



Use of the aProximate™ Proximal Tubule Cell Model for the Evaluation of Safety and Renal Accumulation of Radioconjugates and Large Molecules

The aProximate™ Proximal Tubule Cell (PTC) model offers a cutting-edge platform for the evaluation of drug-induced nephrotoxicity, radioconjugate retention and the accumulation of large molecules (ADCs, ASO, antibiotics, peptides, etc) in the proximal tubule. This white paper outlines the advantages of using the aProximate™ PTC model in predicting and assessing renal safety in the early stages of drug development, focusing on its application in the context of renal radioconjugates and large molecule dynamics.

and the pharmacokinetics of new therapeutics, not only of traditional small molecules, but also of the new modalities of large molecules and particularly radioconjugates, which are prone to renal accumulation. The cells in the model maintain differentiation, expressing high levels of renal transporter, including Megalin and Cubilin, which mediate protein and large molecule uptake in the nephron (Figure 1.). This model has been validated against various parameters, including Trans Epithelial Electrical Resistance (TEER), uptake and flux of small and large molecules and specific biomarker expression of toxicity, ensuring its physiological relevance and accuracy in safety evaluations.

Model Overview

The aProximate™ PTC model replicates the physiology and function of human proximal tubule cells. These cells are crucial for assessing kidney toxicity, drug-drug interactions

Safety Evaluation & Radioisotope Accumulation

The very specific, targeted and focused exposure of tumour cells to radioligand with minimal collateral damage represents a major breakthrough in cancer therapy. Unfortunately, this

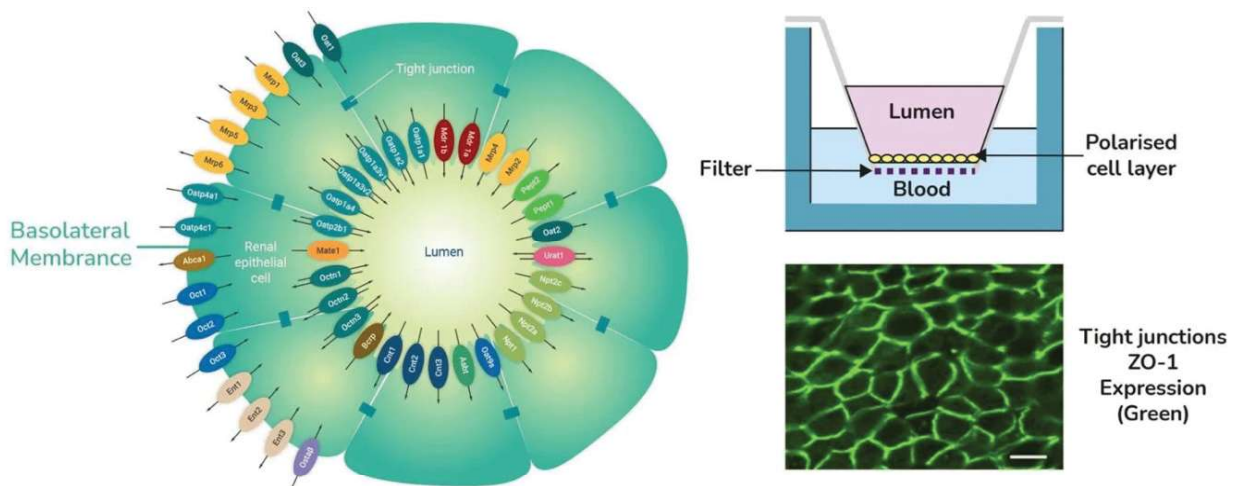


Figure 1: (A) Schematic representation of the aProximate™ PTC cell model representing the high expression of all key transporters, the formation of an epithelium with tight junctions and polarity, and the use of Transwells™ for cell culture to recreate a lumen and a blood compartment to assess renal transport directionality.

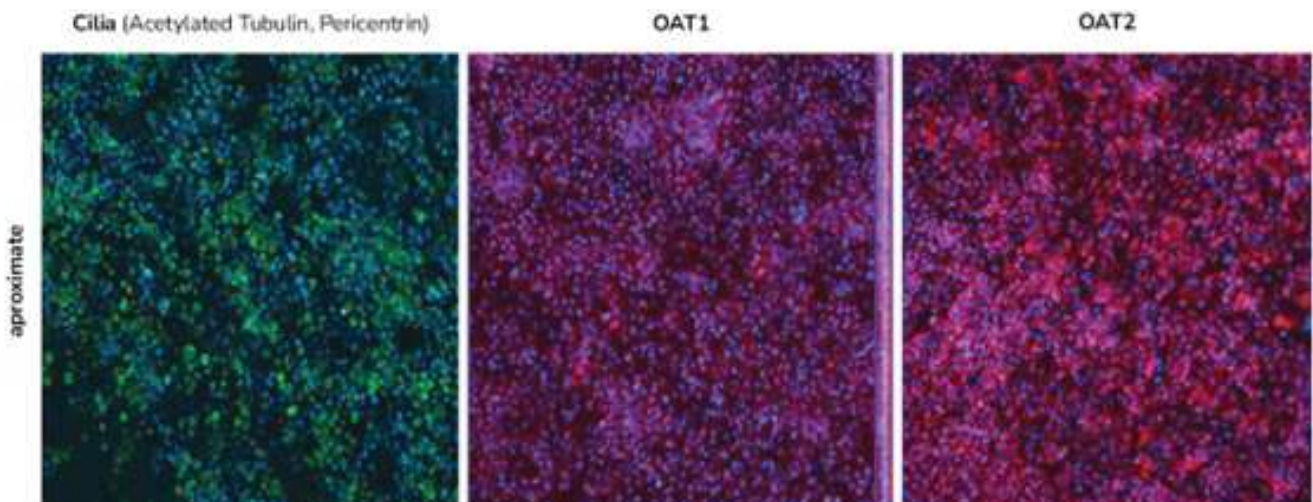


Figure 1: (B) Immunofluorescence staining of aProximate™ proximal tubule cells for Tubulin, Pericentrin, OAT1 and OAT2.

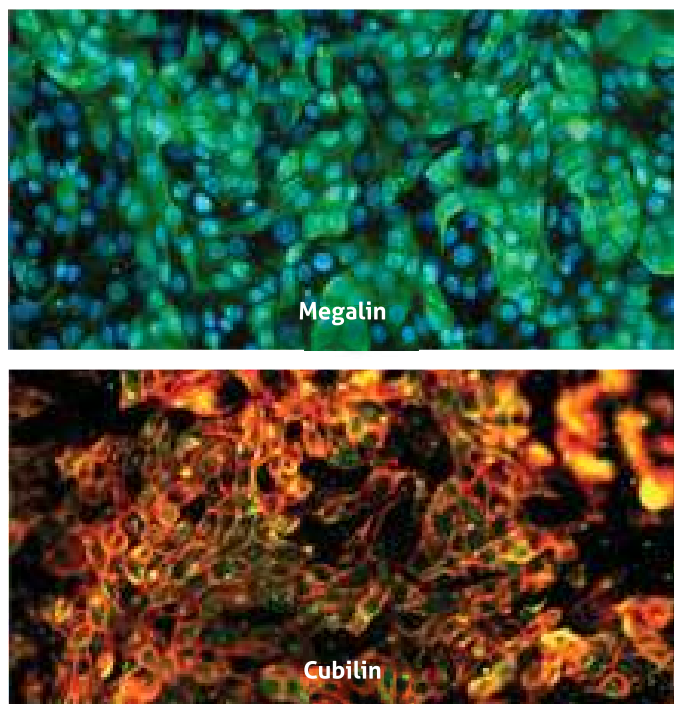


Figure 1: (C) Immunofluorescence staining of aProximate™ proximal tubule cells for of Megalin and Cubilin.

modality has the potential for severe off-target damage. The kidney is particularly at risk due to its potential accumulation of radioconjugates within the cortex of the kidney, leading to severe nephrotoxicity. The mechanisms behind this have been under intense scrutiny and the Megalin-Cubilin receptor-mediated endocytic pathway has been implicated (for review see: Pahir *et al.* 2021, Translational Oncology). With the kidney as a major target, it is therefore crucial to fully assess the renal clearance and retention of novel radiopharmaceuticals and their impact on kidney function at an early stage in drug development. Screening for the renal retention and subsequent renal toxicity can be greatly accelerated with cutting-edge *in vitro* models such as aProximate™. The aProximate™ PTC model can be instrumental in evaluating this risk by being a predictive model of the proximal tubule environment and crucially expressing the key Megalin-Cubilin receptors. The aProximate™ model provides an ideal platform to screen renal accumulation of radioconjugates and define strategies to reduce renal retention and de-risk candidate radioconjugates for renal retention and renal toxicity.

Assessment of Large Molecule Accumulation

Radioconjugates include a range of structures, including Antibody-Drug Conjugates (ADCs), peptides and bispecific antibodies. A common feature of these constructs is a chelator, a short half-life radioisotope. The short half-life of the isotope has proved a roadblock in studying the renal retention and renal damage of radioconjugates due to the difficult logistics inherent

in their use. Newcells Biotech has addressed this by developing a number of different labelling techniques to overcome this hurdle (Table 1). These include measuring a non-radioactive form of the radiolabel, labelling the radioconjugate with 3H or a fluorescent probe.

Cade Studies & Applications

Flux of a Radioconjugated Peptide

The aProximate™ PTC rat model was used to measure the apical and basal uptake of a 3H radiolabelled peptide. This data was fundamental in understanding the kinetics and dynamics within the renal cortex, supporting the development of safer therapeutic agents with minimised renal side effects. The data was complemented with an inhibition study to confirm the active role of Megalin and Cubilin in this transport (Figure 2b).

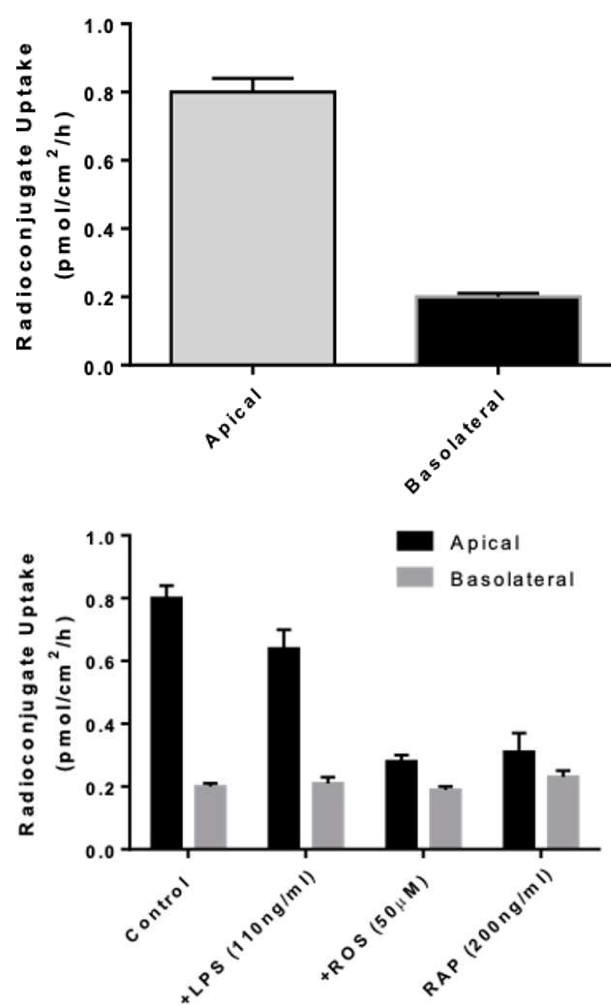


Figure 2: Characterisation of apical uptake of radioconjugate peptide using a 3H radiolabelled peptide in rat aProximate™. (A) Quantification of Radioconjugate uptake showing apical uptake. (B) Uptake inhibitor study with 3H radiolabelled peptide. Flux inhibition and apical uptake is reduced in the presence of known inhibitors of Megalin/Cubilin by LPS, ROS and RAP.

Modality	Detection Method	End Point
Peptide	3H-label	Measurement of Apical uptake, transepithelial flux, inhibition of uptake by RAP, single TA
ADC	Alexa568 Dye	Measurement of Apical uptake, inhibition of uptake by RAP, 3 Tas
Bispecific AB	Lu metal LC/MS IPC	Measurement of Apical uptake, inhibition of uptake by RAP, rank order of TAs

Table 1

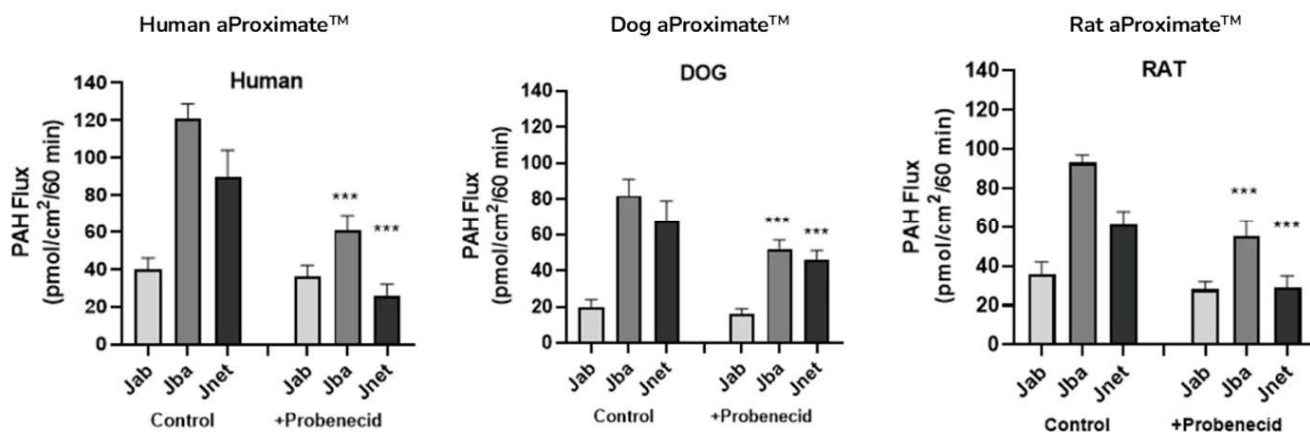


Figure 3: Cross-species comparison of PAH, (para-aminohippurate), a derivative of hippuric acid used as a diagnostic agent for measuring renal plasma flow. Upon IV infusion, PAH is extracted from the blood by OAT1 renal transporter in the proximal tubule. PAH excretion was compared in different species, showing a net secretion of PAH in human, dog and rat.

Cross-species Safety Analysis of Large Molecules

We have developed a number of cross-species models of the proximal tubule; these include human, rat, dog and mouse. These models have been used extensively to understand cross-species differences in the renal handling of small molecules. Figure 4 shows the handling of the prototypic anionic molecule-PAH (para-aminohippurate) and the ability to interrogate different preclinical species using the aProximate™ PTC models. This type of study is crucial to ensure the safety and efficacy of therapeutics across different biological systems before clinical trials. This work has also been extended to validate the handling of large molecules – including radioconjugates in rat, mouse and dog.

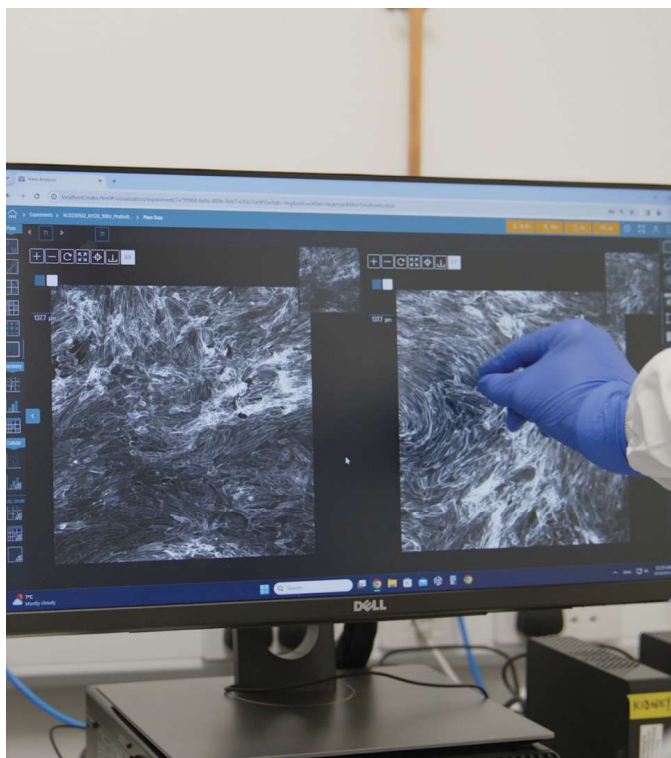
Conclusion

The aProximate™ PTC model represents a significant asset in the field of renal safety evaluation for large molecules, including radioconjugates. By providing a robust, physiologically relevant

cross-species platform, it enables the detailed analysis of nephrotoxicity, drug interactions, and the specific dynamics of radioisotope and large molecule retention. This white paper underscores how this technology can advance different types of therapy modalities, including peptide receptor radionuclide therapies (PRRTs) and others that utilise radioisotopes. It highlights the model's utility in enhancing drug safety profiles and optimising therapeutic strategies to mitigate renal risks in drug development.

Future Directions

Continued advancements in this area are expected to focus on refining the model to include more complex kidney structures and applying it to a broader range of therapeutic molecules. Additionally, integrating this model with systemic pharmacokinetic models can provide a more comprehensive assessment of drug behaviour across multiple organ systems, further improving the value of *in vitro* models to define the predictability of human clinical outcomes.



Dr. Colin Brown

Colin was born in Edinburgh, grew up in Fife and was awarded his PhD by the University of St Andrews. He was then a Royal Society European Postdoctoral Fellow at the University of Zurich. Following on from this, Dr Brown was a Wellcome Senior Research Fellow at Manchester University. He was then an Associate Professor in the Institute of Cell & Molecular Biosciences for nearly 30 years. Dr Brown is a leading expert in kidney transport, with research interests in renal, hepatic and GI drug transporters. He has developed and commercialised several primary cell-based assays for measuring kidney transport and toxicity, has authored over 100 publications and several book chapters and has been an invited speaker at numerous conferences around the world. He has been a mentor to 16 PhD students and numerous undergraduate and Masters students. Dr Brown is a past Chair of the AAPS Drug Transporter Focus group and a member of the PPDM executive. He was involved in the organisation of four AAPS DTFG Bench to Bedside workshops, including two as Chair.