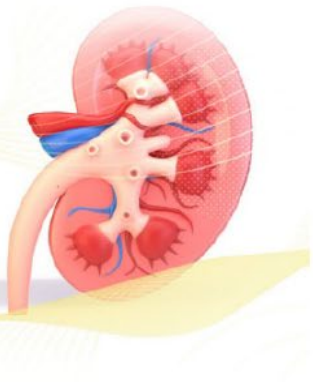
aProximate™

KIDNEY

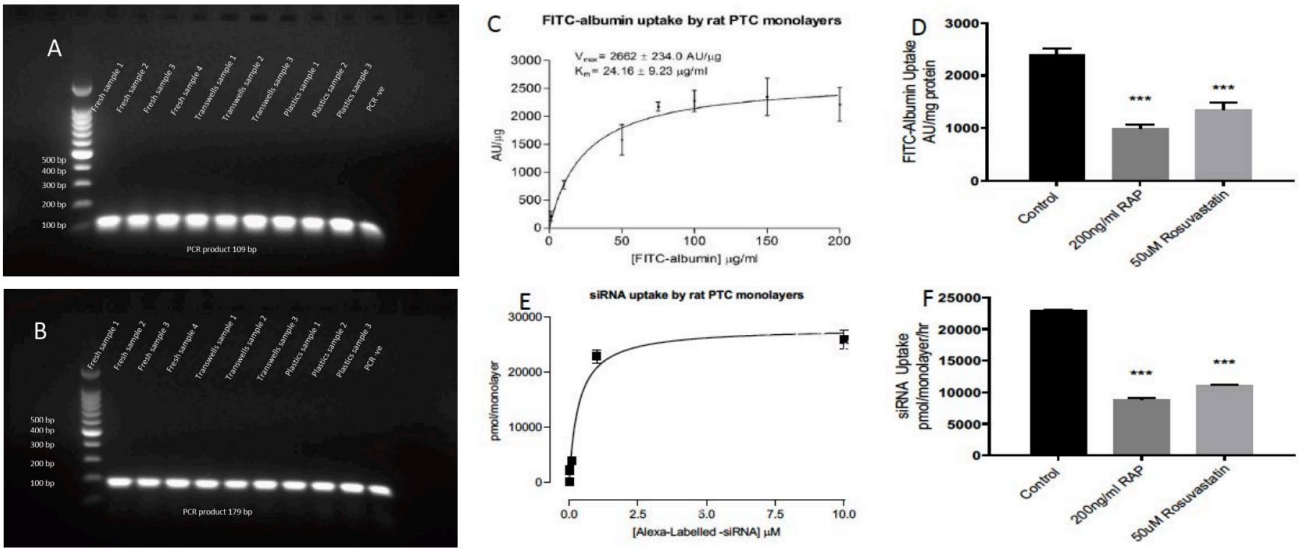


A predictive model for the transport and toxicity testing of biologics

The development of aProximate™ was led by Industry leading expert, Dr Colin Brown. Our renal platform is engineered using proximal tubule epithelial cells with the correct physiological transporters to allow our *in vitro* model to predict in vivo outcomes. Many biologics accumulate in the kidney and can lead to unforeseen kidney injury. Nephrotoxicity is a significant barrier to many drug development programs. Newcells Biotech renal platform is one of the first to enable cross-species comparisons of renal drug toxicity and transport. Using our scientific expertise we have engineered highly differentiated assay platforms using primary renal proximal tubule cells (PTCs). The PTCs are derived from five key animal species to measure both drug transport and drug-induced kidney injury using a range of biomarkers.

Albumin and siRNA uptake by megalin - cubulin

Megalin and Cubulin are key transporters in the reabsorption of endogenous large molecules such as albumin and of a range of large molecular weight xenobiotics including antibiotics and biologics. The aProximate™ PTC model expresses both megalin (A) and cubulin (B) and demonstrates saturable uptake of FITC-labelled albumin(C). In addition, we could measure megalin-cubulin mediated uptake of biologics such as siRNA (D) and antisense oligonucleotides (AONs) making the aProximate™ the ideal model to investigate renal biologics handling.



Megalin (A) and Cubulin (B) were expressed in rat PTC cell monolayers. Functional expression of Megalin-cubulin was demonstrated by showing saturable FITC-Alubumin uptake (C) that was inbited by exposure to Rosuvastatin or RAP (D). Uptake of a labelled siRNA showed similar saturable uptake kinetics (E) and uptake was significantly inhibited by Rosuvastatin or RAP.

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BEST BIOLOGY DRIVING *IN VITRO* INNOVATION

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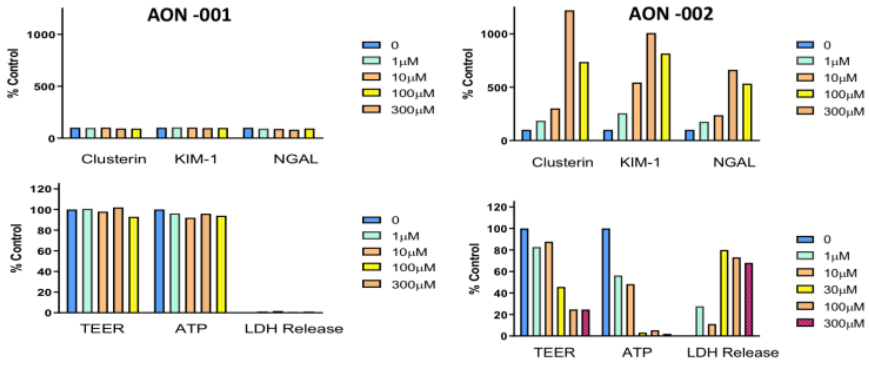
Examples and Supporting Data

Biomarker response to antisense oligonucleotide (AON) challenge

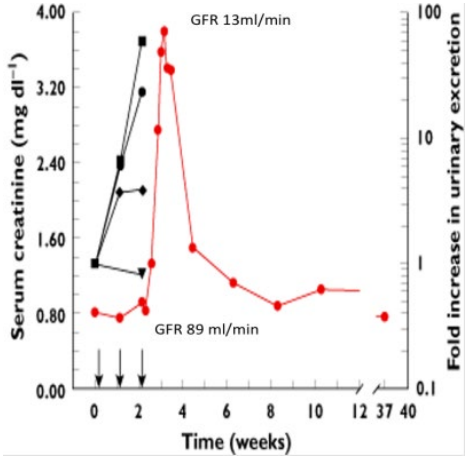


To validate the aProximate™ PTC model as an accurate predictor of *in vivo* toxicity of AONs, a series of AONs were screened using a range of markers of kidney injury including the biomarkers; Clusterin, KIM-1 and NGAL along with measures of tissues integrity; TEER, ATP and LDH release. The results for two AONs are shown in the figure. With the AON toolkit tested to date, aProximate™ PTCs are very predictive of *in vivo* outcome.

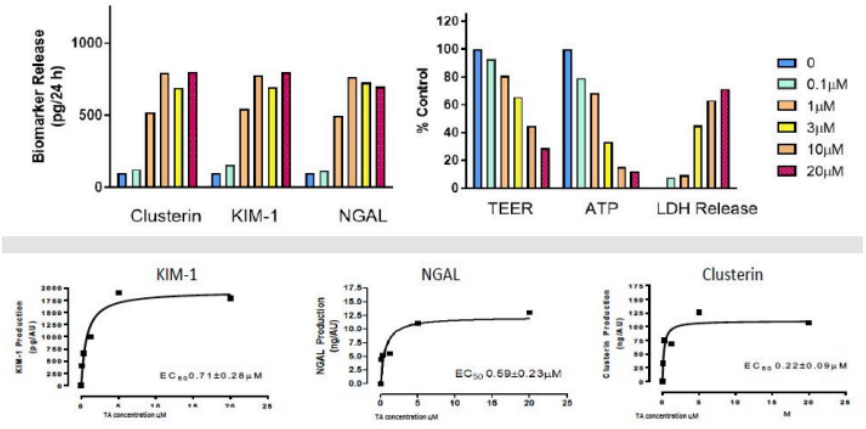
Comparison of challenge of human PTC cells to 72 hour exposure to 2 AONs. AON-001 had no effect on any markers of kidney injury. In contrast, AON-002 resulted in significant increases in all markers of kidney injury.

SPC-5001 challenge resulted in nephrotoxicity in Man

SPC-5001 was an antisense oligonucleotide that reached early clinical trials. However, at first in man, SPC-5001 demonstrated a severe kidney liability. This study demonstrated the power of using biomarkers versus creatinine, the standard marker of renal toxicity. The data clearly show that increases in creatinine were only detected after the 3rd dose of SPC-5001 at which point severe renal damage had already occurred. In contrast biomarkers, Clusterin, KIM-1 NAG indicated kidney injury after 1 dose. In aProximate™ human PTC monolayers, SPC-5001 led to a significant increase in kidney injury markers. These data confirms the utility of the aProximate™ platform to predict in vivo outcomes.



Data from Van Poelgeest et al Am.J.Kid. Dis 62 (2013). The red line shows creatinine levels and the black line shows Clusterin, KIM-1and NGAL levels in urine samples.



In response to SPC 5001 challenge human PTC cells generated a significant increase in markers of kidney injury. The response of KIM-1 Clusterin and NGAL gave apparent IC 50 values of 0.7µM for KIM-1 , 0.6µM for Clusterin and 0.2 µM for NGAL . Data n=6 from single kidney.