cyprotex
AN EVOTEC COMPANY

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)

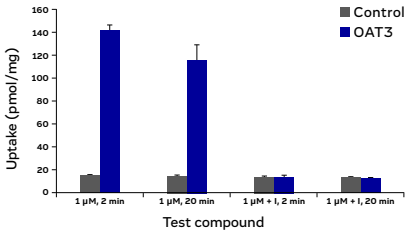
Cell uptake assay

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

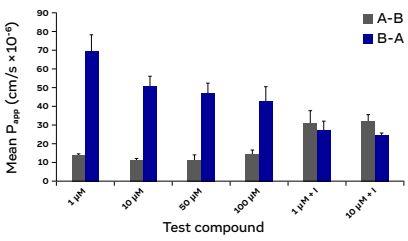
ABC transporters (P-gp, BCRP)

Bidirectional transport assay
(MDCK-MDR1 - P-gp)
(Caco-2 with verapamil – BCRP)

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



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 (^{3}H 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)

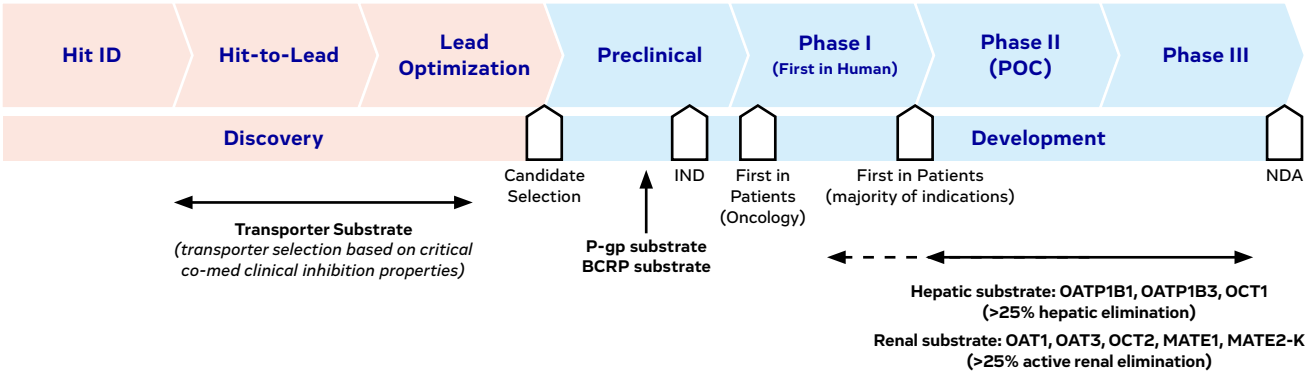
- 1 or 2 concentrations, 1 timepoint (duplicate or triplicate wells)
  – *multiple test articles (medium to high throughput)*
- 2 concentrations, 2 timepoints (triplicate wells)
  – *single test article (SLCs/BSEP/MRPs & P-gp/BCRP vesicles)*

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

enquiries@cyprotex.com | www.cyprotex.com

2024/07 | V1