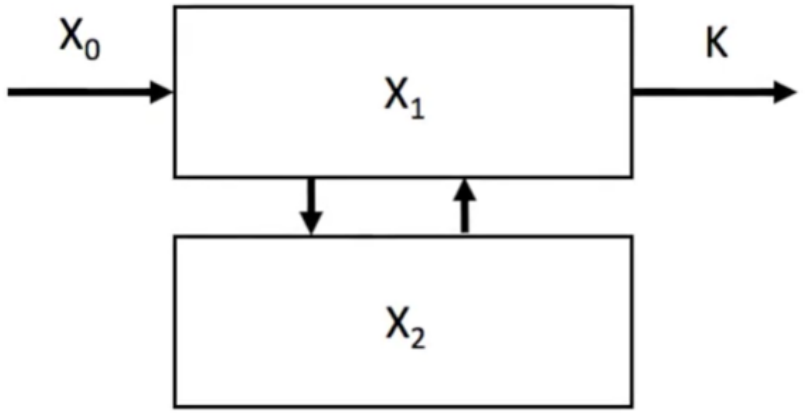
X_0 = Dose of drug X_1 = Amount of drug in the body K = Elimination rate constant



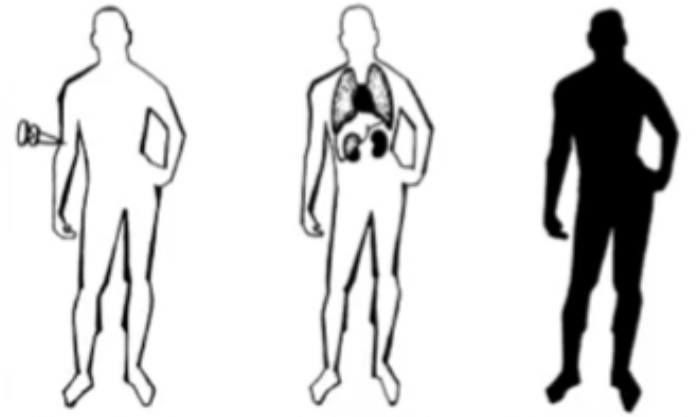
One-compartment model before administration One-compartment model immediately after administration



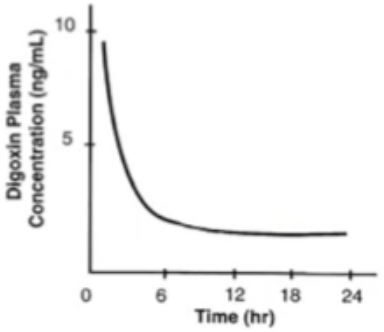
Two compartment model



X_0 = Dose of drug X_1 = Amount of drug in the central compartment X_2 = Amount of drug in the peripheral compartment
K_{12} = Rate constant of drug from central compartment to peripheral compartment
K_{21} = Rate constant from peripheral compartment to central compartment
K = Elimination rate constant from central compartment to outside the body



Two-compartment model before administration Two-compartment model immediately after administration Two-compartment model after distributive equilibrium



Physiology Based PK (PBPK) models represent of patient population

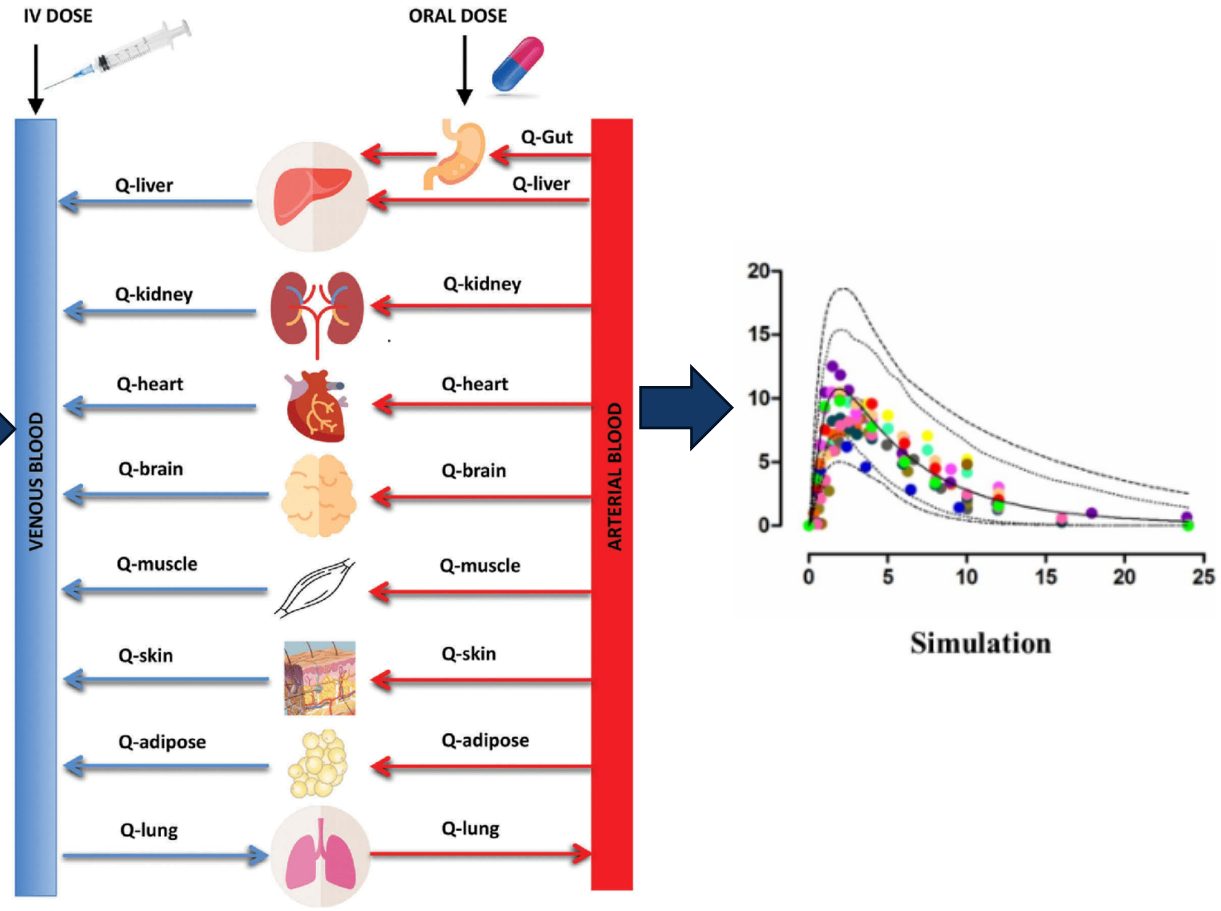
Compartment modeling **not** representative of physiology

Physiology based PK (PBPK) models

Input parameters

In vitro / In silico

- Physicochemical
- Solubility
- Permeability
- Binding
- Metabolism
- Transporters



Collaboration between Pharmacology, Mathematics, Statistics and Computation

EDDC Experimental Drug Development Centre

Thank You

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