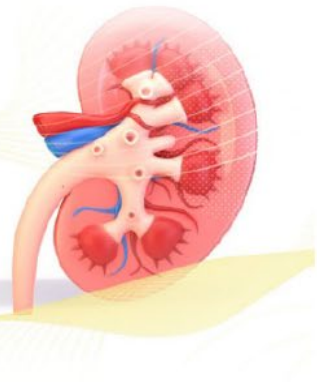
aProximate™



KIDNEY

Kidney Transporter Assays

The most advanced near-physiological high throughput kidney proximal tubule cells (PTC) model to Investigate drug transport modalities *in vitro*.

For new drugs, early understanding of drug handling in the kidney PTCs is a common strategy to mitigating the risk of failure during preclinical and clinical development. More so, as several drugs and metabolites are handled by the same transporters. For example, creatinine, an endogenous metabolite, and the immunomodulating drug pyrimethamine are both substrates for basolateral OCT2 and apical MATE transporters. OATs and MATE transport organic anions, organic compounds like creatinine but also organic cationic drugs, such as metformin, a common drug used to treat Type 2 diabetes.

The excretion of creatinine can be blocked by administering transporter inhibitors such as cimetidine and pyrimethamine. In addition, inhibition of OCT and MATE transporters by cimetidine and pyrimethamine *in vivo* also reduces metformin renal clearance. Cimetidine interferes with the uptake of metformin by proximal tubule cells and pyrimethamine with efflux of metformin. *In vivo*, this leads to a significant increase in systemic exposure and a decrease in metformin renal clearance because metformin and pyrimethamine compete for efflux mediated by MATEs.

These interactions are complex but can be predicted *in vitro* in human aProximate™ PTCs: metformin's basolateral to apical flux (JBA) is significantly reduced by OCT and MATE inhibitors cimetidine and pyrimethamine. This demonstrates interactions between new drugs and renal transporters can be evaluated *in vitro*, to shed light on how new drugs are handled by kidney proximal tubule cells.

In vivo

Transporter	Drug	Transporter Inhibitor	AUC fold increased	CL_R decrease (%)
hOCT2, hMATE1, and hMATE2-K	Metformin	Cimetidine	1.5	28
	Metformin	Cimetidine	1.5	45
	Metformin	Pyrimethamine	1.4	35
	Metformin	Dolutegravir	2.5	N.D.

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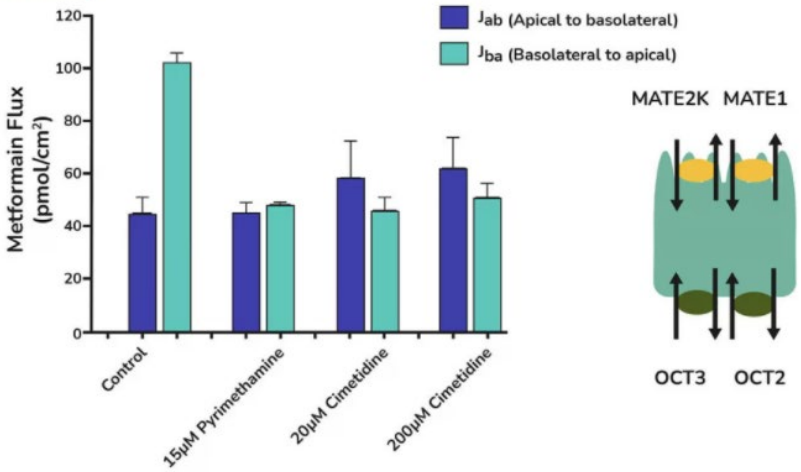

Best in class *in vitro* model to most accurately predict *in vivo* outcomes

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NEWCELLS
BEST BIOLOGY DRIVING *IN VITRO* INNOVATION

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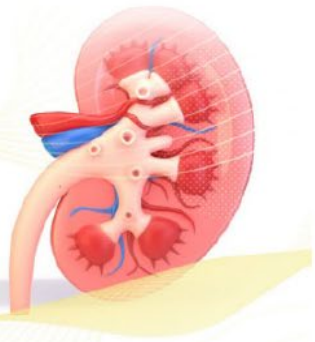

KIDNEY

Validated Model

With most key kidney transporters expressed in the aProximate™ platform, the transport pathway of new drugs can be easily elucidated and validated to support regulatory applications

Gene	Percentage of native kidney expression			
	aProximate™	HK2	REPTEC	HEPTEC
MDR1	65.2 ± 7.1	34	26	28.1
BCRP	31.3 ± 5.5	ND	TBC	TBC
MRP2	31.5 ± 33	1	6	7
MRP4	29.3 ± 4.8	26	24	81
OAT1	20.6 ± 4.6	ND	ND	ND
OAT3	27.8 ± 6.7	ND	ND	ND
OCT2	39.7. ± 4.3	ND	1.8	3.3
OATP4C1	39.0 ± 2.7	28	34	47.6
SLC2A9	27.7 ± 4.8	ND	ND	ND
URAT1	34.6 ± 9.2	ND	ND	ND
MATE1	36.4 ± 4.2	ND	0.6	0.1
MATE2K	15.1 ± 8.8	ND	0.3	ND

aProximate™ PTC remain extremely well differentiated

- ✓ mRNA levels ~ 30% of fresh tissue levels
- ✓ c.f. immortalised human kidney cell lines 1-5% and many at 0% expression
- ✓ Extensive western blot data of protein expression
- ✓ Extensive FUNCTIONAL data of transporter expression

aProximate™ outperforms competition

- ✓ Identification of clinically important transporter-mediated Drug-Drug Interactions (DDI) during drug development and post market in clinic (drug induced AKI)
- ✓ Identification of transporter-mediated renal drug clearance pathways for xenobiotics during drug development
- ✓ Application of renal model to identify renal target and target engagement/efficacy

- ✓ Identification of cross species differences in renal drug handling – de-risking adverse outcomes at first in man
- ✓ Identification of drug induced kidney damage using clinically relevant biomarkers of nephrotoxicity cross species as a predictive tool to improve 'first in man' outcomes
- ✓ Development of screening regime for biologics transport and toxicity

Example Protocol

Assay Format

- Primary isolated kidney proximal tubule epithelial cells cultured on a 24-well Transwell® plates

Species Available

- Human, rat, mouse, dog and NHP

Time points and replicates

- 0, 30, 60, 90 minutes
- Triplicates per concentration

Measurements

- Apical to Basal (Ja-b) and Basal to Apical (Jb-a) flux
- Intracellular accumulation
- Trans-epithelial electrical resistance (TEER)

Test article requirements

- Radiolabelled test article or GC/LC analysis
- Volumes added typically 0.2 ml (apical) and 1 ml (basolateral) per well
- Six dose concentrations

Controls

- Paracellular flux Is evaluated by measurement of radiolabelled mannitol flux
- Control substrates for transporters of interest

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