Syngene
Putting Science to Work

DMPK and non-clinical safety considerations in the assessment of developability in drug discovery

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Preclinical aspects of integrating developability assessment in drug discovery

- The screening cascade: a DMPK/toxicology perspective
- Understanding *in vitro* and *in vivo* correlations
- Safety assessment in discovery
- Human dose prediction
- Preclinical deliverables for candidate selection and IND-enabling studies

Target product profile

- A target product profile (TPP) outlines the desired 'profile' or characteristics of a target product that is aimed at a particular disease or diseases

- TPPs state intended use: **target patient populations**, **route of administration**, **duration of action**, **posology**, **tissue distribution required for efficacy** and **acceptable safety margins**

- Such TPPs guide drug discovery and development and are used as planning tools towards a molecule the desired characteristics

- TPPs are updated during project execution based on the new data available from the chemical series

Target product profile: ADME

	Assay	TPP	Candidate			
			Mouse	Rat	Dog	Human
In vitro	Microsomal clearance f_u corrected	% LBF		4.5	9.5	8.1	5.7
	Hepatocyte clearance f_u corrected	% LBF	**<30**	10.2	22	<4.2	4.3
	PPB	%		94.6	94.6	96.9	97.1
	Plasma stability, $t_{1/2}$	h	**>24**	>100	>100	88	>100
	Caco-2 cell permeability (Papp; AB)	cmx10^{-6}/s	**> 5**	5.8 (Efflux ratio=0.8)			
In vivo	Clearance *in vivo*	% LBF	**< 30**	12	27	5	-
	Volume of distribution (Vss)	L/kg	-	2.7	2.1	1.2	-
	Bioavailability, oral PK	% F	**> 50**	>100	93	86	-
	Rat distribution i.v.			No relevant tissue distribution			
	Rat and dog excretion i.v.			Mainly hepatic clearance of the parent compound			

Target product profile: Safety

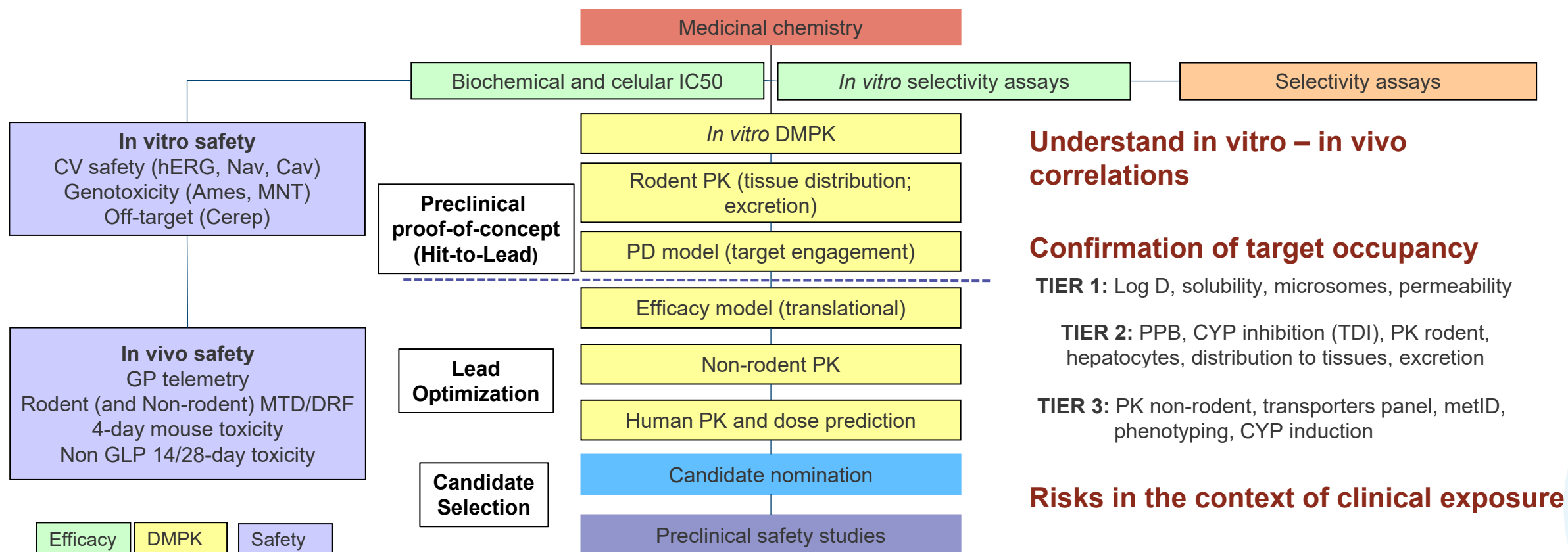|	Assay		TPP	Candidate
In vitro	Cytotoxicity CHO	IC_{50}, µM	**> 10**	54
	Selectivity, nuclear receptors (18 targets)	Fold selectivity vs isoform	**> 100**	> 100
	Selectivity, CEREP panel (55 targets)	Fold selectivity vs isoform	**> 1000**	> 1000
	Selectivity, kinases (75 targets)	Fold selectivity vs isoform	**> 1000**	> 1000
	hERG / Nav1.5 / Cav1.2	IC_{25}, mM	**> 1**	> 30 / > 30 / 21
	In vitro Langendorff heart	Clean dose		3 µM
	Genotoxicity: Ames and micronucleus *in vitro*		**Negative**	Negative
	GSH adduct formation	%	**Negative**	Negative
	CYP450 inhibition	IC_{50}, µM	**> 25 µM**	No inhibition
	CYP induction at 5 µM (1A2, 2B6, 3A4)	Fold induction	**≤ 2**	No induction
In vivo	CNS Irwin test in rats, 1 admin, po	Safety margin*	**≥ 30 fold**	≥105
	Telemetry in guinea pigs, 1 admin, po	Safety margin*	**≥ 30 fold**	≥73
	4-day hepatotoxicity study in mice, po	Safety margin*	**≥ 30 fold**	107
	14-day toxicity study in rats, po	Safety margin*	**≥ 30 fold**	≥106

DMPK/Safety strategy

Progression of compounds meeting the TPP

- Potency and selectivity

- Physicochemical properties

- Solubility and permeability

- Metabolic stability

- Preclinical PK and PKPD

- Plasma protein and tissue binding

- Low potential DDIs

- Adequate safety margins
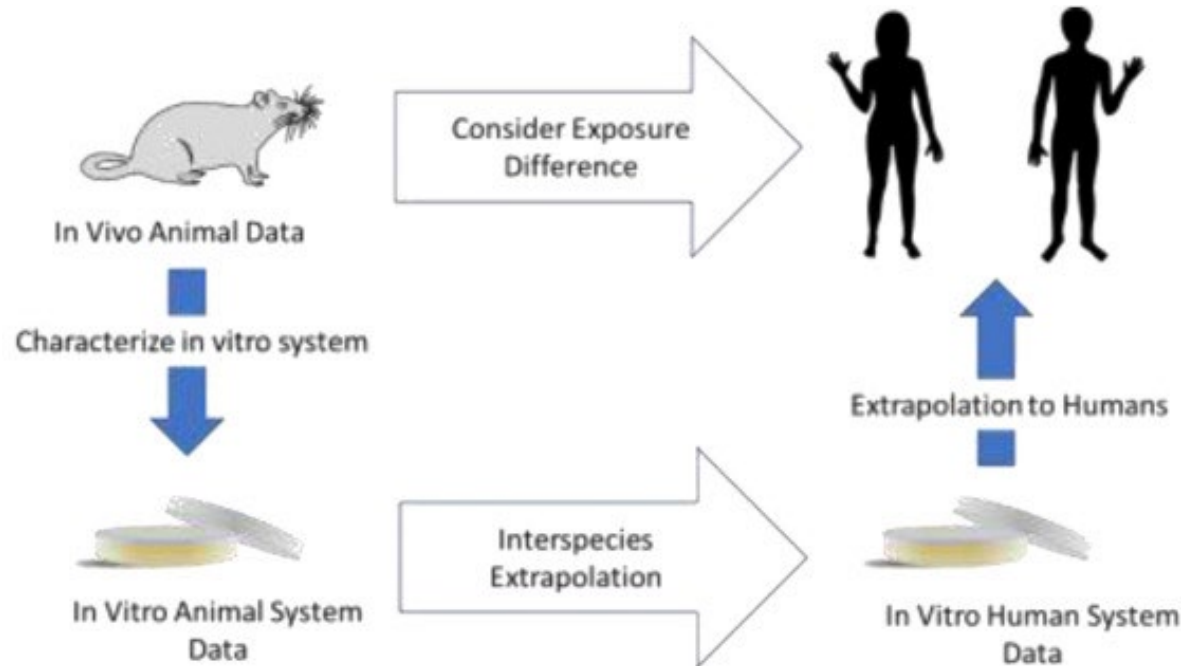
Adapted screening cascade strategy

- <u>Identify problems and formulate hypothesis</u> to address issues. Select molecules and assays to test the hypothesis

- <u>Measure physicochemical</u> properties to design appropriate DMPK for target engagement

- <u>Integrate ADME, PKPD and safety</u> data for human dose prediction Understand which properties need to be optimised

- Review strategy based on new knowledge and issues

The screening cascade: Critical path activities

Medicinal chemistry

Biochemical and celular IC50 | In vitro selectivity assays | Selectivity assays

In vitro safety
CV safety (hERG, Nav, Cav)
Genotoxicity (Ames, MNT)
Off-target (Cerep)

Preclinical proof-of-concept (Hit-to-Lead)

In vitro DMPK

Rodent PK (tissue distribution; excretion)

PD model (target engagement)

Efficacy model (translational)

Lead Optimization

Non-rodent PK

In vivo safety
GP telemetry
Rodent (and Non-rodent) MTD/DRF
4-day mouse toxicity
Non GLP 14/28-day toxicity

Human PK and dose prediction

Candidate Selection

Candidate nomination

Preclinical safety studies

Efficacy | DMPK | Safety

(Hughes 2011; Cantrill 2020)

Understand in vitro – in vivo correlations

Confirmation of target occupancy

TIER 1: Log D, solubility, microsomes, permeability

TIER 2: PPB, CYP inhibition (TDI), PK rodent, hepatocytes, distribution to tissues, excretion

TIER 3: PK non-rodent, transporters panel, metID, phenotyping, CYP induction

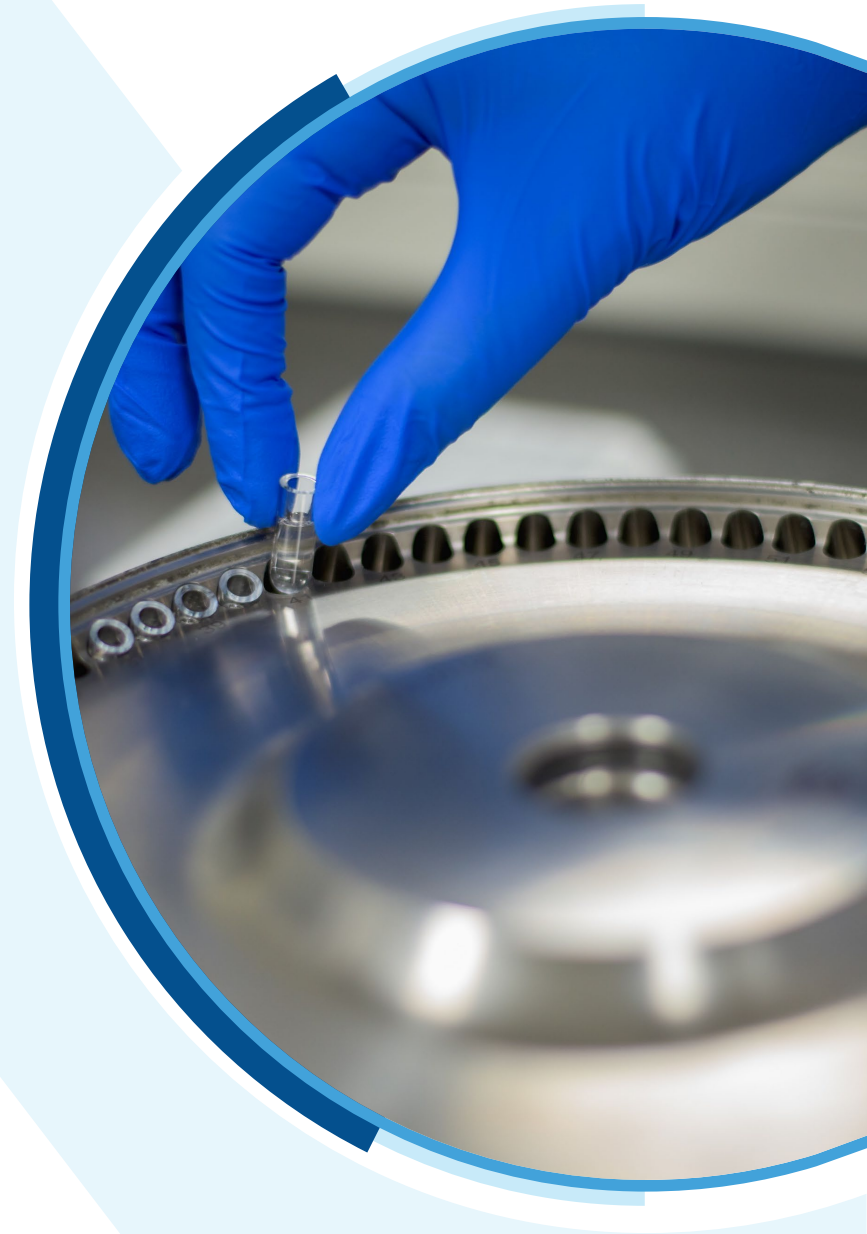**Risks in the context of clinical exposure**

In vitro – in vivo correlations

(Mattes 2020; Pognan 2023)

- Reduce animal experimentation and increase number of tested compounds

- Investigate underlying mechanisms

- **In vitro-in vivo extrapolation (IVIVE)** e.g. calculate *in vivo* clearance from *in vitro* clearance in case metabolism is the main elimination pathway

- *In vitro* three-dimensional cultures and micro-physiological systems for replication of *in vivo* function

Case study 1: Topical drug for dermatology indication

Predicted systemic concentrations of drug candidate are similar to reference

Assay			TPP	Candidate	Competitor
Systemic exposure	Topical PK in non-rodent, Formulation in 10 % BSA	C_{max}, min – max, nM		**3.5 – 9 (total)** **0.2 – 0.6 (free)**	-
	Predicted clearance in humans by allometric scaling	Cl, min – max, L/h	**>50% LBF**	**13 – 50** **(17-66% human LBF)**	19 – 51 (25-67% human LBF)
	Systemic exposure in humans after dermal administration, predicted for 10% BSA and BID	C_{ss}, nM		**8.5 – 32 (total)** **0.16 – 0.6 (free)**	15 – 42 (total) 8.6 – 24 (free)
	Systemic exposure in humans after dermal administration, reported in PhII studies [% BSA]	Range, total, nM		-	0.2 – 8.3 (total)[†] 0.11 – 4.7 (free) [2-20%, mean: 7%]
Brain exposure	Multiple dose oral PK in rats	C_{ss}, nM		**0.16 – 0.6 (free)[‡]**	-

The candidate showed overall good safety profile but inhibited a brain enzyme

Assay		TPP	Candidate
CHO cytotox	IC_{50}, µM	**>30**	**>30**
Selectivity (same class)	Enzyme inhition >80% @ 10 µM	**<20% of enzymes inhibited**	**5% (16 out of 311)** **Brain target IC_{50}= 1 nM**
Selectivity against other targets	Targets with an inhition >80% @ 10 µM	**No critical target inhibition**	**0 out of 55**
Genotoxicity: Ames and micronucleus in vitro	Result	**Negative**	**Negative**
14-day dermal toxicity in rat	Effect	**No skin and systemic toxicity**	**No local alterations** **No systemic alterations** @ max dose C_{max} 302 nM AUC 738 ng*h/mL
Photoirritation in guinea pig, dermal treatment	Result	**Negative**	**Negative** @ max dose
Cardiosafety, dog telemetry	safety margin vs systemic exposure by dermal route	**safety margin ≥30 fold**	**≥715** (vs systemic exposure in non-rodent)
Neurotoxicity, Irwin test in rat	safety margin vs systemic exposure by dermal route	**Safety margin ≥30 fold**	**≥ 990** (vs systemic exposure in non-rodent)

- The brain enzyme inhibited by our drug candidate is involved in different signalling cascades and is thought to be an important mediator of learning and memory

- This finding prior to initiation of regulatory toxicology for IND submission delayed the project:
  - ✓ Discussions with scientists experts in this enzyme
  - ✓ Test the candidate in cellular assays and animal models
  - ✓ Define safety margin (x30?)

- Uncertainties related to de-risking and brain target inhibition led to project closure
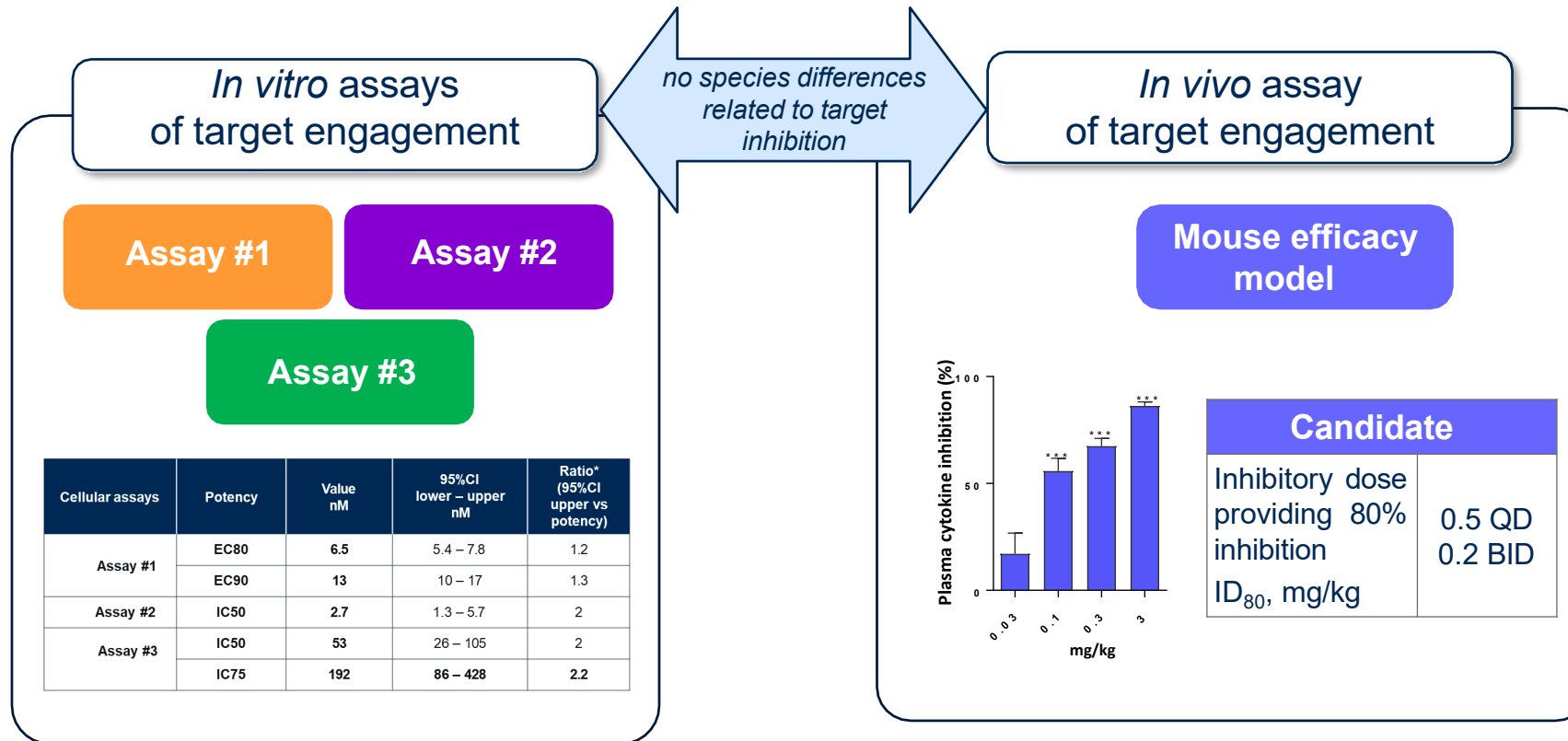
Secondary pharmacology effects of novel drugs and their metabolites is key to identify liabilities as early as possible

(Jenkinson 2020; Brennan 2024)
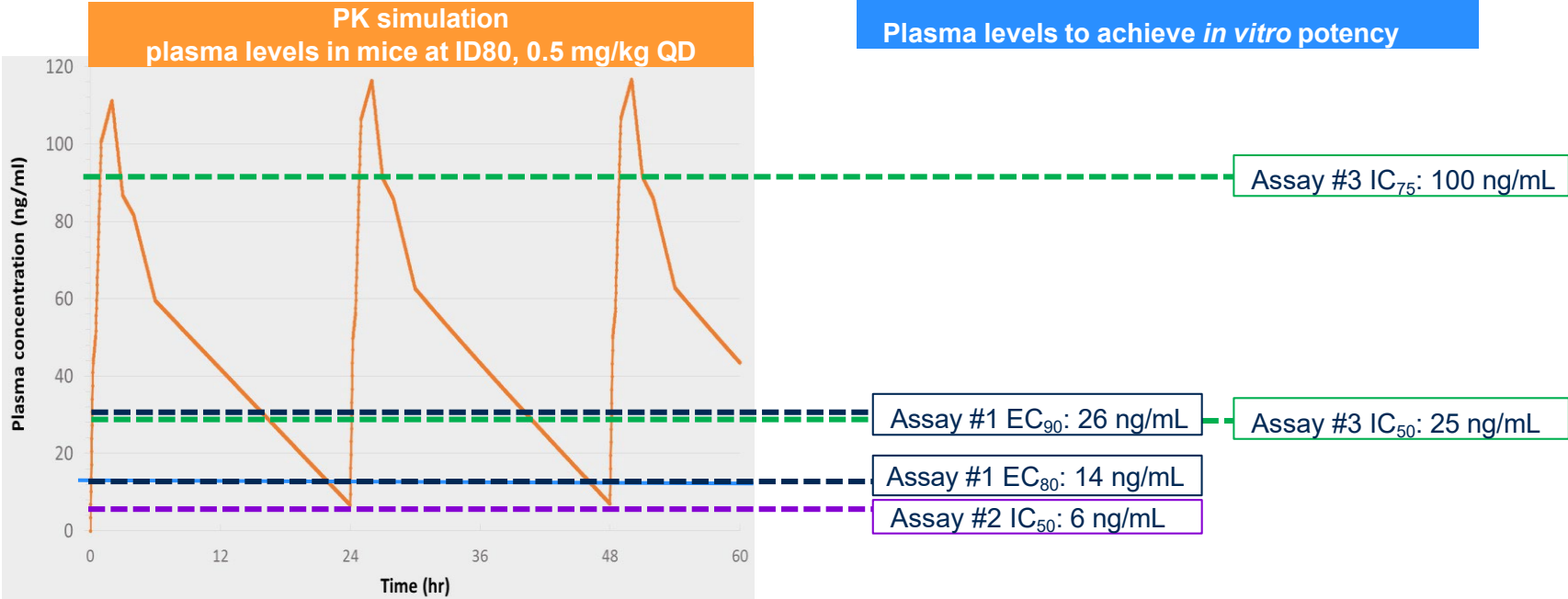
Case study 2: Establishing PK/PD correlation

Case study 2: Establishing PK/PD correlation

In vitro assays of target engagement

no species differences related to target inhibition

In vivo assay of target engagement

Assay #1 Assay #2

Assay #3

Cellular assays	Potency	Value nM	95%CI lower – upper nM	Ratio* (95%CI upper vs potency)
Assay #1	EC80	6.5	5.4 – 7.8	1.2
	EC90	13	10 – 17	1.3
Assay #2	IC50	2.7	1.3 – 5.7	2
Assay #3	IC50	53	26 – 105	2
	IC75	192	86 – 428	2.2

Mouse efficacy model

Plasma cytokine inhibition (%) vs mg/kg (0.03, 0.1, 0.3, 3)

Candidate	
Inhibitory dose providing 80% inhibition ID_{80}, mg/kg	0.5 QD 0.2 BID

- How do *in vitro* target engagement assays fit with in vivo efficacy?
- Which dose (from *in vitro*) is needed to cover the target?
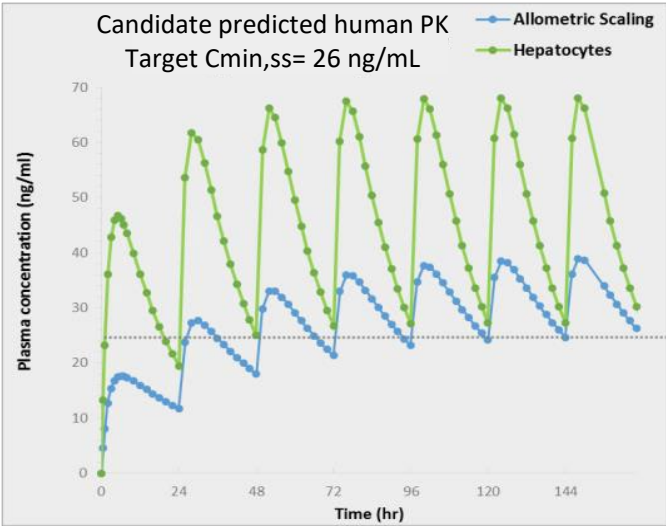- What is the duration of target engagement required for *in vivo* PD?

Case study 2: Establishing PK/PD correlation

PK simulation
plasma levels in mice at ID80, 0.5 mg/kg QD

Plasma levels to achieve *in vitro* potency

Assay #3 IC$_{75}$: 100 ng/mL

Assay #1 EC$_{90}$: 26 ng/mL — Assay #3 IC$_{50}$: 25 ng/mL

Assay #1 EC$_{80}$: 14 ng/mL

Assay #2 IC$_{50}$: 6 ng/mL

*In vitro potency data was corrected by protein content in the assay
**Mouse PPB used to correct plasma concentrations for each potency value

Case study 2: Human dose prediction

Candidate predicted human PK
Target Cmin,ss= 26 ng/mL

Parameter		Candidate PK prediction in humans		
		Human hepatocytes	Allometric scaling	PBPK modeling
Vss	l/kg	NA	1.0	1.9
Half-life	h	13	27	34
Clearance	mL/min/kg	0.86	0.43	0.65
Predicted dose	mg	**9**	**3**	**2.3**
Cmax	ng/ml	67	40	37-42

Assumptions

- Target coverage at Cmin,ss: EC90 from assay #1
- Assumption for human hepatocytes and allometric scaling: Ka= 0.5 h^{-1}; F= 50%
- Prediction from PKPB: F= 97%

> Allometric scaling and human hepatocyte predictions assumed 50% bioavailability (worst case)
> The dose could be lower than predicted if F is higher

Case study 2: Predicted safety margin efficacious dose

Toxicology

The predicted safety margins were calculated by comparing the Cmax and AUC(0-24h) between the NOAELs and the worst case predicted human exposure (case study 3: 9 mg/day (EC_{80} in assay #1))

Study type	Species	NOAEL	HED[a]	Safety margin - $AUC_{(0-24)}$	Safety margin - C_{max}
		mg/kg	mg/day		
Repeat-dose toxicity (2-week)	Rat	80	768	58	91
Repeat-dose toxicity (2-week)	Non-rodent	50	1,764	153	170

[a]The HED was calculated as HED=Animal dose (mg/kg) × [animal weight (kg) ÷ human weight (kg)]$^{0.33}$

Case study 2: Predicted safety margin efficacious dose

Safety pharmacology

The predicted safety margins were calculated by comparing the Cmax and AUC(0-24h) between the NOAELs and the worst case predicted human exposure (case study 3: 9 mg/day (EC$_{80}$ in assay #1))

Study type	Species	NOAEL	HED[a]	Safety margin - AUC$_{(0-24)}$	Safety margin - C$_{max}$
		mg/kg	mg/day		
Cardiovascular	Dog	2	65	6	11
CNS	Rat	1000	9,600	350	278
Respiratory	Rat	1000	9,600	352	258

[a]The HED was calculated as HED=Animal dose (mg/kg) × [animal weight (kg) ÷ human weight (kg)]$^{0.33}$

Preclinical deliverables for candidate selection and IND-enabling studies

ADME

Safety

Development Candidate Profiling

- **Rodent and non-rodent single/multiple ascending dose PK**
- **CYP450 inhibition (incl. TDI)**
- **CYP450 induction**
- **Human dose prediction**

- **hERG full dose response**
- **7-day DRF/MTD, rodent (and non-rodent)**
- **Full receptor profiling for potential off target effects (Eurofins)**

IND Enabling

- **Tissue exposure as needed**
- **Transporter panel substrate/inhibitor**

- **Safety pharmacology** (**Langendorff, telemetered Guinea pig**, Irwin test in rats)
- **14/28-day toxicology rodent** (and non-rodent) – **Safety margins**

Syngene

DMPK at Syngene

Capabilities, capacities and turnaround times

Syngene

Discovery Services: Broad capabilities and excellence in delivery

Discovery Chemistry

- Synthetic chemistry
- Medicinal chemistry
- Niche area: PROTACs, carbohydrates, nucleosides, peptides

Discovery Biology

- Antibody discovery
- Assay biology
- DMPK
- In vivo pharmacology
- Structural biology
- Protein sciences
- Cell and gene therapy

Discovery Services

Computational and Data Sciences

- Computer aided drug discovery (small and large molecule)
- Target ID and prioritization
- Translational informatics
- Artificial Intelligence driven analysis, design, and prioritization

Safety and Toxicology

- Pre-clinical in vitro and in vivo tox studies
- *In silico* predictive tox
- histopathology

Co-located & integrated to enable seamless delivery of client drug discovery projects in a collaborative framework

Syngene

In-vitro ADME capabilities to drive drug discovery

100 + 30
Scientists
BLR + HYD

23 K + 13 K sq ft
lab space

- Automation
- High-throughput
- State-of-the-art

- **Solubility** : Across pH, FaSSGF, FaSSIF & FeSSIF
- **Stability** : Chemical, Plasma, Chemical Reactivity
- Log P, Log D, Chrom Log D and pKa [Sirius T3]

- LCMS based HT RP-chiral methods
- Chiral inversion study in stability studies

Binding
(RED, HTD & UC-PPB)
- Plasma, blood, microsomal & tissue
- **B/P Ratio**
Permeability-
- PAMPA, Caco-2
- MDR1-MDCK-II MRP2-MDCK-II [NIH & NKI clones] & BCRP-MDCK-II
- CaLu-3
- Uptake Transporters: OATPs, OCT, OAT & MATEs

- Rapid & sensitive LC-MS/MS methods across biological matrices
- Qualified for purpose [L0 to L1 levels]
- Cassette Bioanalysis

Phys-chem & stability

Absorption & distribution

Chiral inversion in invitro ADME studies

Qualitative bioanalysis

Metabolism & DDI

Metabolite ID & fingerprinting

- Soft spot assessment [microsomes, hepatocytes]
- Metabolite finger printing
- Reactive metabolite screening

Stability/ Intrinsic clearance
- Liver, intestinal microsomes, S9, Cytosol & hepatocytes
DDI :
Reversible CYP Inhibition: 1A2, 2B6, 2C8, 2C9, 2C19, 2D6 & CYP3A4/5

Mechanism based CYP inhibition:
- IC_{50} shift, K_I & K_{inact}
CYP induction:
- PXR gene reporter assay
- Hepatocytes - enzyme activity & mRNA

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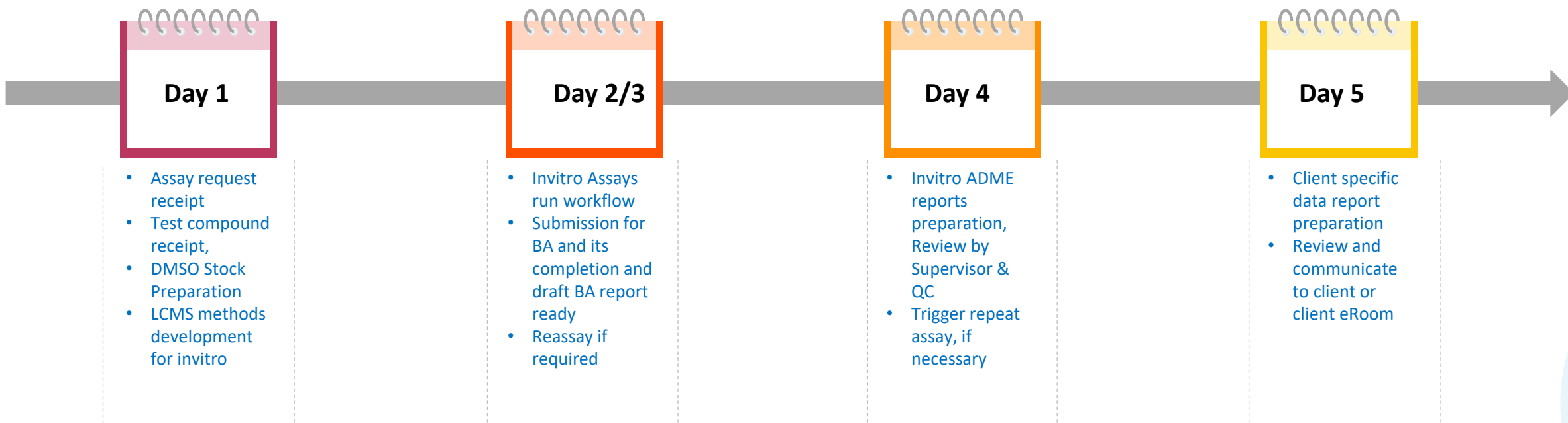
Wave 1 ADME Assays: Capacity and Turnaround times
50, 000 ADME assay points per month

Assay	Matrix Considered	Current Capacity per week	TAT [Days]	Expansion Capacity / week
Kinetic Solubility, LCMS based	pH 7.4, [PBS or phosphate buffer] or specified pH	500	5	1000-1500
Log D – shake flask method	pH 7.4	400	5	800 – 1200
Metabolic Stability – Microsomal, 2 time points	Mouse, Rat, Human	500	5	1000-1500
Metabolic Stability – Hepatocyte, 2 time points	Mouse, Rat, Human	500	5	1000-1500
Plasma Stability, 2 time points	Mouse, Rat, Human	500	5	1000-1500
Protein Binding – Plasma, one concentration	Mouse, Rat, Human	400	5	800 – 1200
CYP Inhibition, One Concentration, cocktails	All Isoforms	500	5	1000-1500
Permeability, Unidirectional	CaCO-2 or MDCK	500	5	1000-1500

Delivering with speed: Compound dispatch to data upload >95% TAT adherence in 5 calendar days with *(Wave 1 Invitro ADME Assays)*

Study time (5 business days)

If compounds are made in-campus or post receipt at Syngene

Day 1
- Assay request receipt
- Test compound receipt,
- DMSO Stock Preparation
- LCMS methods development for invitro

Day 2/3
- Invitro Assays run workflow
- Submission for BA and its completion and draft BA report ready
- Reassay if required

Day 4
- Invitro ADME reports preparation, Review by Supervisor & QC
- Trigger repeat assay, if necessary

Day 5
- Client specific data report preparation
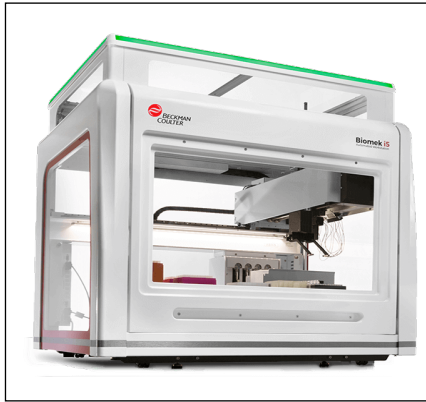- Review and communicate to client or client eRoom

- Wave 1 assays include kinetic solubility, Log D, metabolic stability, plasma protein binding and CYP inhibition assays [cocktail method]
- >95% TAT adherence with no more than 2x TAT [Considering compound reagent related challenges}
- Real-time updates on holidays, & other anticipated TAT impact, communicated to customer for planning operations & study workflow

Syngene DMPK features a state-of-the-art quantitative instrumentation facility

Lab cyte Echo® 555

Biomek i7 Automation

Sirius T3

INSTRUMENTS		BLR	HYD
LC-MS	API 7500	2	2
	API 6500	2	3
	API 5500	1	2
	API 4500 QTRAP	1	0
	API 4500	7	0
	API TRIPLE TOF	1	0
	Total	14	7
UPLC	UPLC	14	7
	UPLC-UV	3	3
BIOMEK	BIOMEK i5	2	0
	BIOMEK i7	0	3
VIAFLO	INTEGRA 96/384	1	2
ECHO 555	LABCYTE	1	1

SCIEX, 7500-QQQ

Triple TOF 5600+

Sciex 5500 QTrap

Syngene

In vivo PK capabilities to drive drug discovery
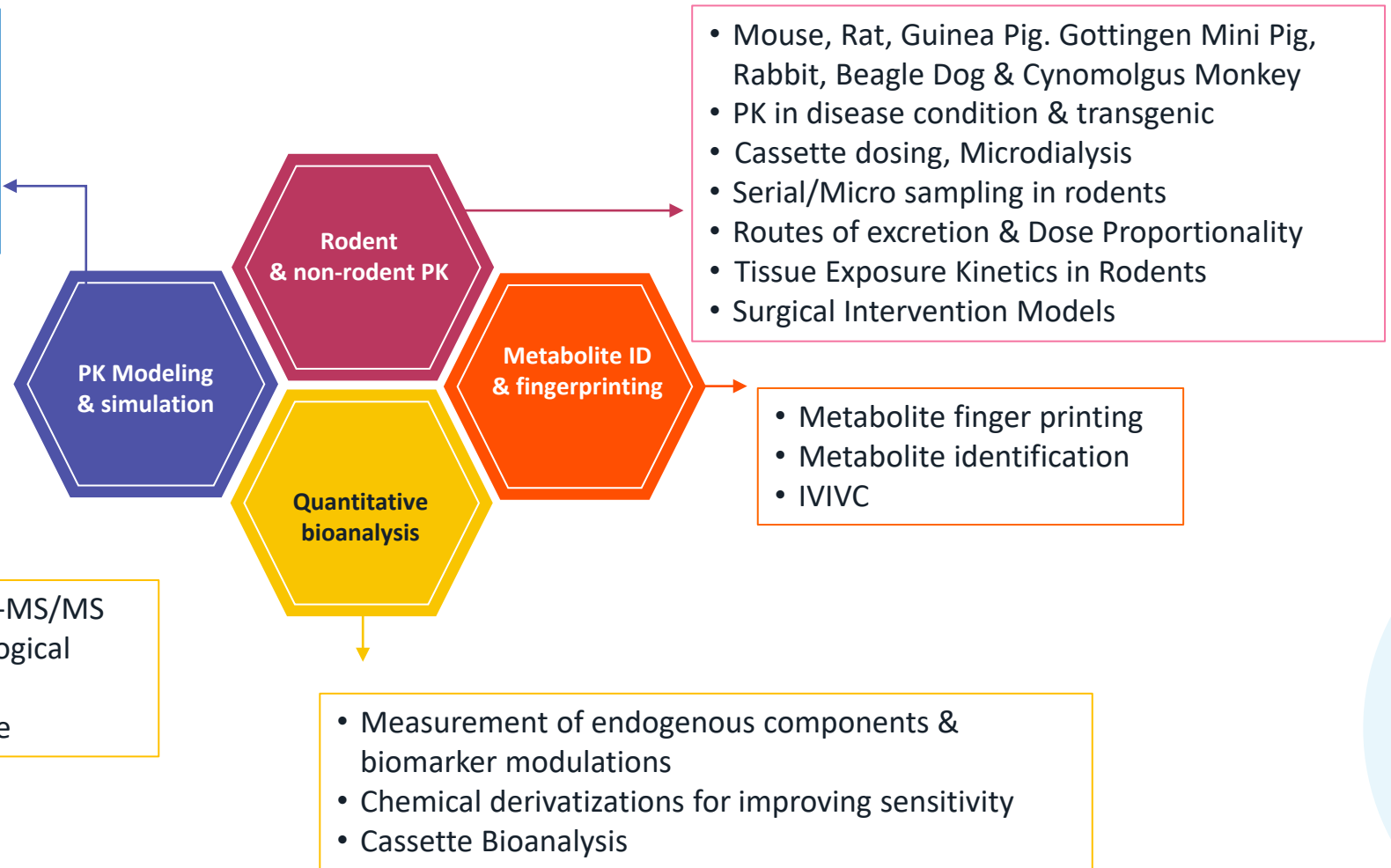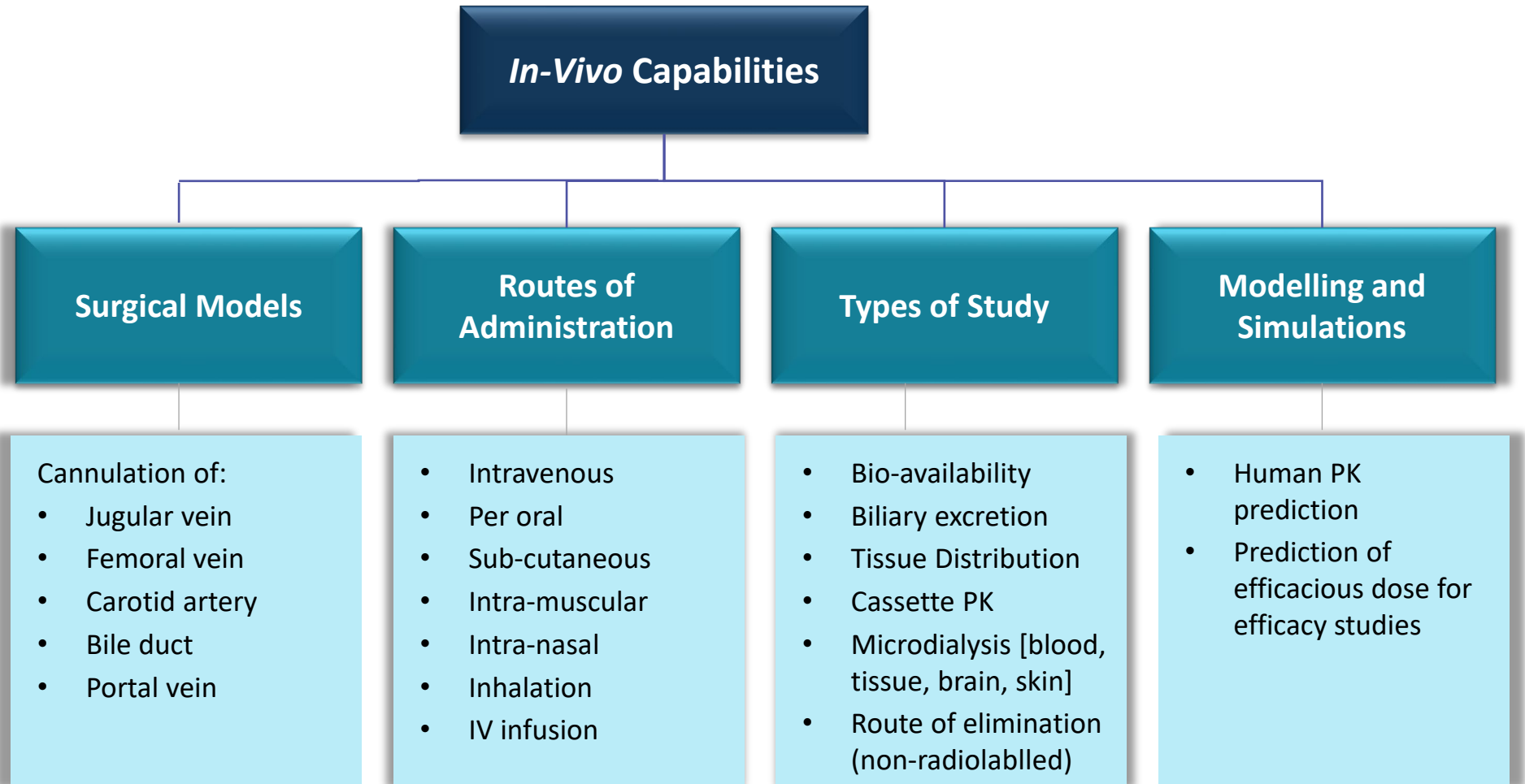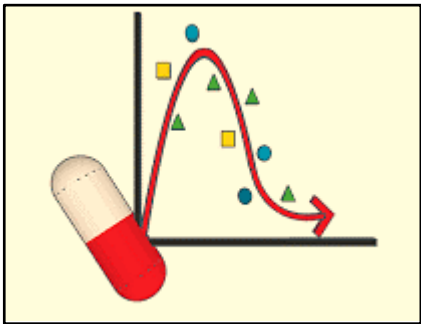
30
Scientists
BLR

26 K
lab space

- Automation
- High-throughput
- State-of-the-art

- PK-PD/efficacy correlation
- Allometric scaling to predict human PK
- Prediction of FIH

PK Modeling & simulation

Rodent & non-rodent PK

Quantitative bioanalysis

Metabolite ID & fingerprinting

- Mouse, Rat, Guinea Pig. Gottingen Mini Pig, Rabbit, Beagle Dog & Cynomolgus Monkey
- PK in disease condition & transgenic
- Cassette dosing, Microdialysis
- Serial/Micro sampling in rodents
- Routes of excretion & Dose Proportionality
- Tissue Exposure Kinetics in Rodents
- Surgical Intervention Models

- Metabolite finger printing
- Metabolite identification
- IVIVC

- Rapid & sensitive LC-MS/MS methods across biological matrices
- Qualified for purpose

- Measurement of endogenous components & biomarker modulations
- Chemical derivatizations for improving sensitivity
- Cassette Bioanalysis

Our *in vivo* PK profiling capabilities encompasses a broad range of species, surgical models, routes of administration and study types

In-Vivo Capabilities

Surgical Models	Routes of Administration	Types of Study	Modelling and Simulations
Cannulation of: • Jugular vein • Femoral vein • Carotid artery • Bile duct • Portal vein	• Intravenous • Per oral • Sub-cutaneous • Intra-muscular • Intra-nasal • Inhalation • IV infusion	• Bio-availability • Biliary excretion • Tissue Distribution • Cassette PK • Microdialysis [blood, tissue, brain, skin] • Route of elimination (non-radiolablled)	• Human PK prediction • Prediction of efficacious dose for efficacy studies

In-life phase will be outsourced to partner CRO; Syngene will be one- point contact for hassle free work; Bioanalysis, PK data and reporting conducted at Syngene

Syngene

Synvent™

A specialized platform for integrated drug discovery and preclinical development

Syngene

SynVent^{TM} will help you make the right decisions, at the right time, to accelerate your drug discovery projects to successful clinical endpoints

SynVent^{TM} - Integrated Drug Discovery platform

- A leadership team of experienced drug-hunters

- Understand the targets and opportunities

- Develop detailed project plans with well-defined milestones

- Drive programs through candidate selection to IND-ready data packages

- Dedicated, cross-functional project management team

- Consultancy services

Meet the SynVent Team

SynVent Senior Leadership

Project Management

Jayashree Aiyar
VP Discovery Biology & Interim
Head SynVent Biology

BOB MARQUIS
Executive Director SynVent
Medicinal Chemistry
>33 years experience
GSK, Merck
Based: PA, USA

MIQUEL SALVA
Executive Director SynVent DMPK
>30 years experience
Almirall, Consultant
Based: Barcelona, Spain

HELEN JOSEPHINE
SynVent Portfolio Manager
>18 years experience
X-Chem Inc, FORMA Thera.,
Brandeis University
Based: Boston, USA

SynVent Team : Biology/Chemistry/DMPK/CDS

- 15 member scientific leadership team
- Each with >15 years drug discovery and project leadership experience in pharma & biotech

SynVent PM Team

- Dedicated PM team of 4 staff
- Experienced in multi-disciplinary, global project management

Mainly US- or Europe-based, fostering more frequent engagement with clients at the strategic level

SynVent will help you be successful in a challenging environment

EXPERIENCED DRUG HUNTERS

Combined experience of +300 years as drug discovery scientists and leaders; working across small molecules & biologics, early & late-stage discovery and into development

ADVANCED DATA ANALYTICS

Combination of commercial & proprietary data analysis tools including AI & ML, plus the expertise to leverage the most value, we can make faster progress with less compounds

INSIGHTFUL TRANSLATIONAL APPROACHES

DMPK profiling and predictions that enable rapid evolution of drug like properties, avoiding blind alleys

Understanding of patient populations & disease pathology, leveraging genomic and High Content Imaging at the single cell level for efficacy and patient stratification

RAPID, SEEMLESS EXECUTION

Providing leadership and guidance to the experimental teams in India, appointing scientists with the most relevant expertise to your project. Your points of contact to ensure the most efficient DMTA cycle with high quality, on-time delivery of data

SynVent are experienced at every step from Concept to Clinic

Target Identification and Validation	Hit Identification	Hit to Lead	Lead Optimization	IND Enabling	IND / Ph1 DE

SynVent

Target Hypotheses

Candidate selection

Research Plans & Objectives

Preclinical evaluation

Iterative Data Analysis & Interpretation

IND-ready data packages

Translational Strategies

Human Dose Projections

Success resulting from milestone driven, highly collaborative multifunctional teams

Strategic Leadership	High quality technical execution	Innovation

Choosing to have SynVent Project Leaders

We will assign SynVent Project Leaders to work with you as a primary point of contact

ROLE OF SYNVENT PROJECT LEADERS

- Be your partner to advise & support all scientific, strategic and operational aspects of the project, and ensure successful delivery of project objectives

- Work with you to construct a project plan with milestones and timelines

- Assemble the experimental team with the appropriate expertise for your project

- Identify and apply the most impactful capabilities & technologies

- Oversee the execution of the project through a cross-functional Core Team; resolve any issues and mitigate against delays

- Design experimental plans, conduct multiparametric data analysis, propose optimization strategies and next steps

- Respond to external forces including competitor disclosures, publications and patents

Concluding remarks

- Present-day drug design strategies allow the identification of drug candidates with remarkable drug-like properties

- Inadequate safety and efficacy now constitutes a primary cause of attrition in preclinical development through early clinical development

- Understanding the pharmacokinetics of our drug candidate is essential for success with special attention to:
  - ✓ Rodent and non-rodent single ascending dose and multiple dose PK
  - ✓ Drug-drug interactions
    - ○ CYP450 inhibition/induction
    - ○ Transporter panel substrate/inhibitor
  - ✓ Human dose prediction

- It is essential to establish a preliminary safety profile prior to the initiation of GLP toxicology studies for FIM:
  - ✓ Secondary pharmacology (Cerep panel)
  - ✓ Cardiovascular safety (hERG, Ca/Na channels, Langendorff, telemetered GP)
  - ✓ 14/28-day toxicology in rodents (non-rodents)
  - ✓ Adequate safety margins

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