Development of a predictive in vitro human proximal tubule platform for de-risking nephrotoxicity

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Introduction

- Nephrotoxicity is a major reason for drugs failing during clinical development.
- Currently there is no in vitro platform that enables cross-species comparisons of drug transport or nephrotoxicity.
- Our innovative solution is to develop highly differentiated assay platforms using primary renal proximal tubule cells(PTCs) derived from key animal species to measure both drug transport and drug induced kidney injury a range of biomarkers across species
- Here we showcase a data from our highly differentiated Human Primate proximal tubule model

Methods

- PTCs were isolated from fresh Human kidneys and cultured onto Transwell inserts as outlined below
- Test toolkit was made up of 36 compounds with known nephrotoxicity liability and clinical data were screened using the model Toolkit was made up of 19 Primary PT-toxic and 17 Secondary PT-toxic (3) or non-nephrotoxic (14) compounds
- Monolayer were exposed for 72hours to a range of concentrations (0-300µM) of test compound
- Toxicity was measured used 6 parameters of cell health: Transepithelial Resistance (TEER), Intracellular ATP concentration, LDH release , KIM-1 release, Clusterin release NGAL release

Kidney decapsulated and cortex dissected and minced

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Collagenase digest

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Digested tissue passed through cell strainer separate via density gradients

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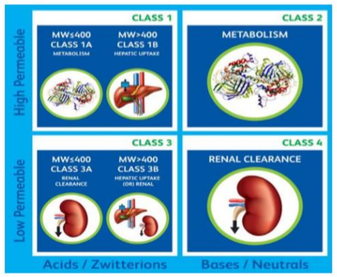
Tubular cell retrieved and cultured on Transwell inserts

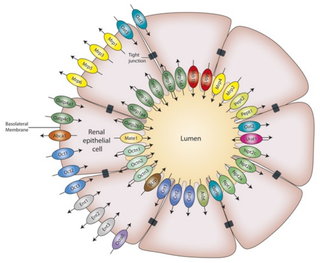
Toolkit of 36 Compounds Tested

Diverse Chemical Structure



Varma et al Pharm Res. 32:3785-3802 (2015)

Range of Mechanistic Exposure Routes



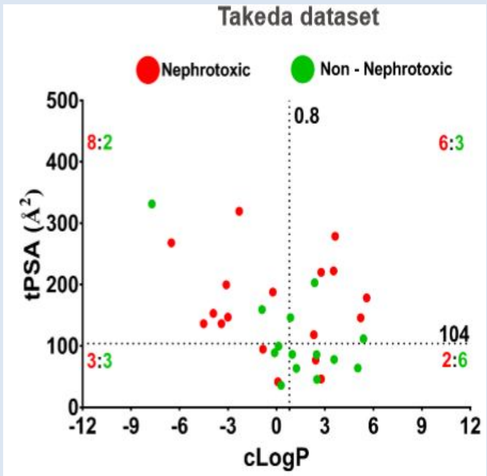
Toolkit was chosen to encompass a diverse range of chemical structures and be substrates for a wide range of proximal tubule transporters

Glomerulus	Cystatin C
Proximal Tubule	KIM 1 Clusterin NGAL NAG Osteopontin

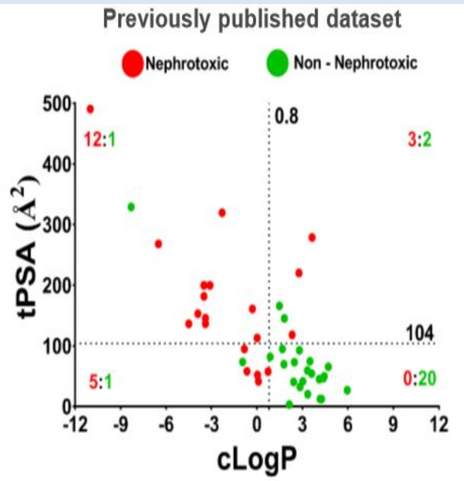
August 2018- FDA approved a safety composite biomarker panel to aid in the detection of kidney tubular injury in phase 1 trials in healthy human volunteers. Biomarkers are detected in the urine

Biomarkers were 3 from 5 qualified biomarkers of proximal tubule damage outlined by FDA

Physiochemical properties of Toolkit of Compounds



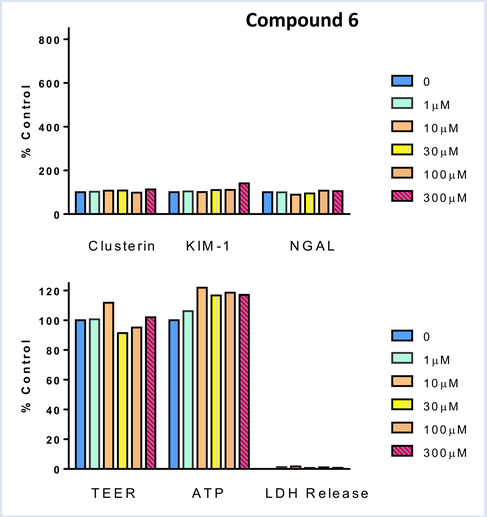
Parameter	Sensitivity (%)	Specificity (%)
cLogP < 0.8	57.9	64.3
tPSA > 104	73.7	64.3
cLogP + tPSA	89.5	42.9

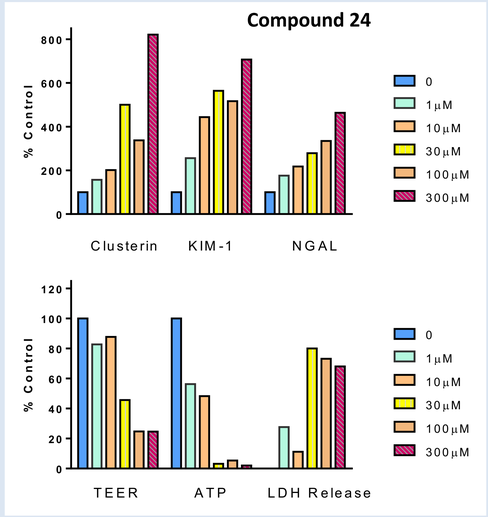


Parameter	Sensitivity (%)	Specificity (%)
cLogP < 0.8	85	91.7
tPSA > 104	75	87.5
cLogP + tPSA	100	83.3

33 Compounds were selected to minimize influence of physiochemical properties in weighting the outcome of assay

Biomarker Response to Nephrotoxic Challenge is Highly Predictive of In Vivo Outcome





Predictive performance of all end-points – 72h of compound exposure

Only AC_{50} value

	TEER	LDH	ATP	KIM-1	NGAL	Clusterin
Cut-off (µM)	< 15	< 10	< 50	< 30	< 22	< 95
AuROC	0.52	0.56	0.61	0.61	0.64	0.66
Sensitivity (%)	5.3	26.3	47.4	47.4	47.4	63.2
Specificity (%)	88.2	88.2	88.2	88.2	88.2	88.2

In vitro safety margin (AC_{50}/C_{max})

	TEER	LDH	ATP	Clusterin	KIM-1	NGAL
Cut-off (X)	< 5	< 10	< 25	< 31	< 30	< 30
AuROC	0.53	0.62	0.64	0.75	0.74	0.78
Sensitivity (%)	15.8	31.5	42.1	57.9	63.2	63.2
Specificity (%)	88.2	88.2	88.2	88.2	88.2	88.2

- Injury-specific biomarkers (KIM-1, NGAL, Clusterin) showed better predictivity than non-specific end points (TEER, LDH, ATP)
- Incorporation of exposure (total C_{max}) improved predictive performance of most end-points than just IC_{50}-value by itself

Conclusions

Human proximal tubule cell monolayers retain a remarkable degree of differentiation and express a range of functional transporters and clinically relevant biomarkers of nephrotoxicity that are sensitive to nephrotoxin challenge over time. Human PTC monolayers show excellent potential as an in vitro predictive screening platform