NEWCELLS
IN VITRO MODELS TO ACCELERATE CLINICAL TRANSLATION

The aProximate™ model for renal drug safety/efficacy and transporter studies

The predictive go-to-model for any proximal tubule cells (PTC)-based study

What you can achieve:

- Accurate prediction of nephrotoxicity with sensitive readouts for kidney injury markers
- Gain insights into drug transport, uptake flux and drug-drug interactions
- Compare species responses *in vitro* prior to animal studies gaining confidence to move into *in vivo* studies

What forms the basis of the study:

- Primary proximal tubule cells (PTCs) with high transporter expression levels
- High-throughput format (96-well) for rapid nephrotoxicity screening
- Assay-ready plates with freshly isolated PTCs available for shipping globally

How can Newcells help

Design and deliver your customized study: renal safety and efficacy studies performed by our in-house experts

Ready-to-order aProximate™ plates: 24-well and 96-well plates available for direct shipment for you to use in your facility

What makes aProximate™ a superior proximal tubule cells model ?

1. Composed of primary PTCs cultured on Transwells™ that remain polarized and form tight junctions even outside their native environment.
2. aProximate™ PTCs retain expression of relevant transporter proteins involved in drug handling, unlike commercially available cell lines, **(see Table 1).**

Table 1: Higher expression of key renal transporters in aProximate™ PTCs compared with commercial cell lines. (TBC: To be confirmed, ND: Not detected).

Transporter Gene	Comparison of aProximate™ PTC with kidney cell lines. Percentage of transporter mRNA expression level normalised to fresh kidney tissue.			
	Human aProximate™ PTC	HK2	REPTEC	HEPTEC
MDR1	65.2 ± 7.1	34	26	28.1
BCRP	31.3 ± 5.5	ND	TBC	TBC
MRP1	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

Megalin and cubilin expression in aProximate™ PTCs

- Megalin and cubilin, two endocytic receptors present at the apical membrane are expressed in aProximate™ PTCs and can be used to model uptake of proteins and aminoglycosides **(Figure 1).**

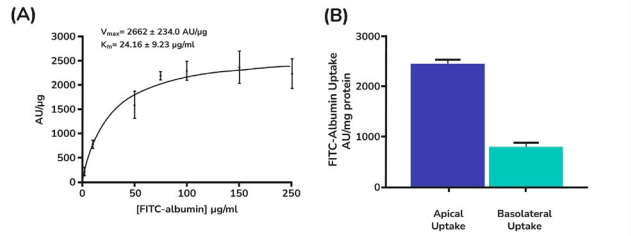

Figure 1: Functional expression of megalin and cubilin in aProximate™ PTCs determined by albumin uptake.

(A) FITC-labelled albumin uptake measured by units of albumin (AU) per microgram of total cellular protein at increasing concentrations of albumin in the media. (B) FITC-labelled albumin uptake represented by apical and basolateral uptake (AU per milligram of total cellular protein). Data is represented as average ± standard deviation.

Transporter-mediated drug-drug interactions (DDI)

- *In vitro* evaluation of complex interactions between new drugs and transporters, in accordance with FDA guidelines.
- Assessment of new compounds as substrates for renal transporters. The aProximate™ model has been used to replicate *in vitro* the decrease in metformin clearance (J_{ba}) in the presence of cimetidine and pyrimethamine **(Fig. 3C)**, which is usually observed *in vivo* **(CLR % decrease in Fig 3A)**. Similarly, we can predict in vitro a decrease in renal clearance of para-amino hippurate (PAH) in the presence of probenecid, an OAT transporter inhibitor **(Fig. 3D)**.

aProximate™ for assessing drug-induced kidney injury (DIKI)

- Sensitive *in vitro* nephrotoxicity assessment of compounds (including toxicity undetected in animal studies).
- Functional kidney-injury markers readouts. In a collaborative study with Takeda Pharmaceuticals, the aProximate™ platform was used to evaluate the safety of 9 known compounds with ATP, TEER, LDH release and kidney-injury marker readouts (Bajaj et al Toxicology Sept. 2020).
- High sensitivity and specificity. The model has been shown to correctly classify four of six true positives and two of three true negatives out of a testing panel, validating the *in vitro* model for detection of PTC toxicants **(Figure 2).**

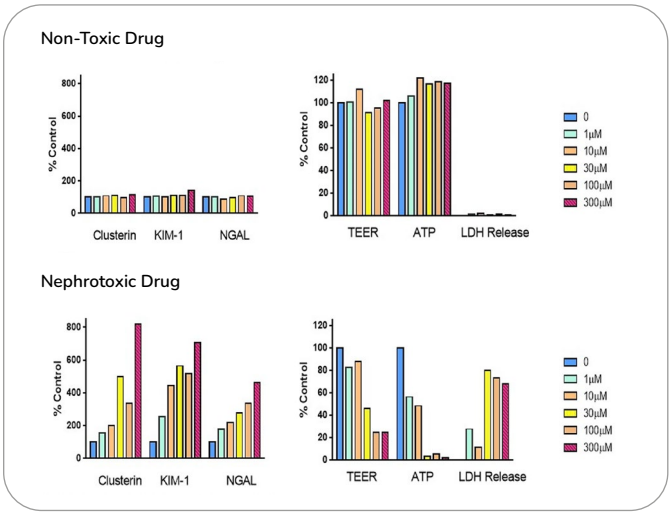

Figure 2: Expression of biomarkers and functionality of aProximate model upon exposure to non-toxic and toxic drugs. Human aProximate™ PTCs were exposed to known non-toxic (top) and toxic (bottom) drugs and reacted as expected when monitored for functionality, integrity and viability. The parameters measured were: approved biomarkers of renal toxicity KIM-1, NGAL and clusterin and the expression of non-specific injury biomarkers, transepithelial electrical resistance (TEER), ATP-based cell viability and LDH release. For the cytotoxic drug (bottom panel), kidney injury-specific biomarkers increased in a dose dependent manner while non-specific kidney markers such as TEER and ATP decreased. For the non-toxic drug (top panels), both specific and non-specific biomarkers were unaffected by increasing concentrations of the drug.

A:

In vivo

Drug clearance studied	Secondary Drug	AUC fold increase	CL_R decrease (%)
Metformin	Cimetidine	1.5	28
Metformin	Cimetidine	1.5	45
Metformin	Pyrimethamine	1.4	35
Metformin	Dolutegravir	2.5	N.D.

B:

In vivo

Drug clearance studied	Secondary Drug	AUC fold increase	CL_R decrease (%)
Parahippurate	Probenecid	3.3	83
Furosemide	Probenecid	2.7	67
Furosemide	Probenecid	3.1	80
Cidofovir	Probenecid	1.8	52
Fexofenadine	Probenecid	1.5	73
Fexofenadine	Probenecid	1.5	70

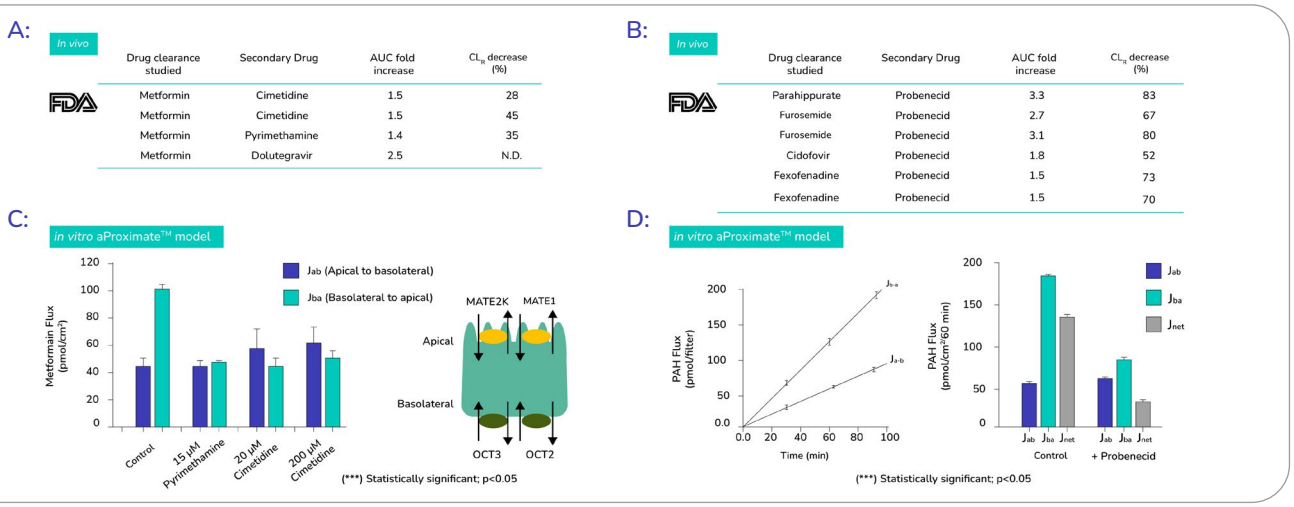

Figure 3: Correlation between drug-drug interaction studies in vivo (A, B) and in vitro (C, D). (A, B) Clinical data compiled by the FDA showing how the renal clearance (CL) of a drug is modified by adding a second drug. The area under the plasma drug concentration (AUC) reflects the body exposure to a drug after administration and is dependent on the rate of elimination. (C) in vitro clearance of metformin (J_{ba}) is decreased in the presence of the same secondary drugs as in vivo (A). (D) in vitro clearance of para-aminohippurate (PAH) showing comparable results to the clinical data (B) with a decrease (J_{net}) upon addition of probenecid, an inhibitor of the OAT transporters in the basolateral membrane.

Cross-species comparison of drug handling

- Testing of drug handling in different species *in vitro* prior to animal studies. aProximate has been used to show in vitro the different efficiency and way in which triclopyr (TCP) is handled via OAT 1 in different species.
- The ability to show *in vitro* that TCP drug handling in dogs differs from humans highlights that aProximate™ is a powerful tool to support the selection of relevant species for preclinical studies.

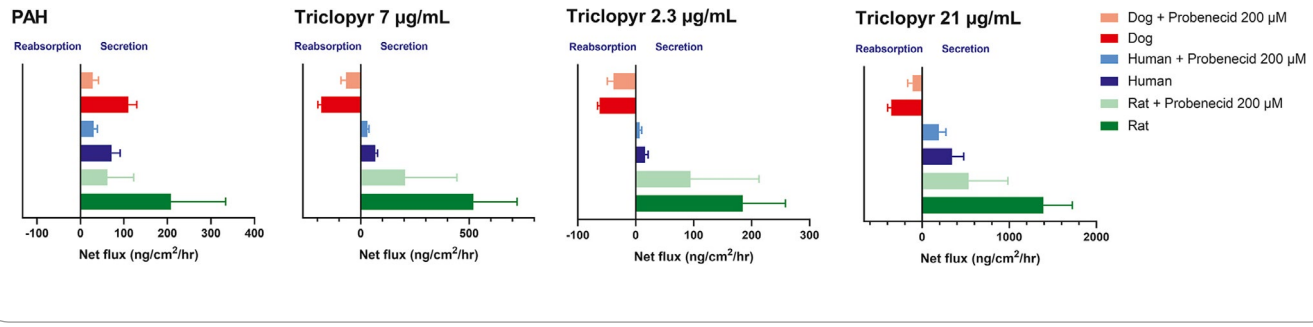

Figure 4: Cross-species comparison of TCP drug handling via OAT1. Transport of TCP is largely through renal transporter organic anion transporter 1 (OAT1) and can be partially inhibited in the presence of Probenecid, a known substrate for OAT1. Measurement of net flux shows a net secretion of TCP in human and rat PTCs, and a net reabsorption in dog PTCs. Inter-species differences in renal transport were measured in aProximate™ PTCs.

For more information:

If you would like further information, please contact our experts or visit our website:

info@newcellsbiotech.co.uk or visit:
www.newcellsbiotech.co.uk/PTC

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aProximate™ model offerings						
SKU No.	Offering	Format	Species	Readouts	Time-points	Inclusions
aProximate™ Assay-Ready Plates						
KP0000HA24	Assay-Ready Plates	24-Trans-wells™	Human	NA	NA	Serum-free maintenance medium for proximal-tubule cells
KP0000RA24						
KP0000HA96		96-Trans-wells™				
KP0000RA24						
Nephrotoxicity Assay						
KSN00000H	Nephrotoxicity	96-Trans-wells™	Human	ELISA/MSD, TEER, ATP assay, LDH production	72-hours	A positive control, vehicle control, 3 compounds and 6 conc. per compound
KSN00000R			Rat			
KSN00000M			Mouse			
KSN00000D			Dog			
Drug Transporter Interactions & Drug-Drug Interactions						
KST00000H	Drug Transporter Assay/ Drug Interactions/ Flux and Net Transporter Measurements/ Measurement of intracellular drug and metabolite concentrations	24-Trans-wells™	Human	Uptake/Flux measurement & imaging	As per customer requests	A positive control, 3 conc. per compound and flux meas. in two directions
KST00000R			Rat			
KST00000M			Mouse			
KST00000D			Dog			
Disease Modelling						
KSD00000H	Calcium and Phosphate Transporters Imbalance/Amino Acid Transporter Impairment/ Urate Transporters Deficiency	24-Trans-wells™	Human	As per customer reqts.	As per customer requests	As per customer requests
KSD00000R			Rat			
KSD00000M			Mouse			
KSD00000D			Dog			
Cross-Species Comparison						
KST00HRMD	Drug transporter assays	24-Trans-wells™	Human, Rat, Mouse, Dog	Uptake/Flux measurement & imaging	0,30,60, 90 & 120 mins	Minimum 2 species and max. 1 compound, 3 conc. per compound
	Drug interactions					
	Flux and net flux drug transport					
	Intracellular drug and metabolite concentrations					
KSN00HRMD	Nephrotoxicity assays	96-Trans-wells™		ELISA/MSD, TEER, ATP assay, LDH production	72-hours	Minimum 2 species and max. 3 compound, 6 conc. per compound
	Renal drug safety evaluation			TEER, ATP assay, FITC-Dextran Permeability, Imaging	72-hours	