Blood Brain Barrier studies in CNS drug discovery projects: Integration and Strategies to maximize their value

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COVANCE SYMPOSIUM
(Harrogate, UK)

3-4 October, 2018

Inspired by patients.
Driven by science.

Framework (1/2)

Main purposes of in vivo studies in Drug Discovery:

- To select compounds that deserve progression (decision making)
- To understand what drives the PK and the PD (drug design and translational)
- To predict (pharmacology study design and translational)

Considerations facing an in vivo study

- 3Rs (reduction, replacement, refinement)
- Cost – Value –Effectiveness (relevance, decision making,…)
- Practicalities (formulation, timeframe, risk of side effects, feasibility, readouts,…)

Framework (2/2)

Main challenges of CNS projects on New Targets:

- **Unknown biology** (RO/TE extension and duration?, biomarkers? Turnover?)
- **Alternative chemistry** (targets hardly druggable, covalent inhibitors,….)
- **Disease modifying** (long term pharmacology, often terminal semiquantitative readout)

Two main goals in early Drug Discovery

- **Non-clinical POC** (preclinical validation of the target)
- **Suitability of chemical series** (likelihood of delivering a candidate from the series)

Some suggestions from experience:

- **Work out what you look for.**
- **Expect the unexpected.**
- **Find creative solutions to deal with unknowns.**

Background

Blood Brain Barrier (BBB) assay is often the first in vivo assay in CNS Research projects.

The progression in technological capabilities can be used to adapt the BBB assay

Why?

Extract additional relevant information from the same experiment (3Rs-cost-time-value).

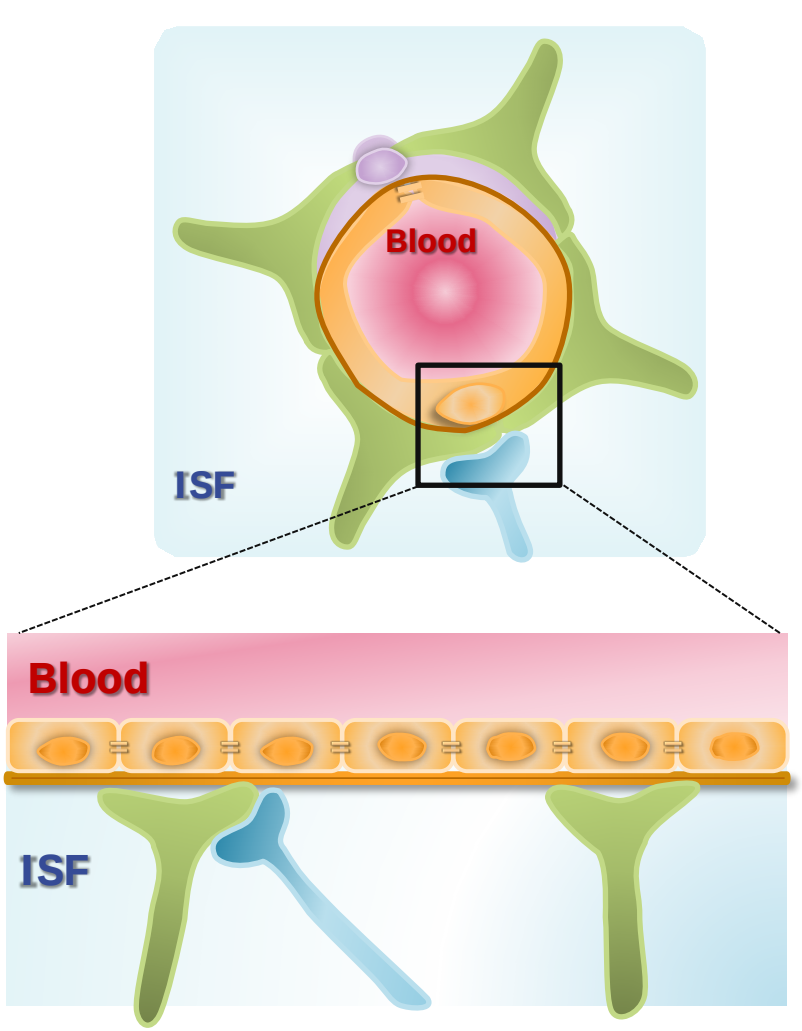
Use complementary information for a more robust decision on compounds to progress and asses their quality

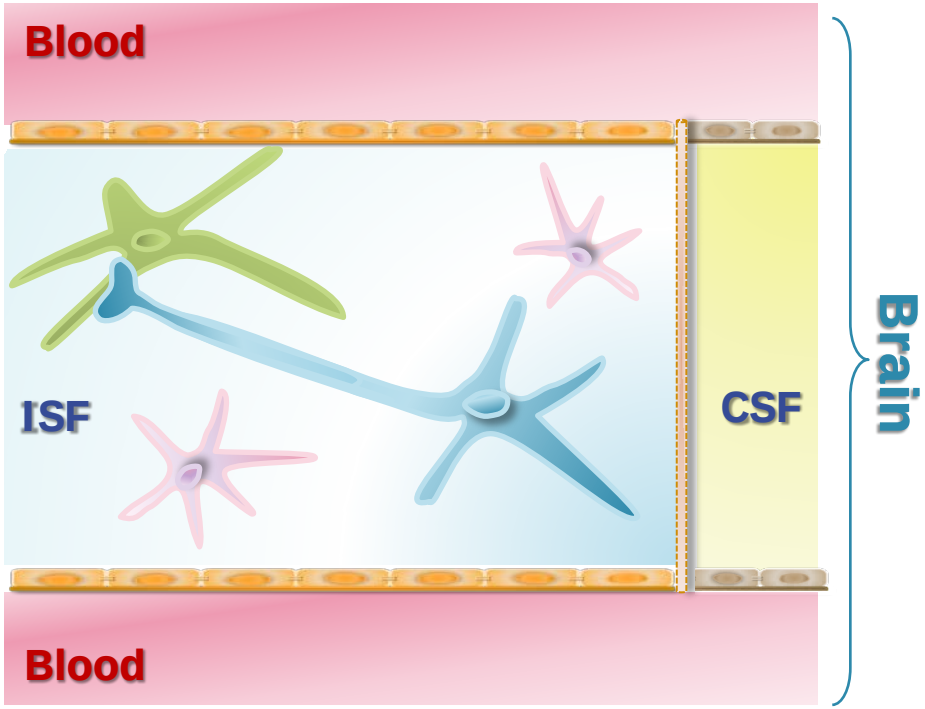
How?

Use cassette dosing and adapt the BBB assay to obtain a rough early estimate of PK parameters and/or a first PK profile of metabolites in plasma and brain

Optimize position of the assay in the screening cascade for timely and holistic data driven decision making

Blood Brain Barrier

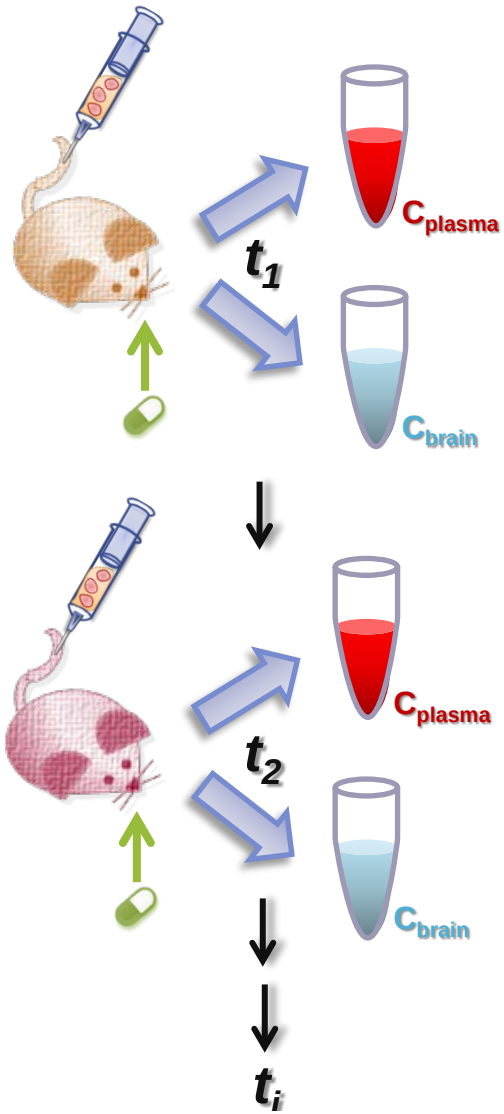




ISF: Interstitial fluid
CSF: Cerebrospinal fluid

Abbot et al. (2006) Nature Reviews 7:41-53
Lange (2013) J Pharmacokinet Pharmacodyn 40:315–326
Reichel (2009) Chemistry and Biodiversity 6:2030-2049

BBB Assay

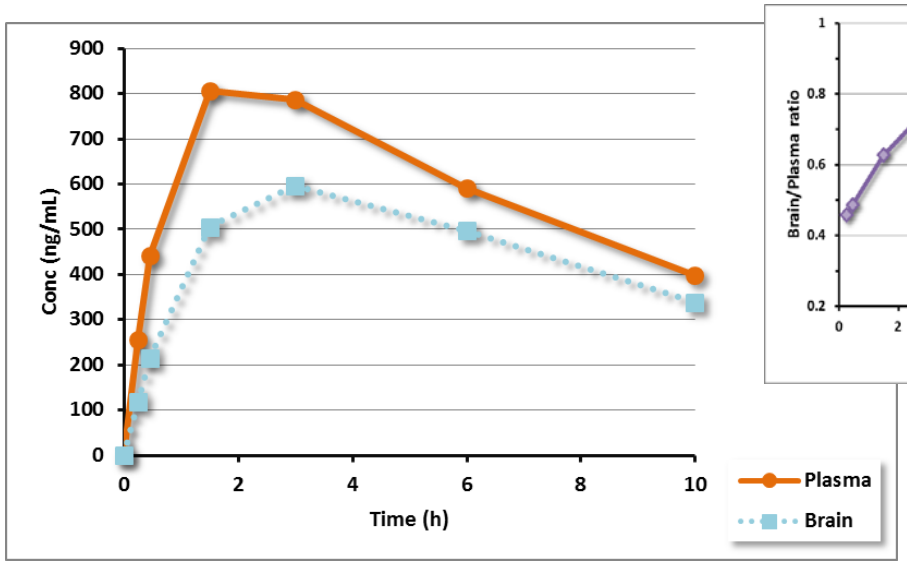


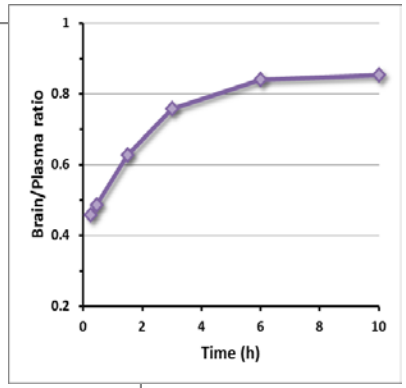
Fu measured by equilibrium dialysis
(plasma and brain homogenate)

$B/P = C_{brain} / C_{plasma}$

$Free\ B/P = C_{brain} \times fu_{brain} / (C_{plasma} \times fu_{plasma})$

$Cu_{brain} = fu_{brain} \times C_{brain}$





$K_p = AUC_{brain} / AUC_{plasma}$

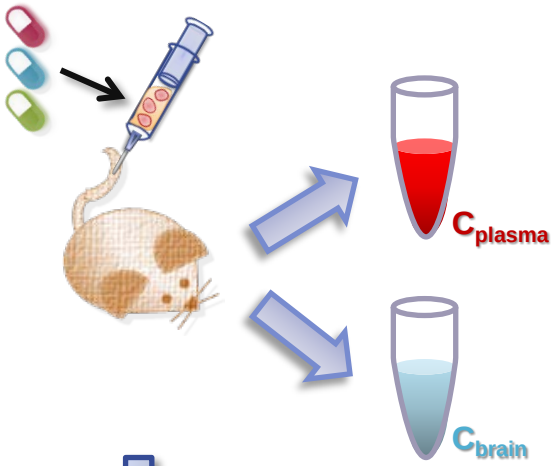
$K_{p,uu} = K_p \times fu_{brain} / fu_{plasma}$

Reichel (2009) Chemistry and Biodiversity 6:2030-2049
Summerfield & Dong (2013) J Pharmacokinet Pharmacodyn 40:301–314

Example (1/2) Refinement BBB assay (1/2)

BBB iv cassette dosing with estimation of PK parameters

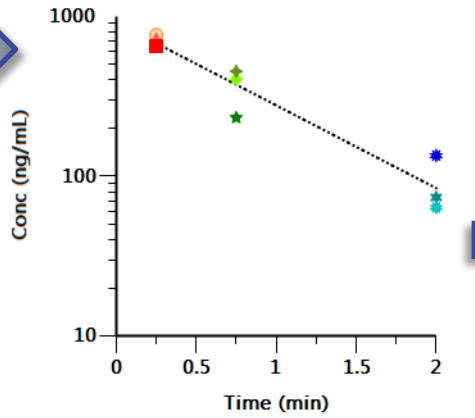
Adapted BBB screening assay for estimation of PK



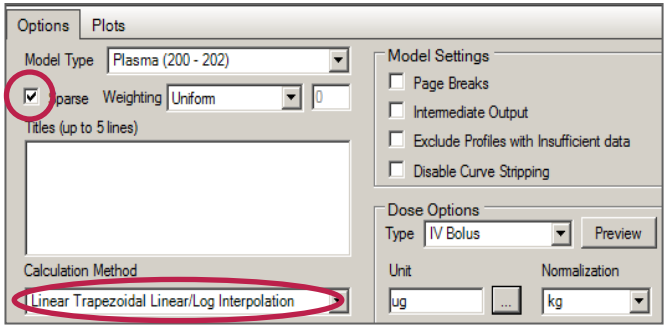
1) BBB iv cassette dosing protocol

- 3 compounds
- Dose: 0.5 mg/kg iv[(1)]
- Rat or mouse
- 3 time points, typically 15, 45, 120 min.
- n=3 by time point (9 animals)
- Whole blood/Plasma ratio (RBP) determined on first time point (15min)

2) Plot Log plasma concentration vs Time with regression line



3) Calculate Cl and Vss from plasma samples (NCA model, sparse method)



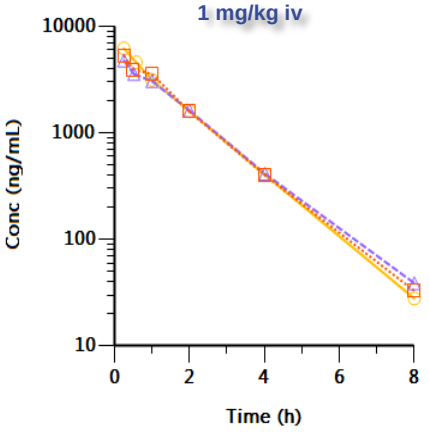
Cl_obs (mL/min/kg)	Vss_obs (L/kg)	t1/2 (h)	Vz (L/kg)	AUCall (h*ng/mL)	AUC∞ (h*ng/mL)	C0 (ng/mL)
9.54	0.56	0.75	0.62	740	874	930

[(1)]Formulation can be challenging

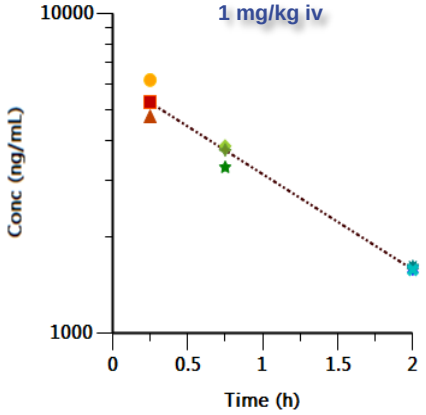
Estimation of PK parameters with 3 time points.

Magnitude of error depends on the PK profile

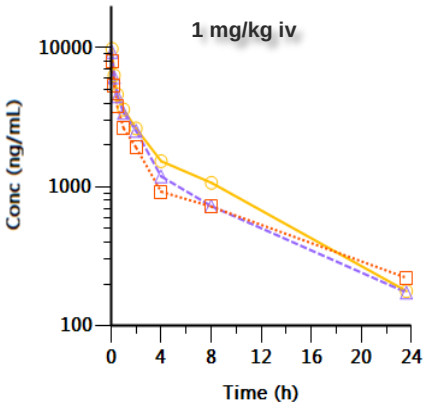
Compound A: One-compartment PK



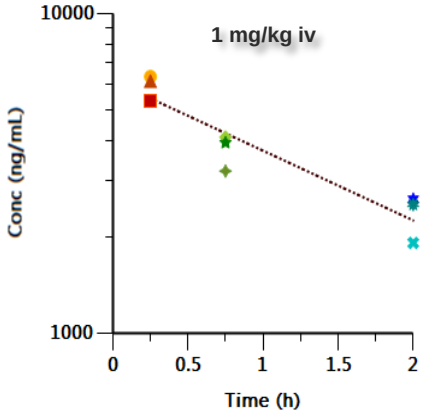
		Full PK	3 points PK
Cl_Obs	(mL/h/kg)	101 ± 5	107
Vss_Obs	(L/kg)	0.15 ± 0.2	0.15
t1/2	(h)	1.1 ± 0.1	1
AUC	(h*µg/mL)	9.96 ± 0.49	9.35
Vz	(L/kg)	0.15 ± 0.02	0.16



Compound B: Two-compartment PK



		Full PK	3 points PK
Cl_Obs	(mL/h/kg)	39 ± 5	79
Vss_Obs	(L/kg)	0.31 ± 0.1	0.16
t1/2	(h)	7.6 ± 1.6	1.4
AUC	(h*µg/mL)	25.9 ± 3.2	12.7
Vz	(L/kg)	0.15 ± 0.02	0.16



Pros and cons of adapted BBB screening assay

PROS

- Get first estimates of Clearance, Vss and half-life together with brain Kp and time to reach equilibirum (for 3 cpds in cassette). Together with RBP (blood-plasma ratio) (suitable for early assessment of IVIVE)
- Higher confidence (than just Kp & in vitro data) in decision making for progression to oral route.
- Allows very early use of in vivo data to evaluate quality of compounds and combination with in vitro data for multivariate analysis

CONCERNS

- Assume monocompartmental PK
- Only 3 time points
- Very rough estimate of Vss and Cl that could be far from reality
- Create confusion in Drug Discovery projects with more definitive PK parameters

MITIGATION PLANS

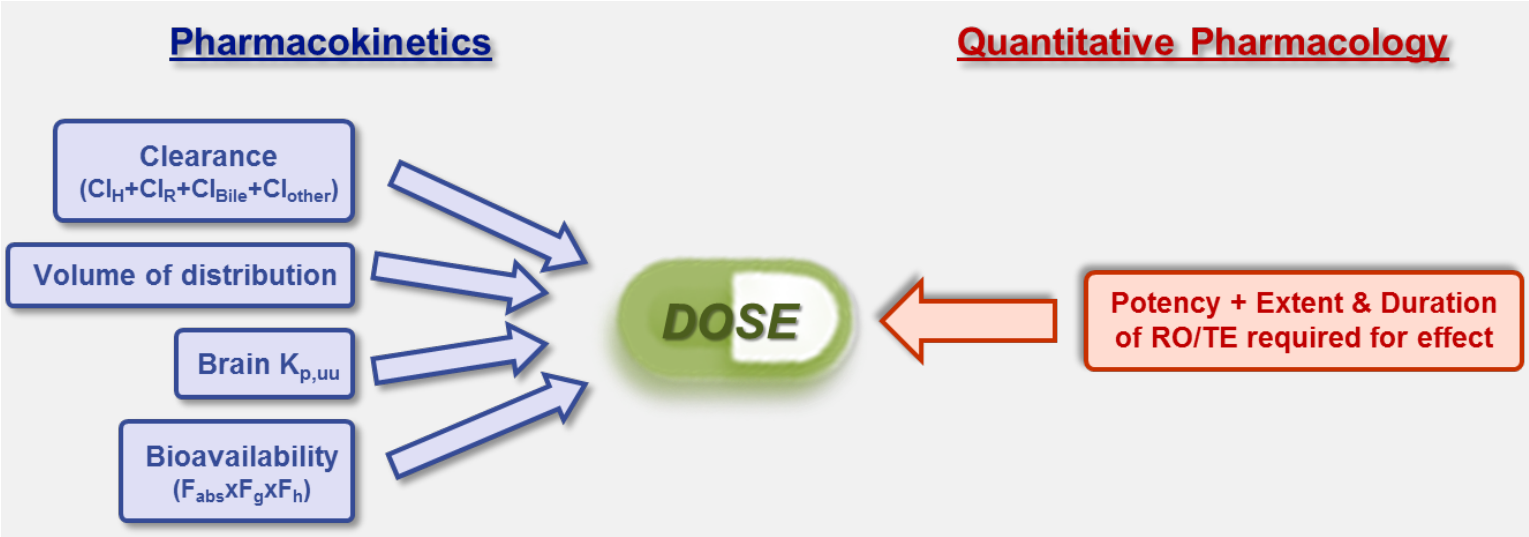
- Explore carefully the PK profile observed in the BBB assay (signs for bi-compartmental model)
- Verify approach in the chemical series with cassette iv PK
- Publish data with different name and different columns to avoid confusion with more definitive PK parameters

Integration of the BBBiv cassette dosing assay into the Drug Discovery screening cascade

The use of early dose prediction in Drug Discovery

Use of early dose prediction in human and rodent as a composite PK/PD parameter to support:

- **More robust decision making (compounds to prioritize, parameters to be optimized)**
- **Head to head comparison (≠ series, early vs advanced compounds, aimed TE,…)**



Bueters et al. (2015) Future Med. Chem. 7: 2369–2351
Mente et al. (2012) ACS Med. Chem. Lett. 3:515-519
Page (2016) Mol. Pharmaceutics 13:609-620
Peterson (2016) SMi 11th Annual ADMET conference

Early calculation of dose and assumptions

$$eDose_{(\frac{mg}{kg}/\text{day})} = \frac{EC_{av,ss\ (nM)} \times Cl \times 1440_{(min)} \times MW}{K_{p,uu} \times F \times 10^9}$$

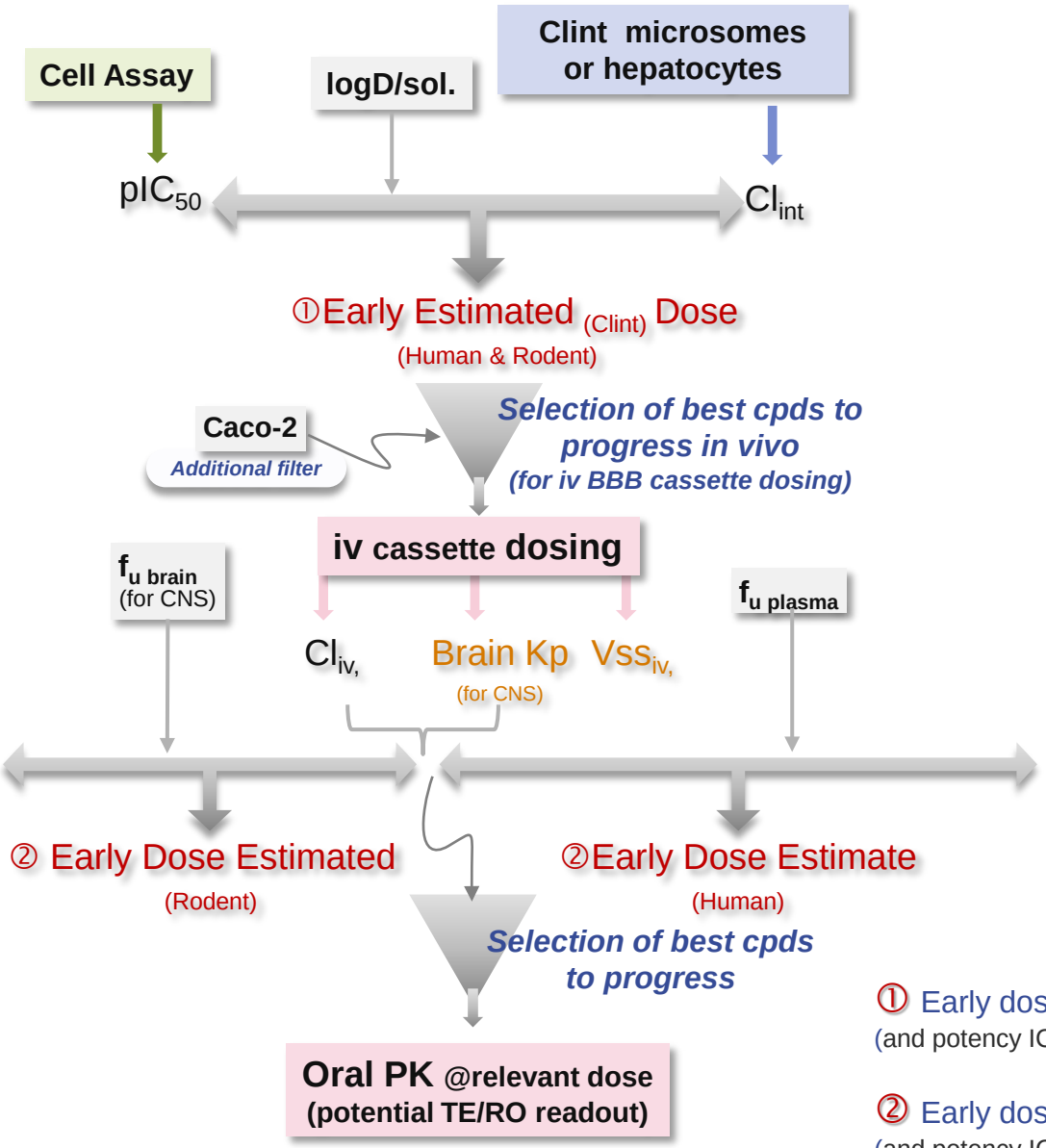
$EC_{av,ss\ (nM)}$ Relates to in vitro IC_{50}

Cl Obtained from Clint in vitro or Cl observed in vivo

Assumptions:

- **Efficacy depends on free Brain $C_{average\ @\ steady\ state} \geq$ In vitro IC_{50}**
- **In vitro IC_{50} accurate and good surrogate of activity in vivo**
- **Rodent cell IC_{50} = Human cell IC_{50}** (if Rodent cell IC_{50} is missing)
- **Fa 100% (full absorption and negligible gut metabolism)**
- **F= 1-Cl/Q_H**
- **$f_{u\ vitro\ brain}$ x total brain concentration = free brain concentration**
- **$Cl_{iv,BBB\ (3points)}$ good surrogate of in vivo Cl**
- **Brain/Plasma ratio measured by AUC good surrogate of brain Kp @ steady state**

Integration in Drug Discovery Screening Cascade

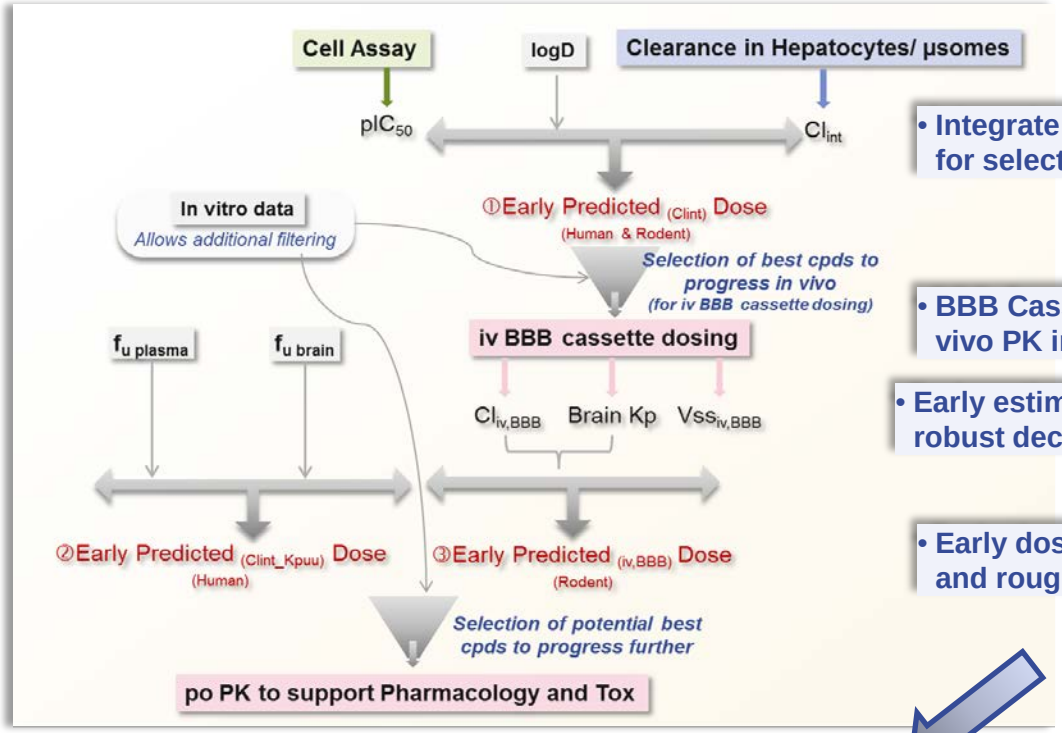


① Early dose estimate from in vitro clearance (and potency IC_{50})

② Early dose estimate from in vivo iv BBB data (and potency IC_{50})

Beyond compound selection (1/3)

Assessment of quality of compounds and project progression

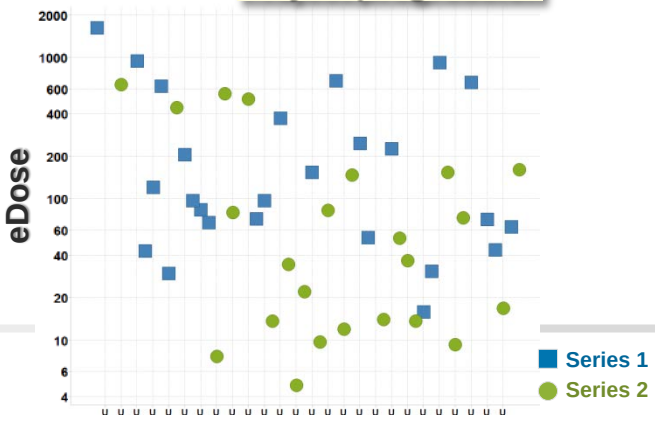


- Integrate quantitatively activity and clearance for selection of first screening in vivo
- BBB Cassette dosing including relevant in vivo PK information
- Early estimate of PK parameters allows a more robust decision which cpds progress further
- Early dose estimate allows comparison between cpds and rough assessment of quality of compounds

Compound ranking

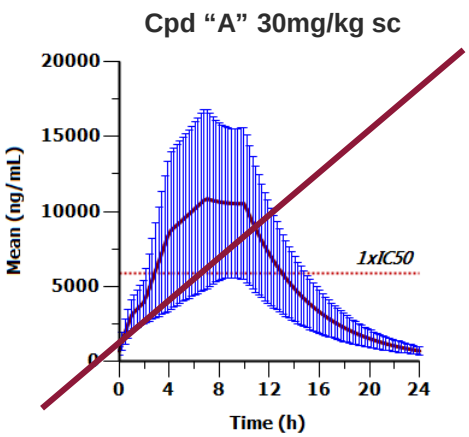
Compound ID	eDose RAT vitro Cl (mg/kg)	Brain Kp,uu	eDose RAT BBBiv (mg/kg)	Cell pIC50 RAT	Clint Hep RAT (µL/min/10^6¢)	Clobs BBBiv (mL/h/kg)
UCBxxxxxxx	2	0.26	15	8.5	51	0.8
UCBxxxxxxy	2	0.68	21	8.8	52	2.1
UCBxxxxxyy	1	0.04	24	9.1	83	1.9
UCBxxxxyyy	4	0.17	81	7.7	11	0.5
UCBxxxyyyy	5	0.14	99	7.5	12	0.9
UCBxxyyyyy	45	1	126	7.4	105	6.1
UCBxyyyyyy	4	0.22	204	7.3	6	0.3
UCByyyyyyy	5	0.81	279	7.3	9	0.3
UCByxxxxxx	15	0.18	369	8.3	254	17.6
UCByyxxxxx	17	0.17	396	7.2	24	0.6
UCByyyxxxx	64	0.56	612	8.3	731	28.2

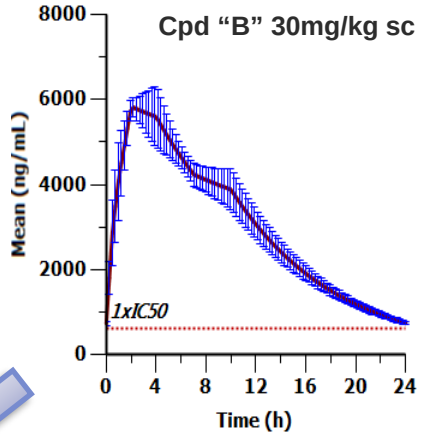
Project progression

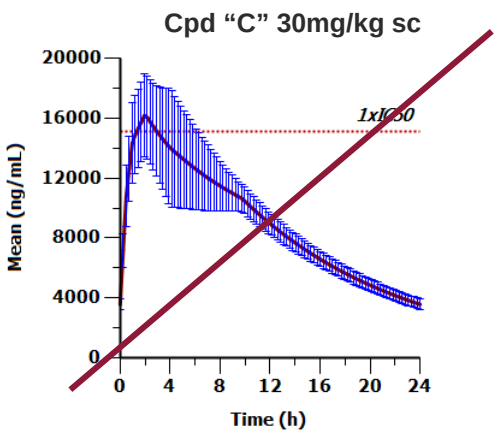


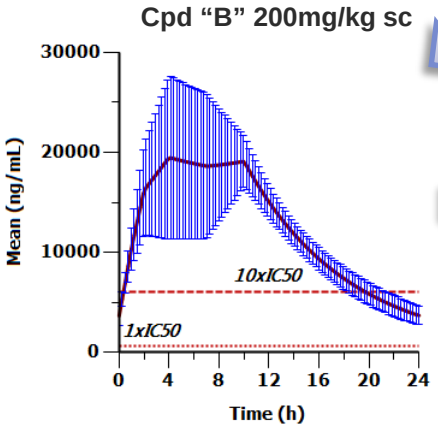
Beyond compound selection (2/3)

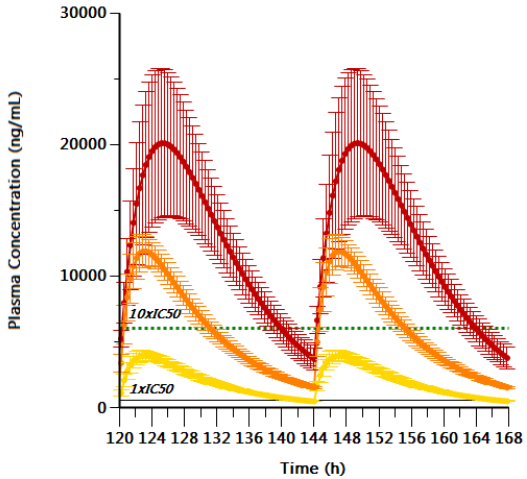
Selection of compound, dose and dose regimen for preclinical POC of a new Target

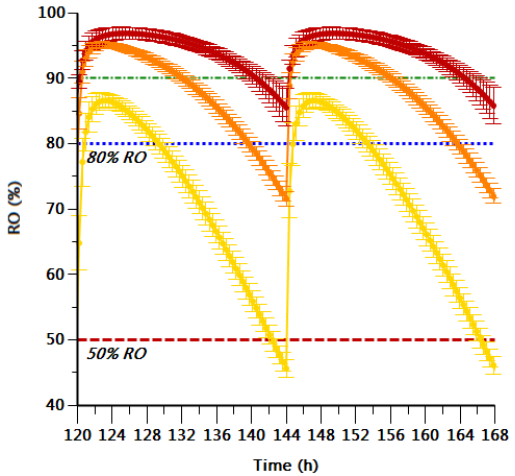








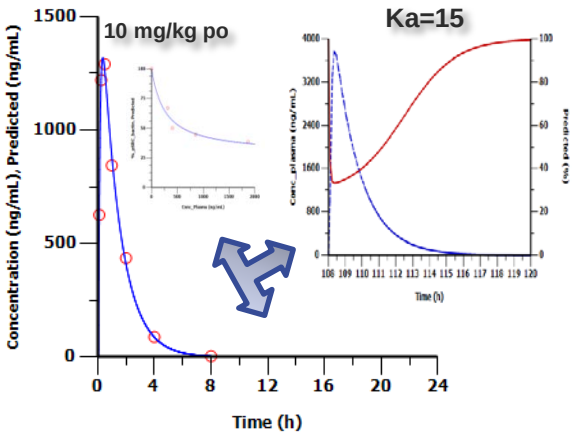




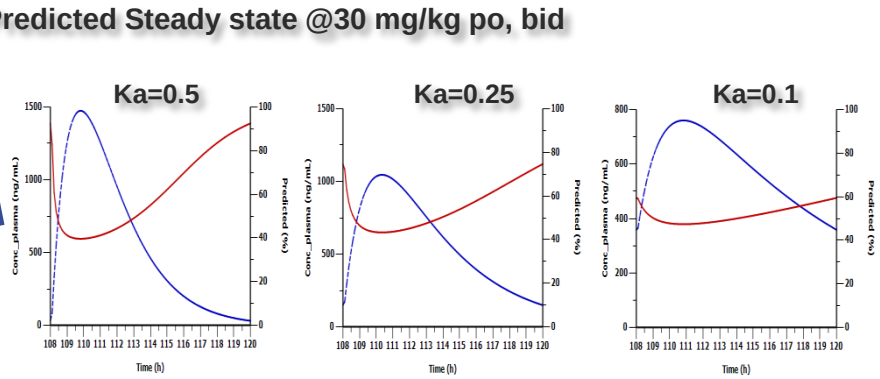
Dose, sc (mg/kg/day)	Min RO @ss (%)	Max RO @ss (%)	Ave RO @ ss (%)	RO>80% @ss (h)
20	46 ± 1	87 ± 1	71	≈ 9h
60	72 ± 1	95 ± 1	87	≈ 20h
200	86 ± 3	97 ± 1	94	24h

Beyond compound selection (3/3)

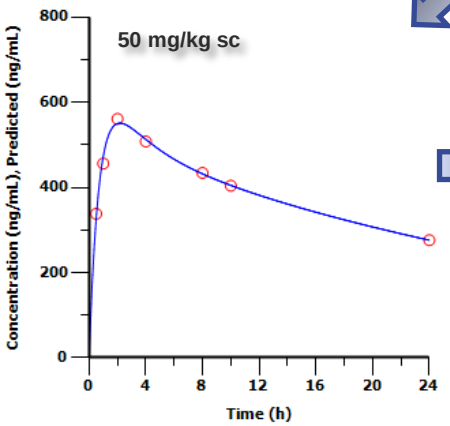
Rescuing a discarded compound for preclinical POC



- Compound “x” has a PK profile that does not allow appropriate duration of TE → it was initially **discarded as tool compound**

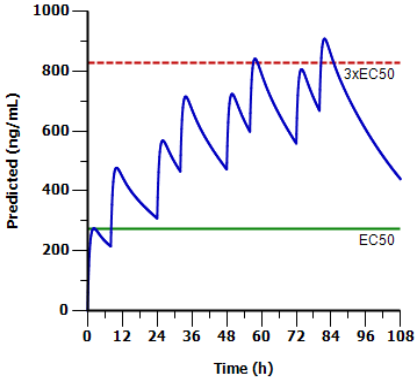


- It is predicted that delaying absorption can be an option to increase duration of TE. Slow release and sc administration were proposed

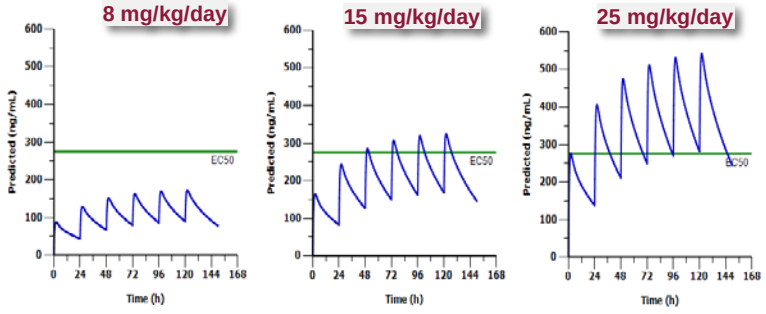


- SC administration leads to a flatter PK profile resulting in sufficient duration of TE → it was **nominated as tool compound**

Estimated exposure in tolerability study
4 days 2x25 mg/kg (50mg/kg/day)



- Appropriate PK profile for TE is observed but the dose regimen (2x25mg/kg/day) was not tolerated

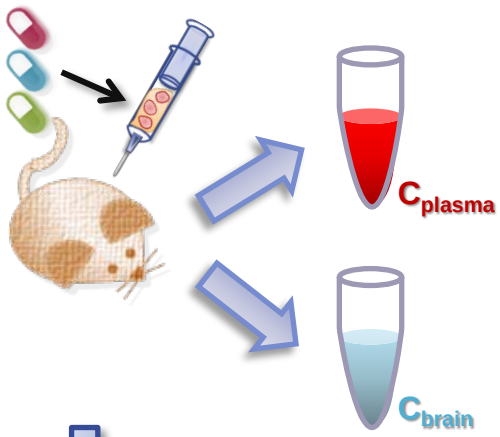


- It is predicted that 25 mg/kg/day sc od is at the limit to have TE≥50%. Lower doses are unlikely appropriate for a reliable POC experiment.

Example (2/2) Refinement BBB assay

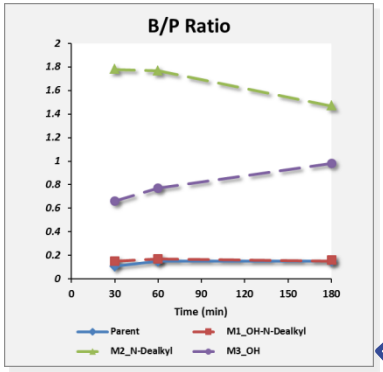
BBB ip cassette dosing with metabolic profiling

Adapted BBB screening assay for metabolite profiling



BBB ip cassette dosing protocol

- 3 compounds
- Dose: 10 mg/kg i.p.
- Rat or mouse
- 3 time points, typically 15, 45, 120 min.
- n=2 by time point (6 animals)
- Metabolic profile determined by HRMS



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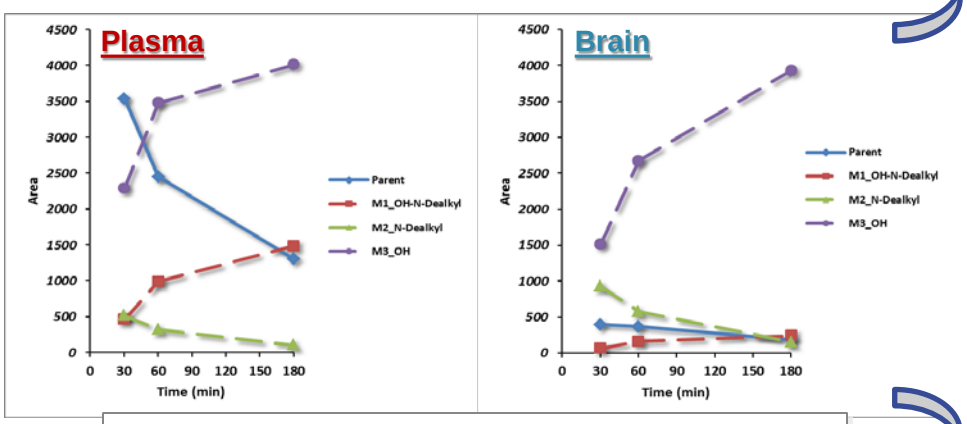
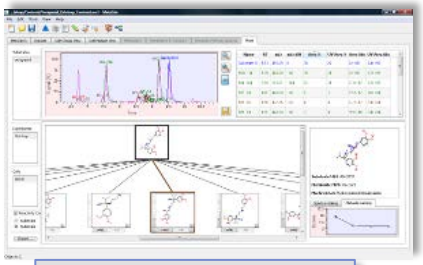
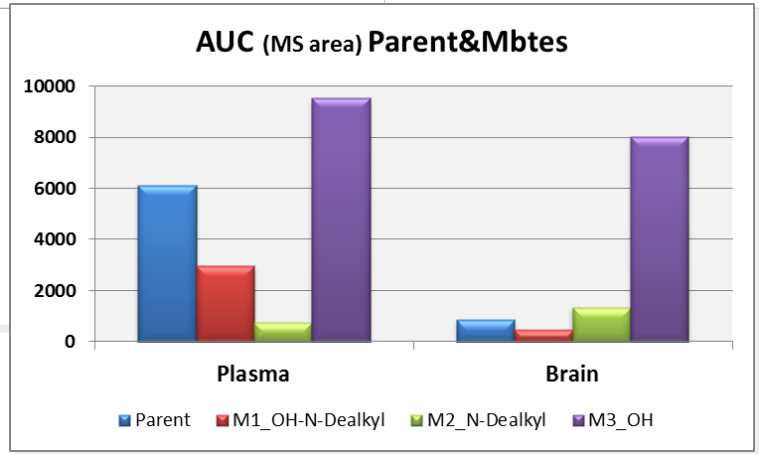
UPLC/HRMS Analysis

3X





Mass MetaSite
(Molecular Discovery)



(1)

(1)Main caveats: Quantification and differences in fu

Integration of the BBB metabolic profiling assay into the Drug Discovery screening cascade

The use of early in vivo metabolic profiling in Drug Discovery

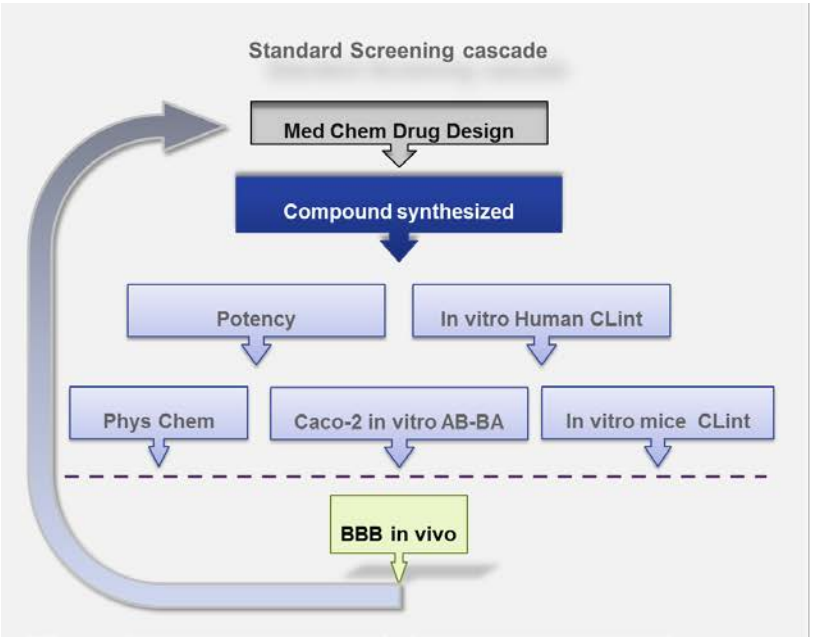
- **In vivo metabolite profile in conjunction with brain distribution can be instrumental to any CNS-Drug Discovery project.**

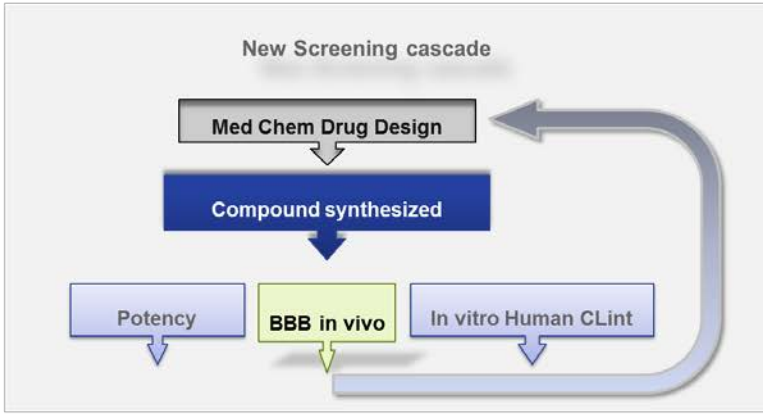
- **The technological advances in HRMS** (High Resolution Mass Spectrometry)**, software and *in silico* prediction of metabolites allow the simultaneous quantitative and qualitative analysis of in vivo samples for several compounds. This opens a window of new opportunities in the way we run Drug Discovery projects, design screening cascades, evaluate the DMPK properties of the chemical series and acquire PK/PD knowledge of the target.**

- **The value of obtaining in vivo brain distribution, identification of major metabolites and time-course profile (parent and metabolites) simultaneously for several compounds is of clear value:**
  - **3Rs**
  - **Get earlier understanding of the in vivo properties of the chemical space to impact more effectively drug design**
  - **Expand chemical space through exploiting information on metabolites**

Example of Integration of BBB cassette dosing with metabolic profiling in screening cascade & output

Exploiting HRMS technology to overcome the constraints on compound availability (challenging synthesis) and timelines for a focused CNS project





Information reported from BBB studies

- Brain/Plasma ratio time course of parent (3 cpds) and metabolites
- Comparison B/P ratio parent and metabolites
- Time course and AUC of parent and metabolites (MS) in plasma and brain
- Identification of main metabolites in vivo

- **The approach led to a better understanding of the relationship between structure, brain penetration and exposure in the chemical series.**
- **The qualitative/quantitative focus resulted in the identification of some metabolites with better CNS drug-like properties than the parent molecules.**
- **The in vivo cassette dosing identified some compounds as substrates and inhibitors of PgP and BCRP efflux transporters which had not been observed in preliminary in vitro experiments**

Summary

- **BBB assay is typically the first in vivo assay for CNS Drug Discovery projects.**
- **Technological evolution allows application of a different paradigm in the way screening cascades are designed, leading earlier to relevant and robust decision-making information and consistent with the 3Rs spirit**
- **The optimal design of the BBB assay and its integration in the screening cascade depends on several factors like the target, the chemical series, the challenges to be solved,… and the potential impact of the caveats in the approach.**
- **The ivBBB assay can provide an early rough estimate of PK properties (Cl, Vss,..) highly useful for decision making and in vitro/in vivo evaluation**
- **The ipBBB assay with metabolic profiling can give insight in the chemical species entering into the brain, their disposition, and the identification of main metabolic pathways in vivo.**

Acknowledgement

UCB

Claude Delatour

Pierre Bonnaillie

David Boucaut

COVANCE

Lisa Patterson

John Kendrick

Questions?