

sanofi



ADME,
our challenges in 2023

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DMPK, Vitry-sur-Seine



Alternatives To Animal Experimentation
07.06.2023

1 Introduction

Department and missions

2 Biologics Pharmacokinetics (PK)

Our new goal

3 Tissue penetration

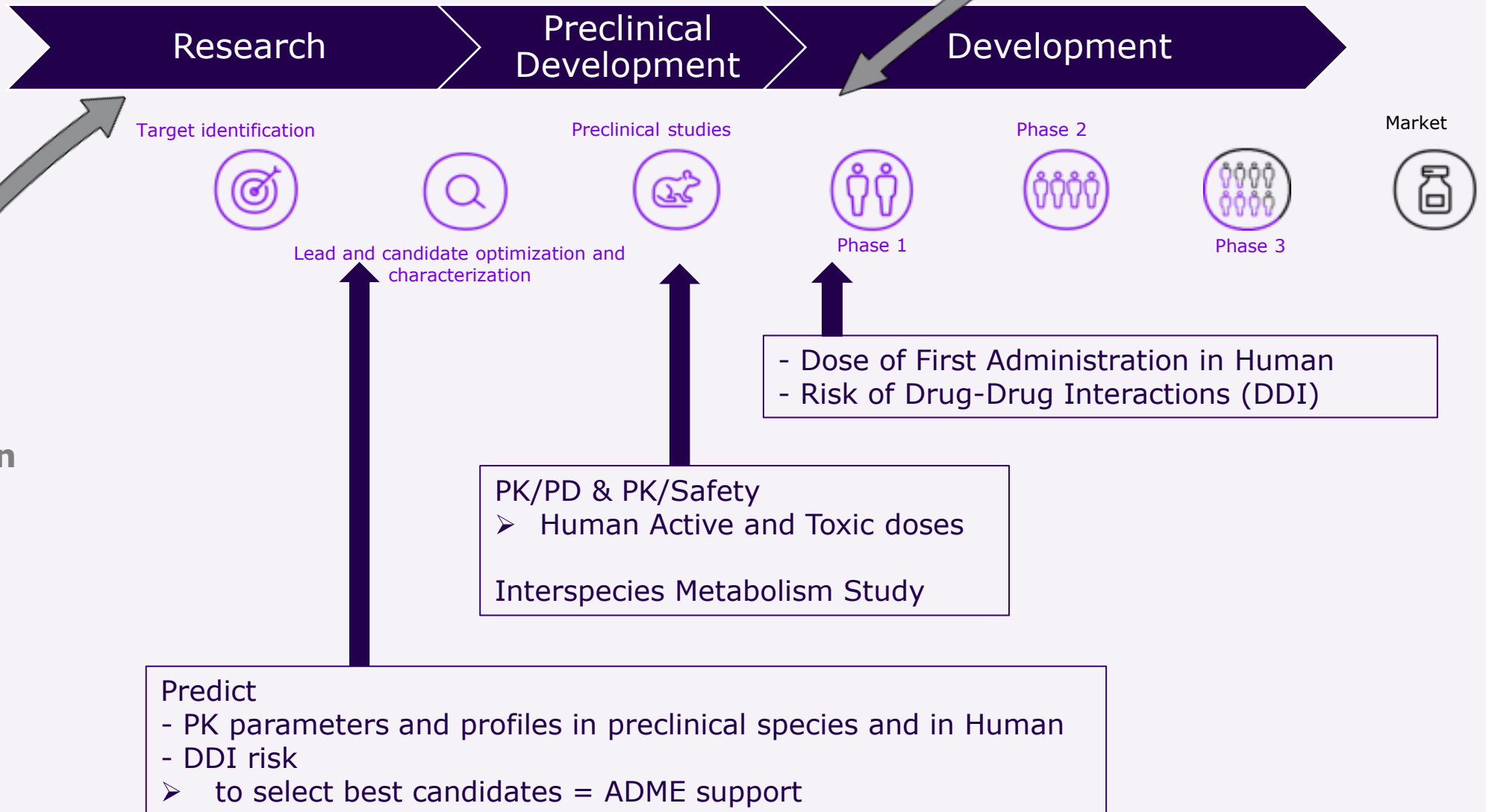
Brain, Tumor



Drug Metabolism and Pharmacokinetics

To First In Human

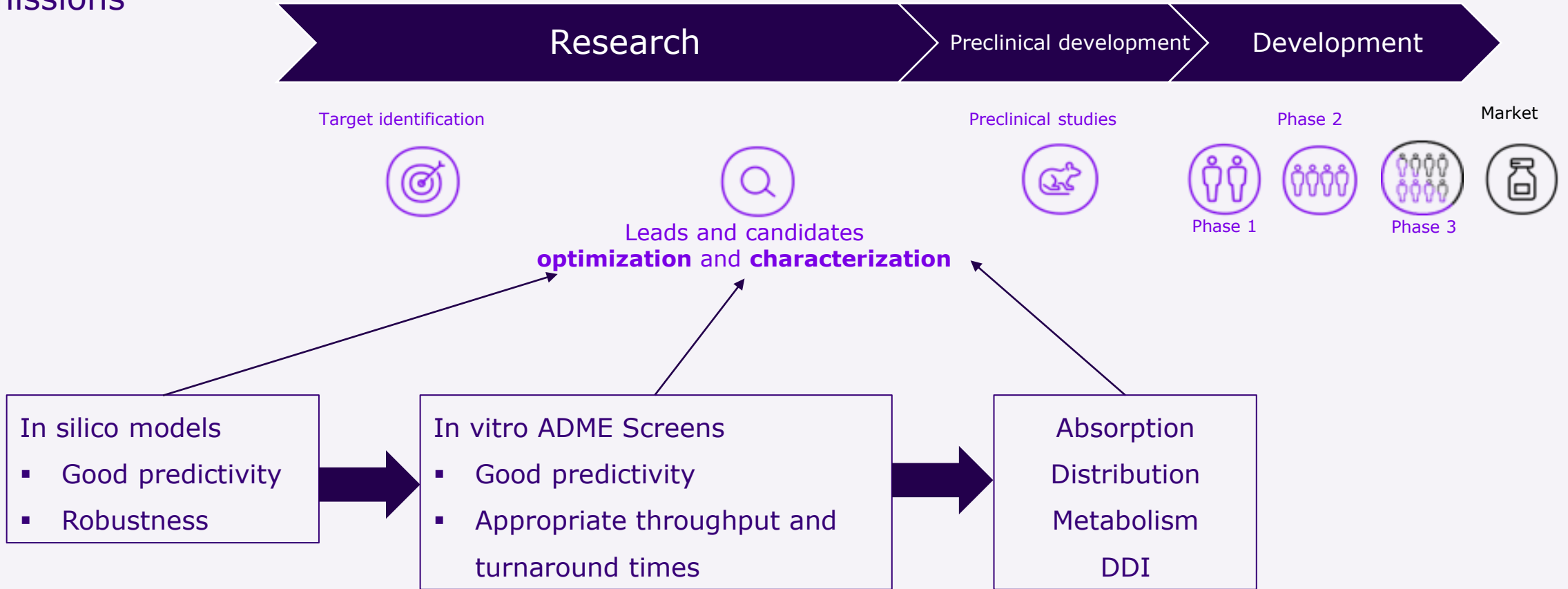
Our missions



From Chemical Optimization

Absorption Distribution Metabolism & Elimination (ADME)

Our missions

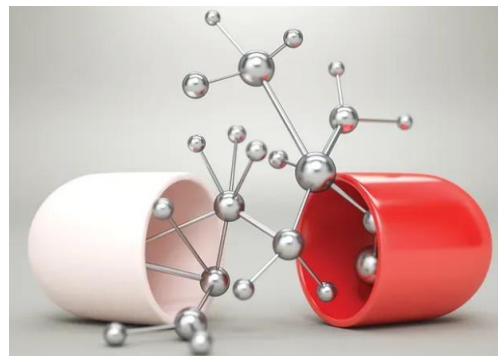


From Small Molecules to Biologics

One of our greater challenges

Small Molecules

Historically established criteria



- Phys chem properties
 - pKa
 - Solubility
 - Lipinski's rule of 5
- ADME properties
 - Metabolic Stability
 - Permeability
 - Protein binding

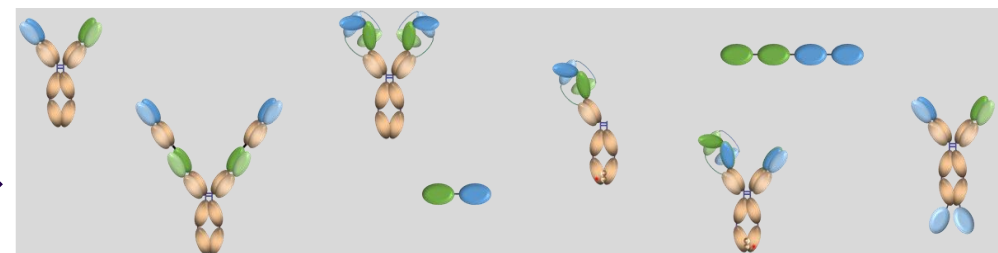
Evolving Portfolio's



BioTherapeutics
Modalities

Biotherapeutics

Emerging knowledge



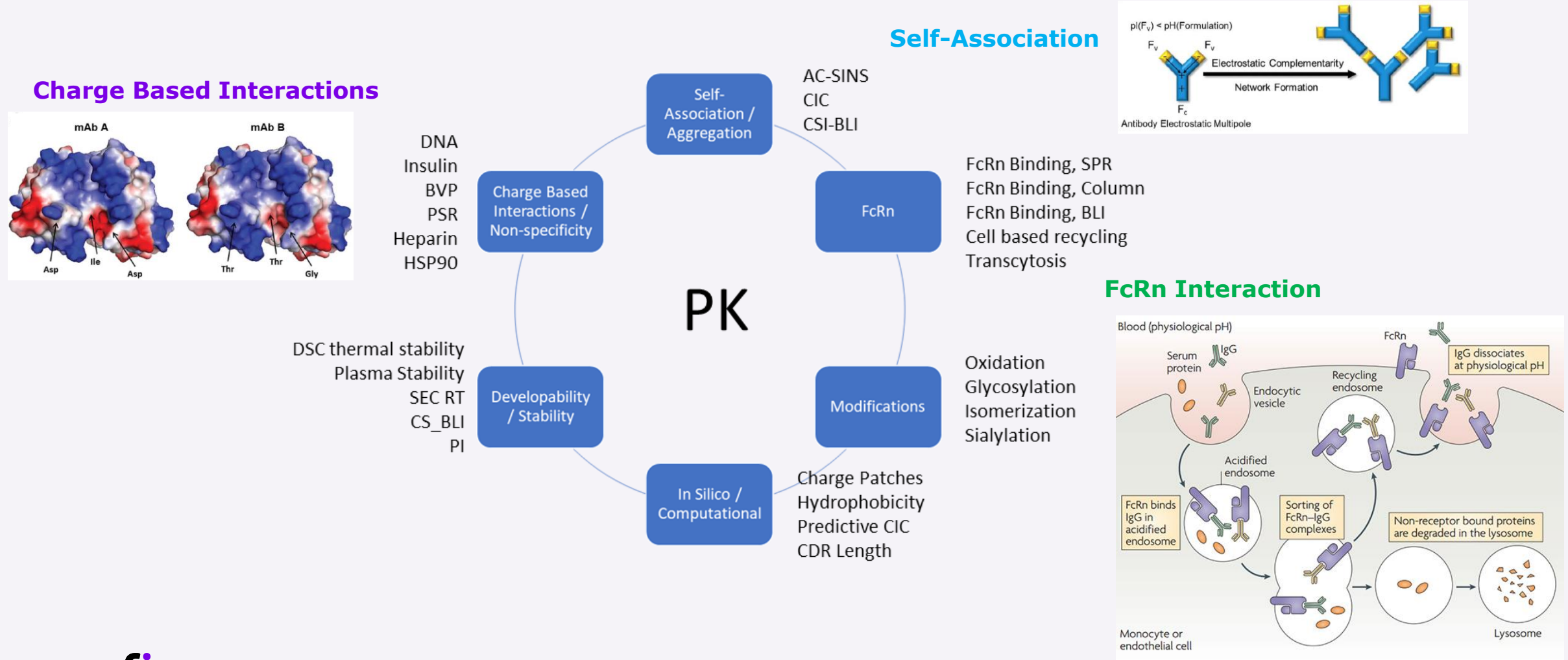
- Phys Chem attributes
 - pI, Hydrophilicity,...
- Nonspecific interaction
 - Charge based interactions
 - Self-association / Aggregation
- Specific interactions
 - Fc:FcRn

Early identification of the right Phys-Chem & ADME properties

➤ to predict optimal PK and Drugability

Physicochemical Properties & Biological Mechanisms Influencing PK

3 Categories identified in the literature with the strongest correlative potential to PK for biologics

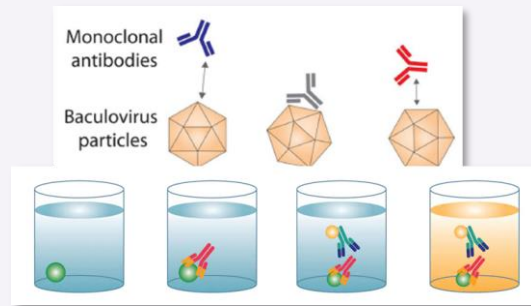


In Vitro Assay Overviews

4 in vitro assays developed to evaluate the physicochemical properties of biotherapeutics that have demonstrated to have an impact on pharmacokinetic properties

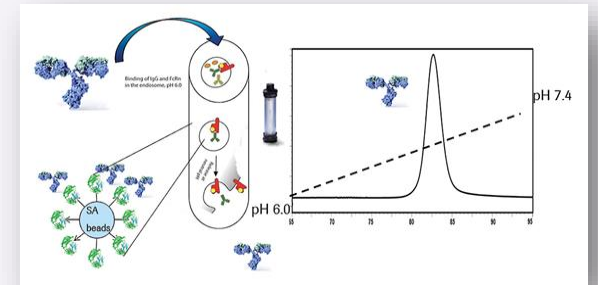
BVP ELISA (non-specific binding / charge-based interaction)

- Undesirable binding interactions with a complex protein/lipid/virus particle mixture



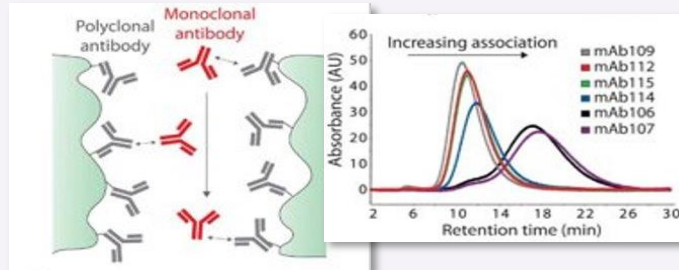
FcRn Column (FcRn interaction)

- Affinity chromatography column with immobilized human FcRn



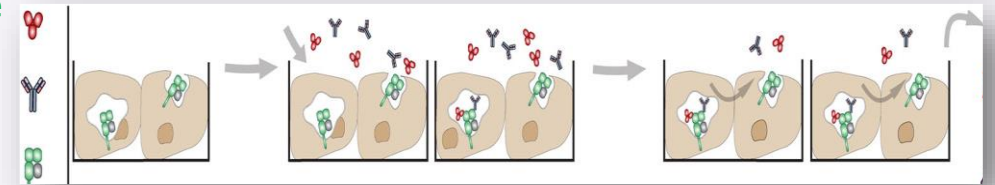
CIC Column (Self-association / interaction)

- Propensity for self- and cross-interactions



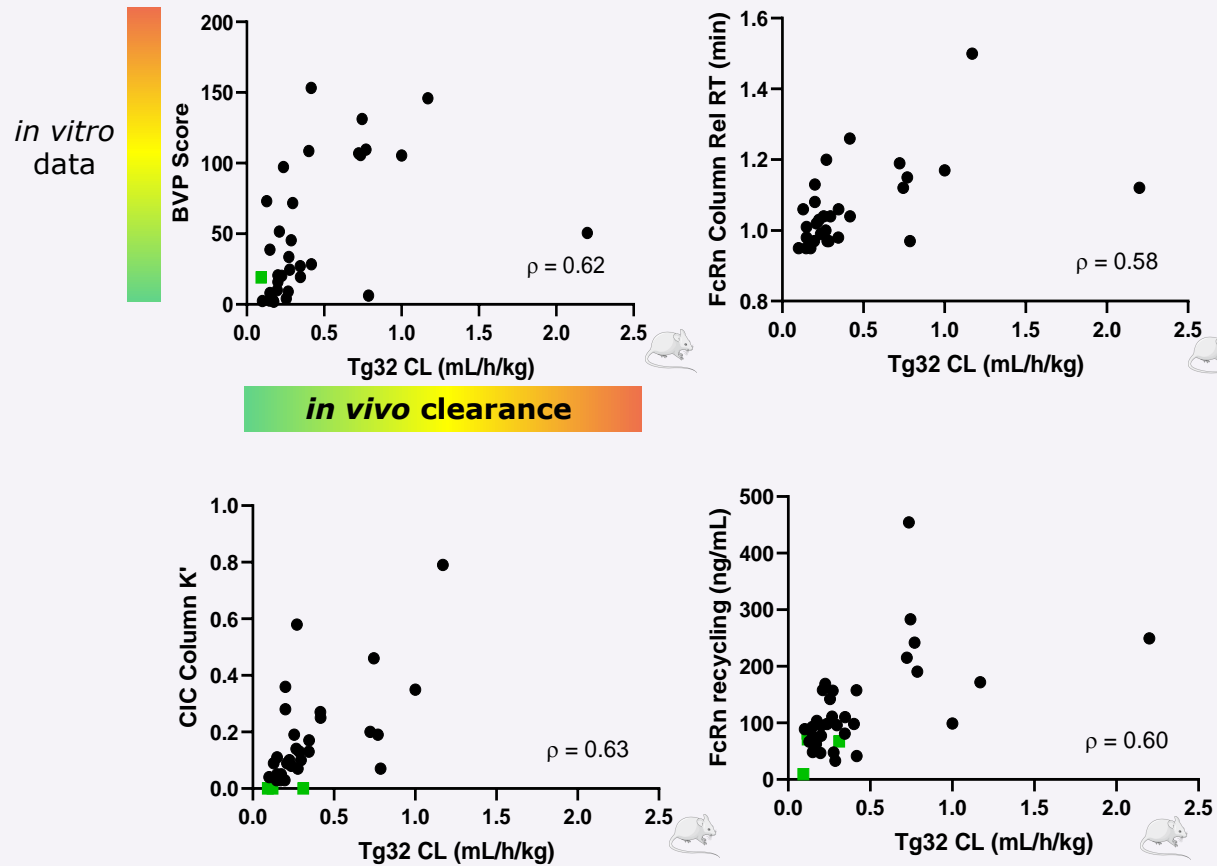
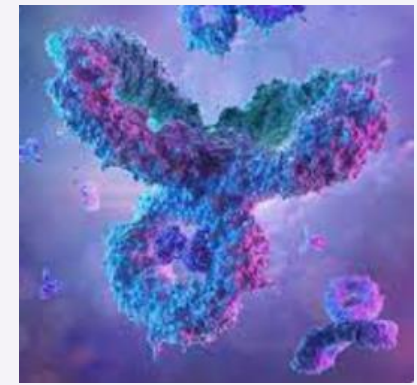
Cellular Recycling (FcRn interaction / biological mechanism)

- Cellular uptake-recycling-transcytosis using huFcRn transfected cell-line

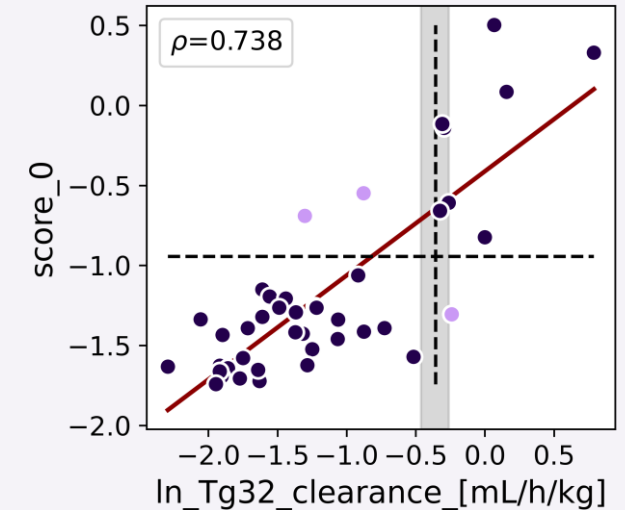


Validation Overview – Monoclonal AntiBody Dataset

Individual In Vitro Assays and Relation to In Vivo PK



Multidimensional linear fit with *in vitro* data



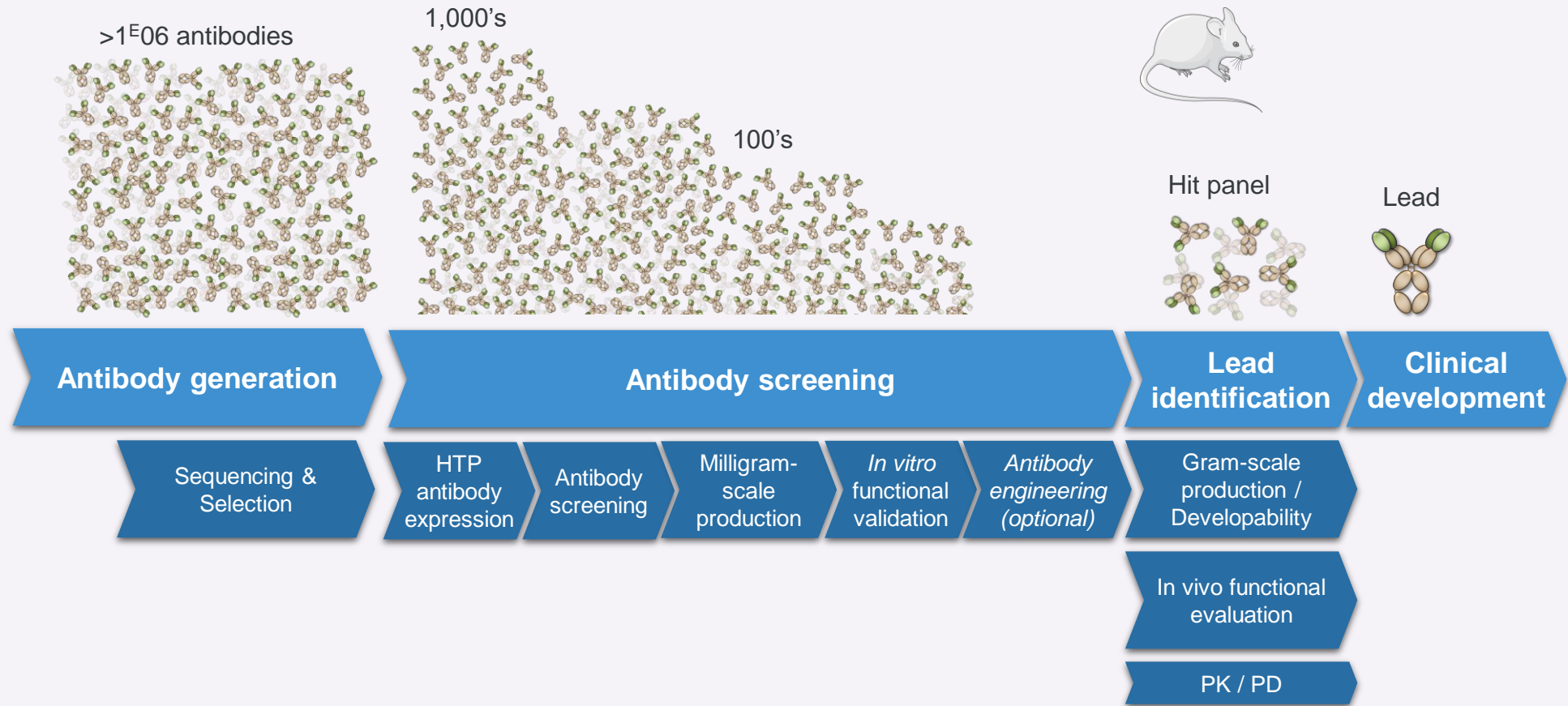
Clinical mAbs (n=7)

43 mAbs, with +/- drugability descriptors



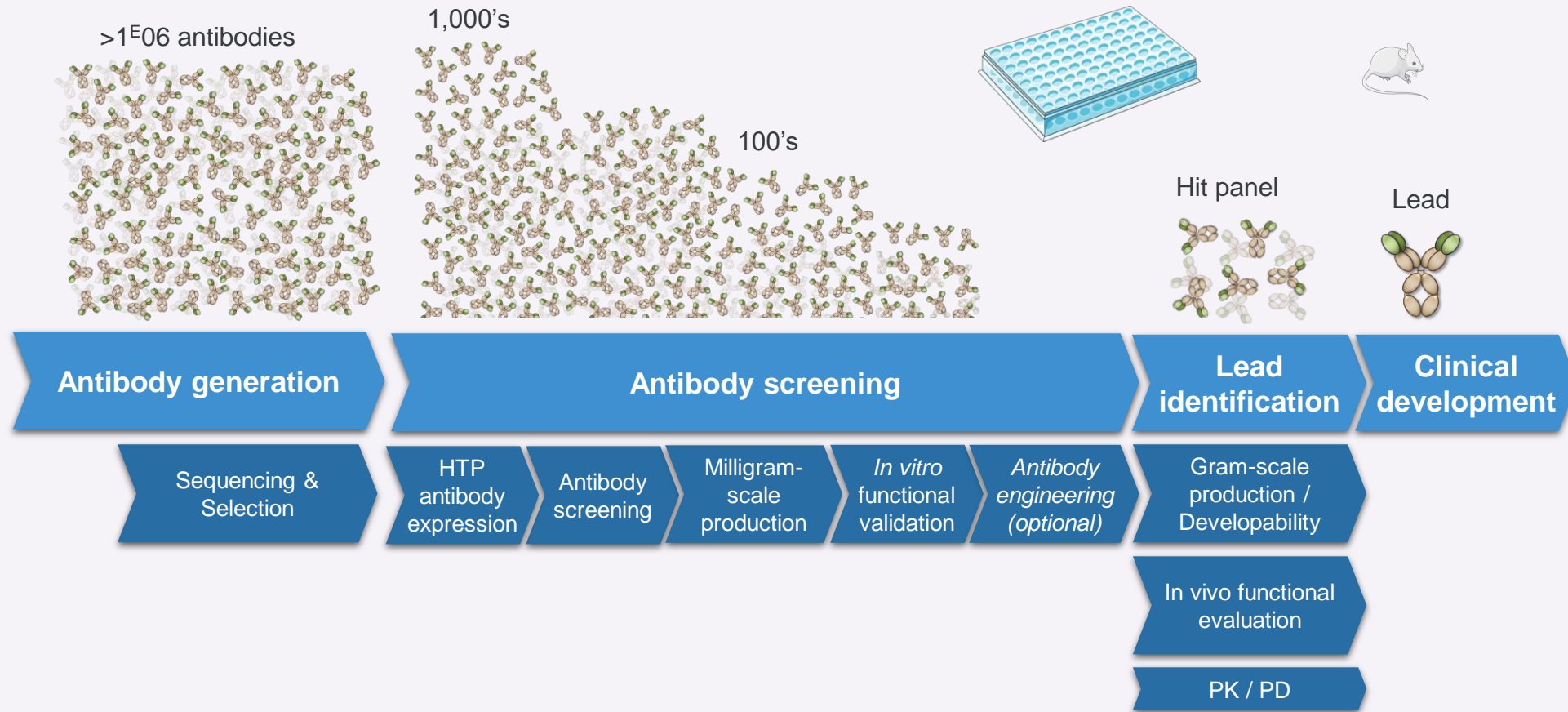
Reduce the number of PK studies

Today



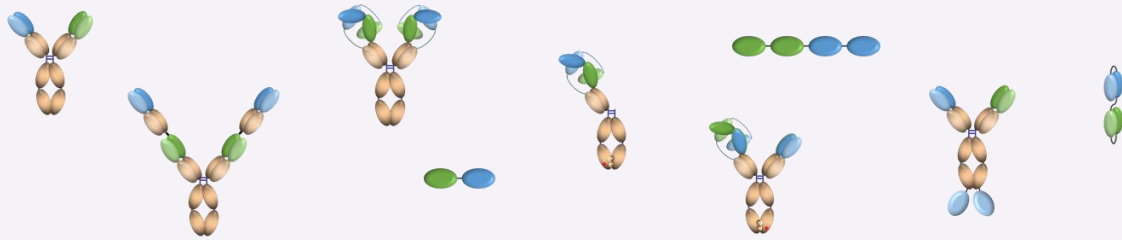
Reduce the number of PK studies

Tomorrow

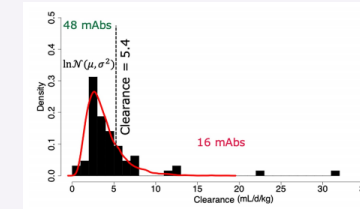
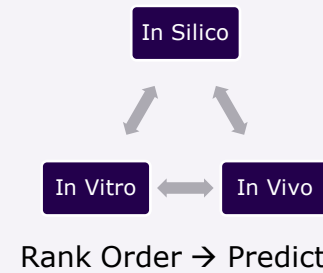


Next steps

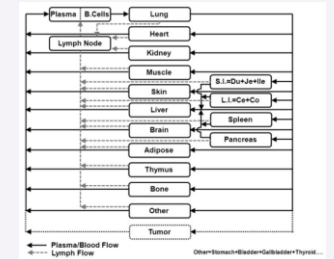
Complete data set for complex modalities



Introduce in silico models to enhance predictivity



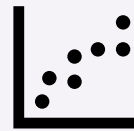
Multi-Parametric & Computational



Model-Based

Define rules for assays and results

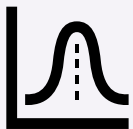
Automatize assays
➤ From request to data registration



Define & communicate rules for data interpretation



Develop the multiparametric approach



Online optimization

New assays

➤ Literature + benchmark

Increase knowledge on phys-chem parameters governing biologics PK



Small molecules, still some challenges

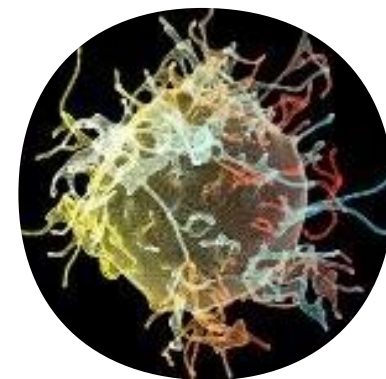
The blood-brain barrier (BBB)



- Currently no in vitro BBB predictive model
 - Internal in silico tool
 - Phys chem properties + QSAR
 - Screen on Caco2 cells in dedicated conditions
 - in vivo determination

The tumor penetration

- As a key factor for antitumoral activity
 - Unbound fraction
 - Plasma concentration
 - in vivo determination



Brain penetration in silico models

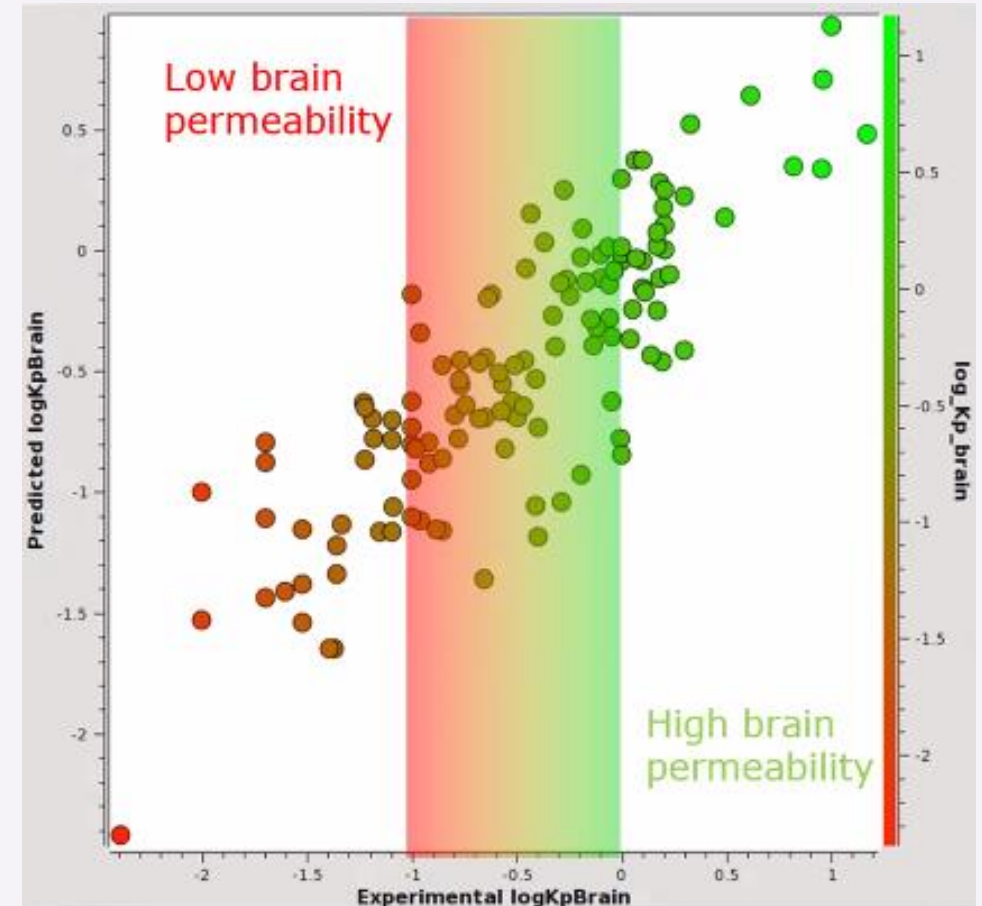
Example from one project

Experimental descriptors:

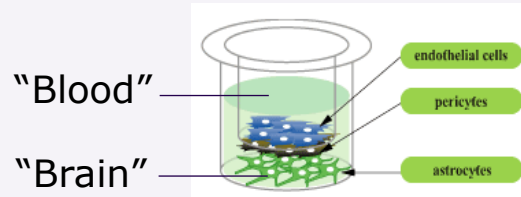
In vitro permeability on Caco2 cells:
Flux & efflux with & without cyclosporine

Physicochemical descriptors:

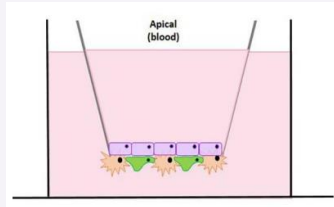
Fraction ionized at pH7.4
Number of aliphatic rings
Fraction charged surface area
logP, PSA, pKa, ...



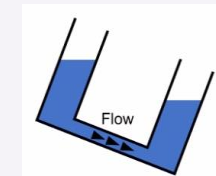
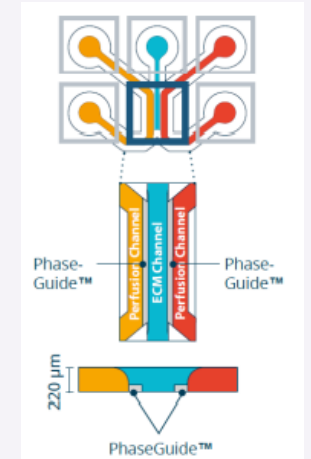
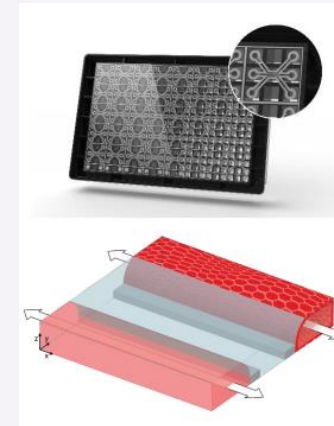
Brain penetration models under evaluation



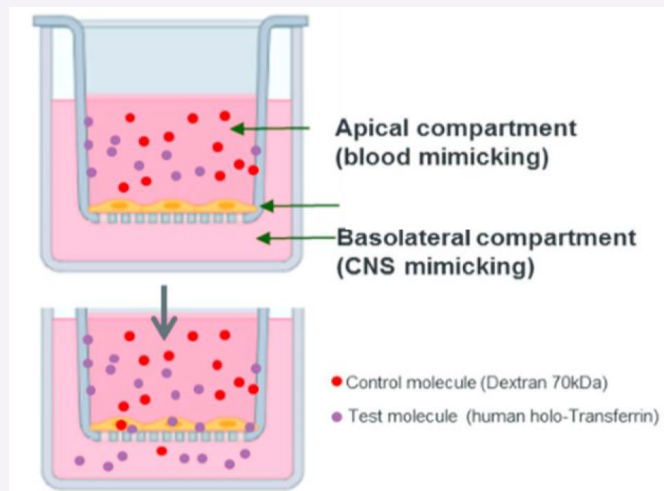
Primary **Non-Human-Primate** Brain Microvascular Endothelial Cells, Astrocytes and Pericytes



IPS derived Human Brain Microvascular Endothelial Cells, Astrocytes and Pericytes

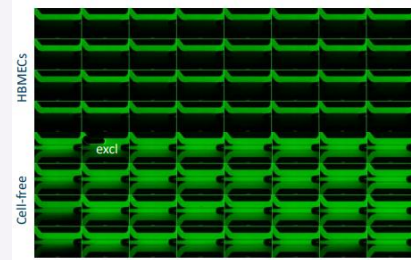
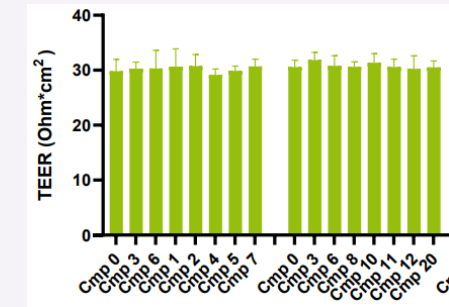
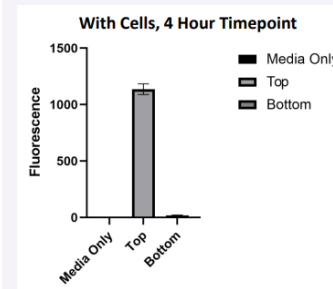
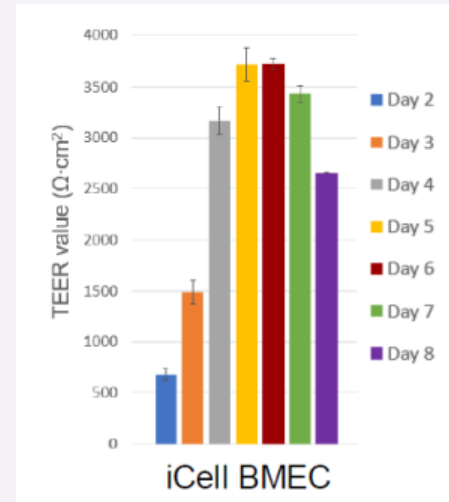
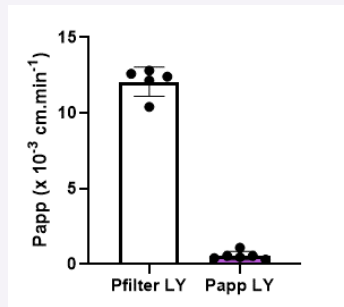
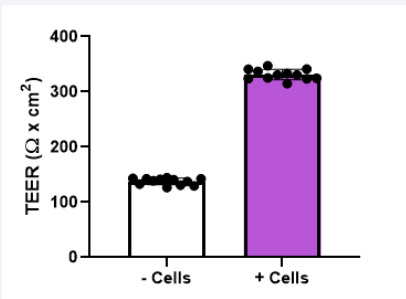


Primary **human** brain microvascular endothelial cells



Brain penetration models under evaluation

Barrier integrity assessment



- Consistent and **reproducible TEER values** across assays
- **Low permeability of Lucifer Yellow** in cell-containing inserts

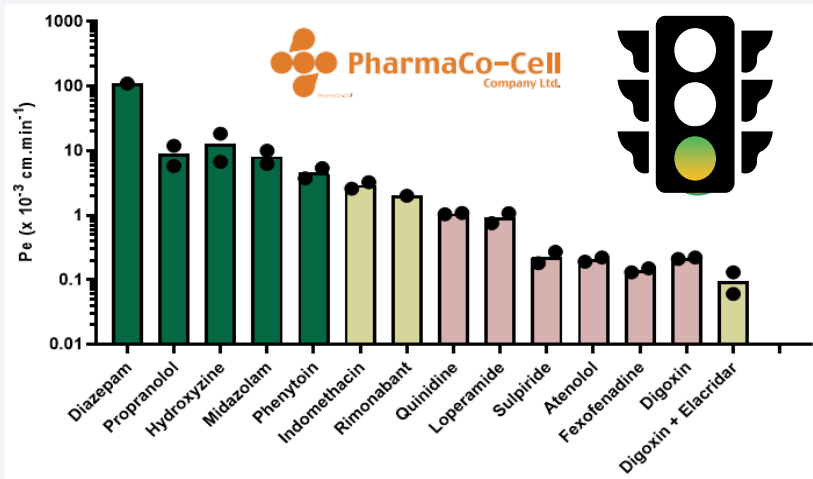
- **Higher TEER values** in accordance with expected data
- **Low permeability of dextran** in cell-containing inserts

- **Lower TEER** values
- **Low variation of TEER** across chips
- **Functional barrier that limit paracellular leakage** of small molecules

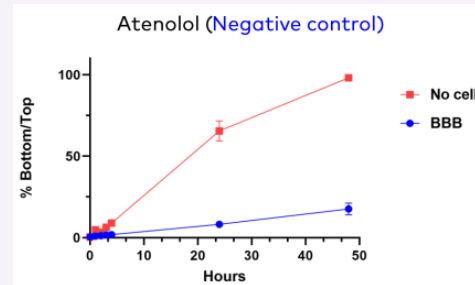
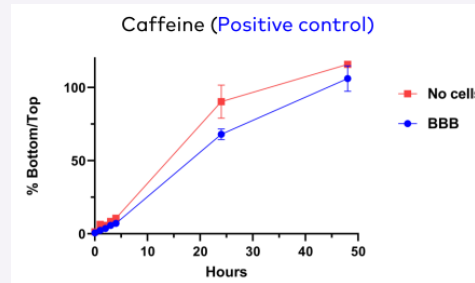
Brain penetration models under evaluation

In vitro permeability of small molecules: reference compounds

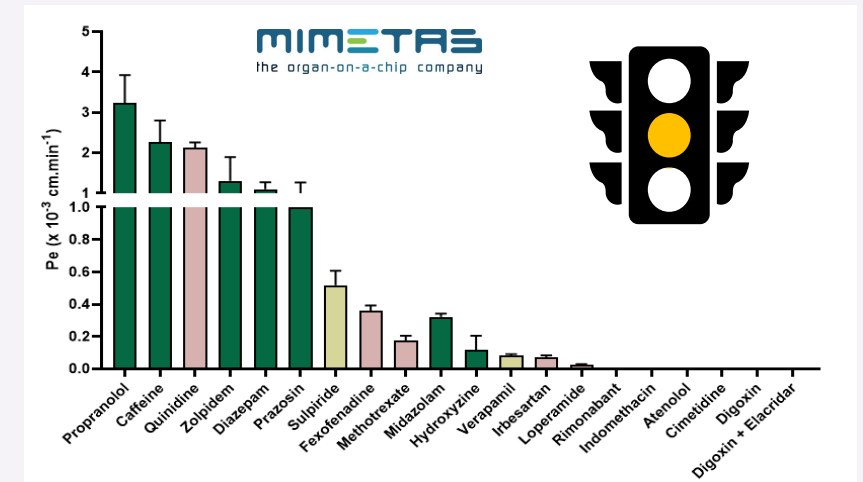
- Brain penetrant
- Mild brain penetrant
- Non-brain penetrant



Good in vitro/in vivo correlation
But efflux pump polarization issue?



Internal evaluation
will start soon



Lower in vitro/in vivo correlation
Efflux pump polarization issue?

Brain penetration models under evaluation

Next steps

- **Expand** the validation for small molecules
- **Characterize the model for antibody transcytosis** evaluation and prediction of CNS penetration for therapeutic proteins
- *For Mimetas: Optimization of barrier tightness with **co-culture** of HBMEC with astrocytes and/or pericytes*

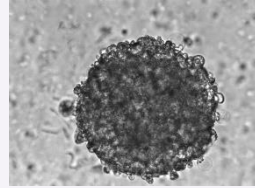
Tissue penetration

Tumor penetration model

Internal work + 2 collaborations on going



2D



Monoculture

Different tumor cell lines

Impact of efflux pumps

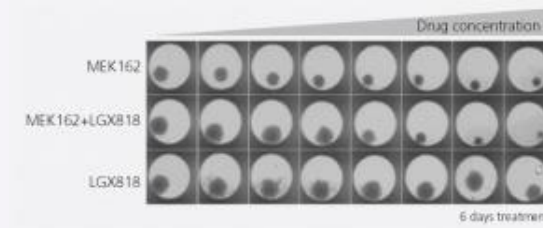
3D



Spheroids

Mono-culture

Co-culture



3D MPS

Spheroids

μ fluidics

Mono-culture

Co-culture

VASCULARIZED 3D LIVER

Aims

- Obtain a fully differentiated endothelium layer
- Build a vascular/liver interface
- Quantify functionality of the model
- In a 24 multiwell with gradient generation

Results

- Endothelium monolayer
- Co culture HUVEC-HepG2 more functional
- Physiological liver gradient mimicking

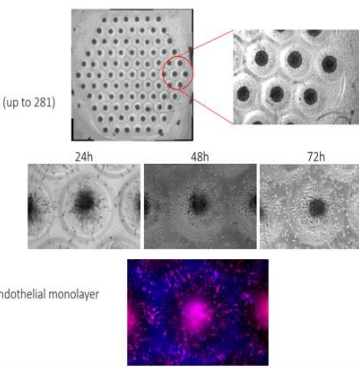
HIGH THROUGHPUT SPHEROIDS IN A WELL – BREAST CANCER

Aims

- Multiplexing the number of spheroids per/well (up to 281)
- Controlling the size homogeneity of spheroids
- Embedding in ECM (collagen)
- Triggered invasive phenotypes
- >7d in a multiwell

Results

- MDA-MB-231 spheroids maintained for 7days
- Invasion phenotype obtained quickly
- Automatization of the medium change
- Next step: add fibroblasts, macrophages and endothelial monolayer



Tissue penetration

Tumor penetration model

Internal work + 2 collaborations on going

Where we are:

Definition of experimental conditions:

- incubation medium (impact of protein binding)

- kinetics timepoints

- lysis method

- ...

Comparison of different cell lines

- no clear difference yet, except the impact of efflux pumps

In vivo data collection → ivivc → new in vitro assay to better select candidates for pharmacology studies

Acknowledgments

DMPK TEAM

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Thank you



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