Transporter Inhibition

Determine whether drug candidates are transporter inhibitors

Transporters play a major role in absorption, distribution, and excretion of endogenous and exogenous compounds and are found in the intestine, liver, kidney, brain, and other tissues. It is critical to evaluate and understand the role of transporters in drug disposition to predict the pharmacokinetics and toxicology of drugs. Moreover, if a drug candidate is a transporter substrate or inhibitor, there is a potential for transporter-mediated drug-drug interactions (DDIs) with concomitantly administered drugs.

Regulatory agency guidance documents emphasize the importance of evaluating the potential for transporter-mediated DDIs and recommend determining the substrate and inhibition potential of drug candidates for clinically relevant drug transporters. Data from *in vitro* transporter assays are used to assess whether clinical DDI studies are required and can ensure patients are not unnecessarily excluded from clinical trial participation.

BioIVT offers definitive transporter studies that use validated test systems and comply with the recommendations of regulatory agencies.

Fit-For-Purpose Study Designs

Tier 1: Basic Program - Minimal study design with customization options for regulatory agency compliance.

Tier 2: IND-Enabling Program - Provides IND submission-ready data and comprehensive standard reports.

Tier 3: IND-Enabling Program with eCTD Report - All the study rigor of Tier 2, with eCTD readiness and the option of GLP.

Three standard study designs are available in addition to customized research programs. BioIVT's ADME experts offer guidance to ensure studies are designed to meet clients' R&D objectives.

Study Design

Standard Study Design

Element	Feature	Tier 1	Tier 2	Tier 3
Design	Appropriate test systems based on the transporter: Caco-2, MDCKII, HEK293, membrane vesicles	✔	✔	✔
	Transporter panel as recommended by regulatory guidelines as shown in Table 1	✔	✔	✔
	Drug candidate concentrations	2	≤7	≤7
	Time points	1	1	1
	Replicates	n=3	n=3	n=3
	Positive control inhibitors	1 or 2	1 or 2	1 or 2
	Inhibition potential based on % inhibition	✔	✔	✔
Deliverables	IC_{50} determination (if applicable)	Follow-up option	✔	✔
	Data meeting FDA/EMA/ICH submission standards	Possibly[1]	✔	✔
	Standardized report - summary, materials/methods, results/conclusions, data tables, figures, supporting information	✔	✔	
	Fully submissible report meeting FDA/EMA/ICH submission standards and formatting for eCTD submission			✔

[1] Meets FDA/EMA/ICH standards if no inhibition is observed. If inhibition is higher than 50%, IC_{50} determination is required to meet regulatory guidelines.

Customizations to the standard design*:

- Preliminary evaluation of stability, toxicity, and non-specific binding
- Complex drug candidate preparation
- Low binding materials
- Modifications to standard assay conditions or procedures
- Customized deliverables
- Follow-up and non-standard transporter assays

**Customization options depend on the tier*

Transporter Inhibition

Methodological Considerations and Test Systems

BioIVT's transporter inhibition study design includes the following test systems dependent on the type of transporter assessed:

- ABC (efflux) transporters P-gp and BCRP are tested in Transwell plates using Caco-2, MDCKII-MDR1 or MDCKII-BCRP cell lines. Probe substrates are added to either the apical or basal side of the monolayer. The efflux of the probe substrate is measured in the presence of different drug candidate concentrations to evaluate the inhibitory effect of the drug candidate.
- MRP2 and BSEP (and occasionally P-gp and BCRP) are evaluated using an inside-out membrane vesicle prepared from insect cells transiently transfected with a single efflux transporter or from mammalian cells chemically selected for the expression of a transporter. The inhibitory effect of the drug candidate is evaluated by measuring the uptake of the probe substrate in the presence of multiple concentrations of the drug candidate.
- SLC (uptake) transporters are assessed in HEK293 cells expressing human transporters. To determine if a drug candidate is an inhibitor of an uptake transporter, its ability to block the uptake of probe substrates is measured in the presence of multiple concentrations of the drug candidate.

Regulatory agencies recommend evaluating the inhibition potential for the transporters listed in Table 1, and additional clinically-relevant transporters as necessary.

Study Deliverables

Transporter inhibition study reports include graphic and tabular illustrations of transporter inhibition. IC_{50} values are calculated if applicable and presented in a table as illustrated in Table 2.

Table 1. List of transporters recommended by regulatory guidelines to be evaluated for inhibition

Transporter	Clearance	FDA	EMA	ICH M12
P-gp	Efflux	✔	✔	✔
BCRP		✔	✔	✔
BSEP			Consider	
OATP1B1	Hepatic	✔	✔	✔
OATP1B3		✔	✔	✔
OCT1			Consider	
OAT1	Renal	✔	✔	✔
OAT3		✔	✔	✔
OCT2		✔	✔	✔
MATE1		✔	✔	✔
MATE2-K		✔	✔	✔

Additional Considerations

With over 100 transporters available, BioIVT offers the largest selection of single-transporter assays using its proprietary OPTI-EXPRESSION® Technology which allows the transient transfection of polarized cell monolayers. Panels are available to evaluate transporters in specific areas such as neurological diseases or adverse drug reactions. Furthermore, OPTI-EXPRESSION Technology allows co-expression of transporters in polarized mammalian cell monolayers and BioIVT offers models with up to five transporters to investigate how various transporters work together to move drug candidates throughout the body.

Table 2. OCT2 inhibition in HEK293 cells and calculation of IC_{50}

Probe substrate	Inhibitor	Inhibitor (mM)	Uptake rate (pmol/mg/min) (mean ± SD)		Background-corrected uptake rate (pmol/mg/min)	% of control	IC_{50} parameters
			Control	OCT2			
[^{14}C]-metformin (10 µM)	No solvent control	0	6.14 ± 0.31	91.5 ± 4.4	85.3	NA	NA
	Solvent control	0	4.79 ± 1.41	92.5 ± 8.3	87.7	100	IC_{50}: 3.64 mM Slope: 1.67 Min: 0% Max:105%
	Drug candidate	0.025	4.67 ± 0.28	94.6 ± 3.7	89.9	103	
		0.075	5.08 ± 0.12	99.8 ± 7.4	94.7	108	
		0.25	4.92 ± 1.02	94.9 ± 6.3	90.0	103	
		0.75	5.12 ± 1.50	91.7 ± 4.2	86.5	99	
		2.5	3.57 ± 0.25	62.6 ± 6.6	59.1	67.3	
		7.5	2.55 ± 0.70	25.5 ± 3.1	22.8	26.0	
		25	5.81 ± 1.74	3.55 ± 0.93	0	0	