

Drug Transporter Substrate ID

Understand whether your investigational drug is a potential victim (object) of transporter-mediated drug-drug interactions by evaluating if it is a substrate of drug transporters *in vitro*. In addition, learn whether your drug is a substrate of a pharmacogenetic transporter (e.g. BCRP, OATP1B1) to inform on any potential impact on PK. Cyprotex has well-validated transporter substrate ID assay methodology and test systems that conform to the recommendations highlighted by regulatory authorities.



Intestine

P-gp*, BCRP*, OATP2B1, OCT1, MRP2, MRP3



Kidney

OAT1*, OAT3*, OCT2*, MATE1*, MATE2-K*, P-gp*, BCRP*, OAT2, OAT4, PEPT1, PEPT2, OCTN2, MRP2, MRP4



Liver

OATP1B1*, OATP1B3*, OCT1*, P-gp*, BCRP*, BSEP, OATP2B1, OAT2, NTCP, MRP2, MRP3, MRP4, preclinical Oatp1b



Blood Brain Barrier

P-gp*, BCRP*, OATP1A2, OATP2B1, MRP4

* regulatory required transporters

ABC transporters
(BSEP, MRPs, P-gp, BCRP)

Vesicle transport assay

- ▶ Compare uptake rate (+ATP) versus uptake rate (+AMP)
- ▶ Uptake ratio = $\frac{\text{uptake rate + ATP}}{\text{uptake rate + AMP}}$

SLC transporters
(OATPs, OATs, OCTs, MATEs, OCTN2, PEPTs, NTCP)

Cell uptake assay

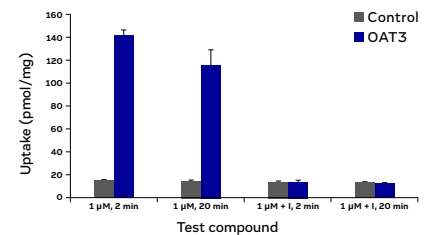
- ▶ Compare uptake rate in transporter expressing cells versus uptake rate in control cells
- ▶ Uptake ratio = $\frac{\text{uptake rate transporter cell}}{\text{uptake rate control cell}}$

ABC transporters
(P-gp, BCRP)

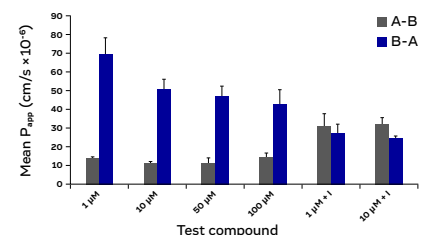
Bidirectional transport assay

(MDCK-MDR1 - P-gp)
(Caco-2 with verapamil - BCRP)

- ▶ Compare B-A P_{app} versus A-B P_{app}
- ▶ Efflux ratio = $\frac{B-A P_{app}}{A-B P_{app}}$



Transporter substrate
Uptake/Efflux ratio ≥ 2 , & reduced by >50% towards unity in the presence of inhibitor with corresponding decrease in +ATP uptake rate/transporter cell uptake rate/B-A P_{app} as appropriate





Your Partner in Transporter Drug-Drug Interaction Studies

- ▶ **Extensive experience:** Our team of experts have decades of combined published experience in transporter-mediated DDIs and contextualisation of *in vitro* data to clinical risk.
- ▶ **From discovery to development:** We offer a comprehensive range of transporter substrate assay formats applicable to either early discovery (screening; 1 or 2 concentrations) or to regulatory profiling stages (4 concentrations) during pre-clinical development, clinical development and on to new drug application.
- ▶ **Regulatory compliance:** We adhere to global regulatory guidance/guideline recommendations.
 - Full panel of transporters (P-gp, BCRP, OATP1B1/3, OAT1/3, OCT1/2, MATE1/2K): as recommended by the regulatory agencies depending on principal elimination route(s) of investigational drug.
 - Choice of analytical endpoint: LC-MS/MS (plus matrix matched calibration curve) or radiolabelled analysis (^3H or ^{14}C).

Assessing Victim (Transporter Substrate) Potential or Disposition along the Drug Discovery/Development Value Chain

Substrate Screening Assays

(if critical co-meds have to be co-dosed)

SUBSTRATE IDENTIFICATION (Screening)

– Assay formats (flexible)

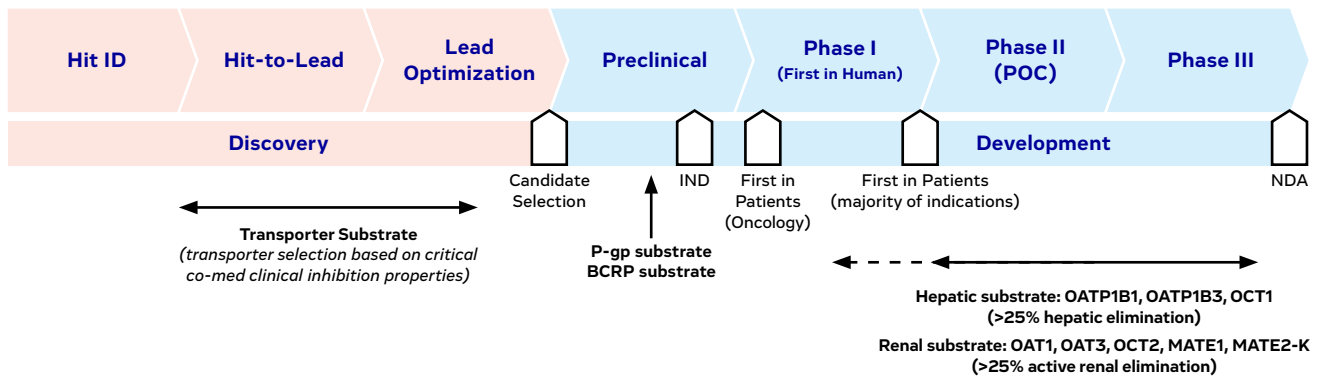
- ▶ 1 or 2 concentrations, 1 timepoint (duplicate or triplicate wells)
 - multiple test articles (medium to high throughput)
- ▶ 2 concentrations, 2 timepoints (triplicate wells)
 - single test article (SLCs/BSEP/MRPs & P-gp/BCRP vesicles)

Substrate ID Profiling Assays

SUBSTRATE IDENTIFICATION FOR REGULATORY REPORTING (Profiling)

– Assay formats

- ▶ 4 concentrations, triplicate wells
e.g. 1, 10, 50 & 100 μM
- ▶ 2 concentrations in presence of reference inhibitor, triplicate wells
e.g. 1 & 10 μM



Cyprotex Europe

Tel (UK): +44 (0) 1625 505100

No. 24, Alderley Park, Mereside, Cheshire SK10 4TG, UK

Cyprotex US

Tel: +1-888-297-7683

200 Staples Drive, Framingham, MA 01702, USA