In vitro platforms for de-risking nephrotoxicity

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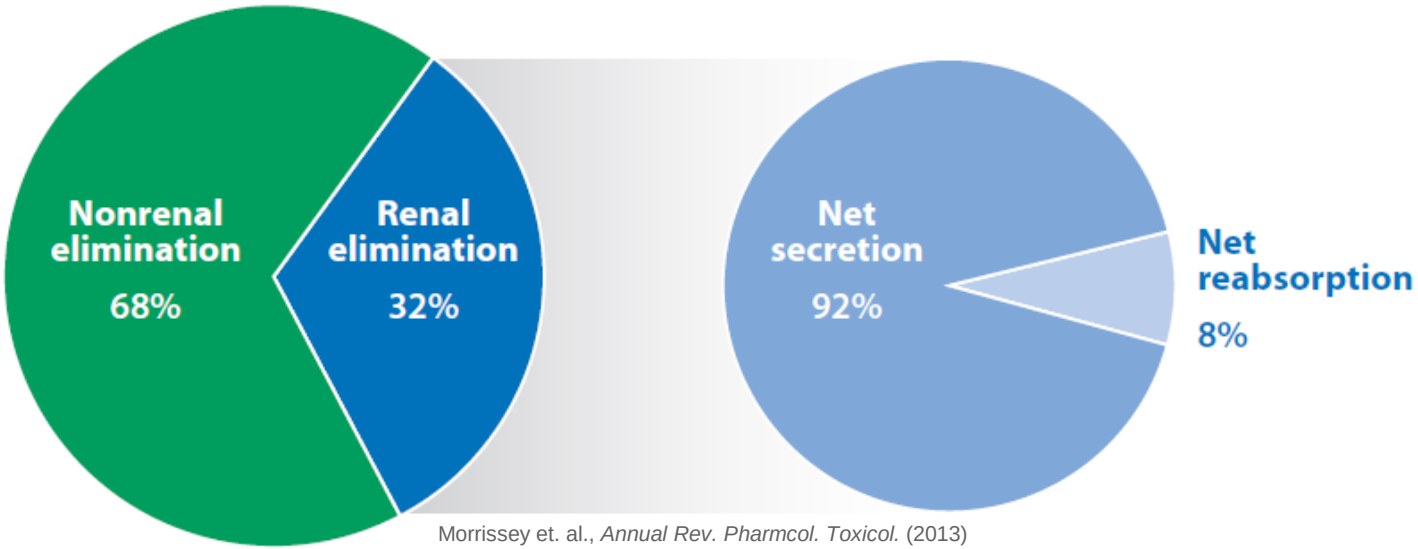
Importance of kidney in drug development

Contribution of kidney to the elimination of top 200 prescribed drugs in US



Morrissey et. al., *Annual Rev. Pharmcol. Toxicol.* (2013)

- Nephrotoxicity is often among the top 5 reasons for drug attrition
- **Nephrotoxicity accounts for only 2% failure in preclinical stages but 19% of all failures in Phase I-III**
- **Post-market: Up to 20% of hospital admissions for acquired acute kidney injury (AKI) are attributable to drug-drug interactions resulting in drug induced kidney injury**
- **Pre-Clinical Testing in Animals is Poorly Predictive of Outcome in Man**
- **Poor Understanding of Drug interactions leads to AKI**

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Current Experimental Models of the Proximal Tubule

Established Cell Culture Models:	MDCK LLC-PK_1 OK	Lack transporters Animal origin
Transfected Cells		Human transporters Single or double transfectants Essentially 'jigsaw' pieces
Human kidney slices		Intact tissue Human Transporters. Limited to basolateral membrane
Human 'proximal tubule cell culture lines	HRPT CaKi-1 RPTEC HPTEC HK-2 HKC-5	Human Tissue. Poor retention of differentiation
Human iPSC renal models		2-3 years away at best
Primary Proximal tubule renal models		Promising data from multiple labs

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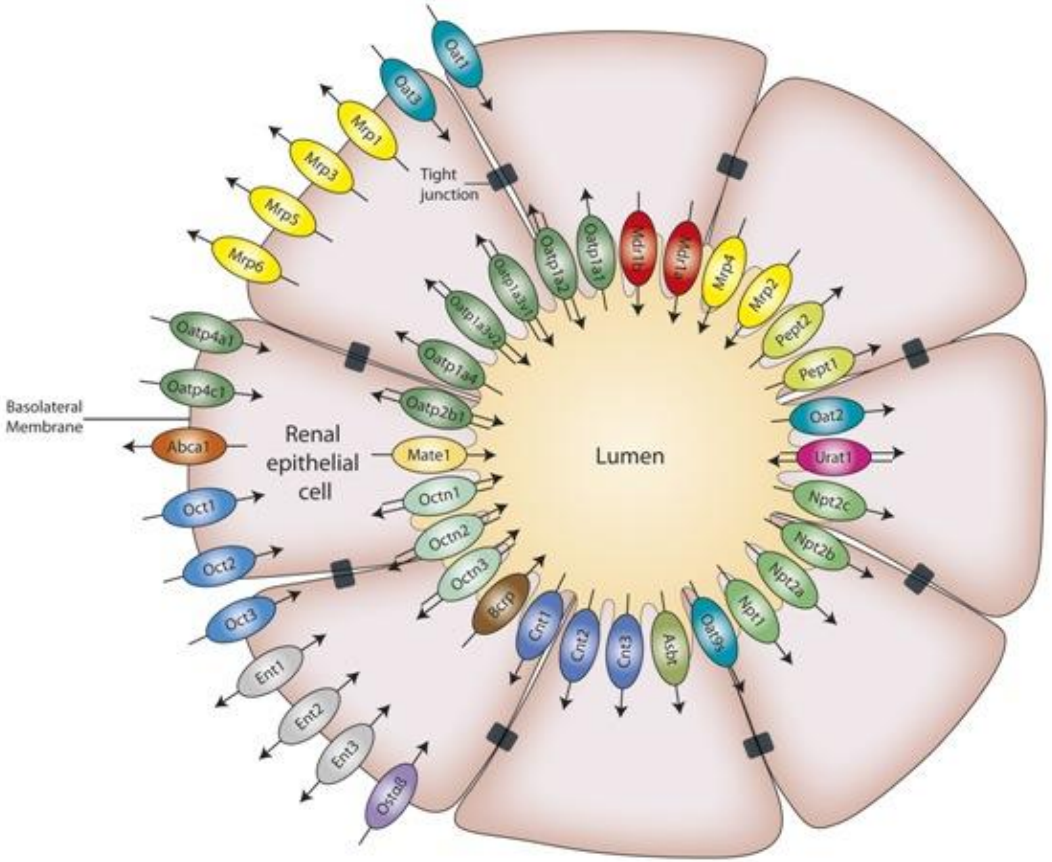
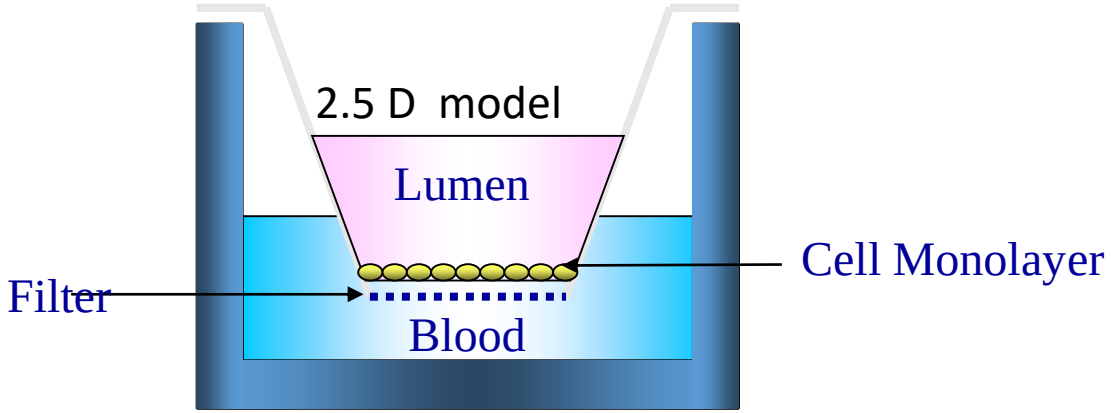
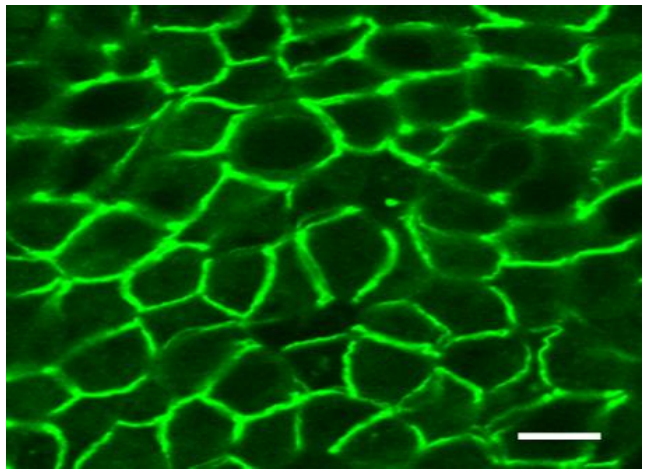
aProximate™ Cells Grown as Polarised Monolayers on Filter Supports Recapitulate Proximal Tubule Physiology and Function







ZO-1 Expression

Screen: Drug secretion & Absorption; Drug toxicity, Drug-Drug interactions

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aProximate™ PT cells Remain Extremely Well Differentiated and Substantially Outperform Competition

Key Transporter Expression

- 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

Gene	Percentage of native kidney expression			
	Human PTC	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	151 ± 8.8	ND	0.3	ND

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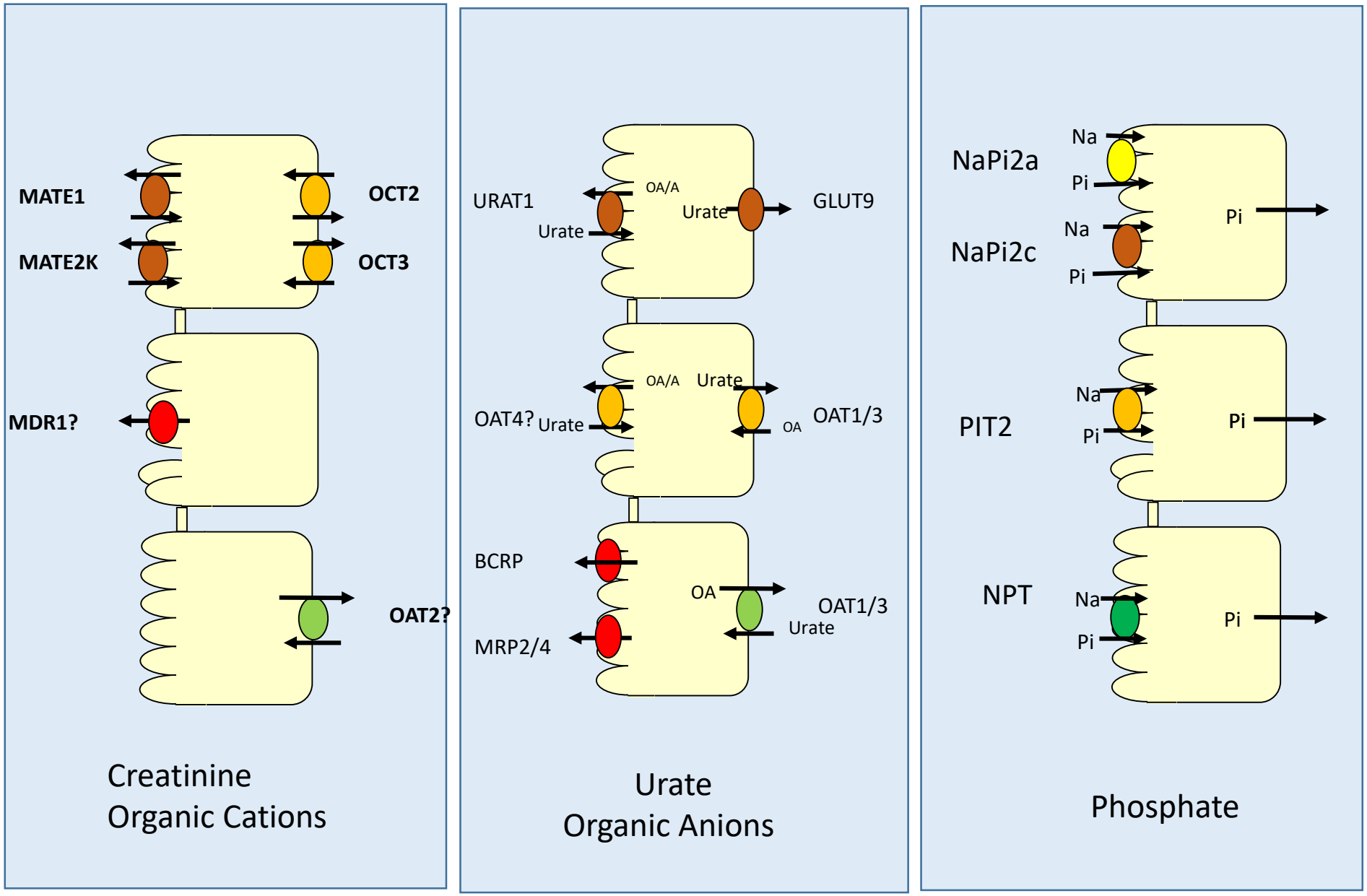
How can we use the Model?

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

Examples of PTCs being Predictive of Renal Handling



Case Study 1: How Well Does in vitro System Reflect in vivo Data?

Transporter	Victim drug	Perpetrator drug	AUC fold increase	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.
hOAT1 and hOAT3	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
P-gp	Digoxin	Quinidine	N.D.	56
	Digoxin	Quinidine	N.D.	33
	Digoxin	Quinidine	N.D.	34

FDA White Paper Decision Tree Examples of Clinical DDI Generated by Transporters in the Proximal Tubule

U.S Food and Drug Administration

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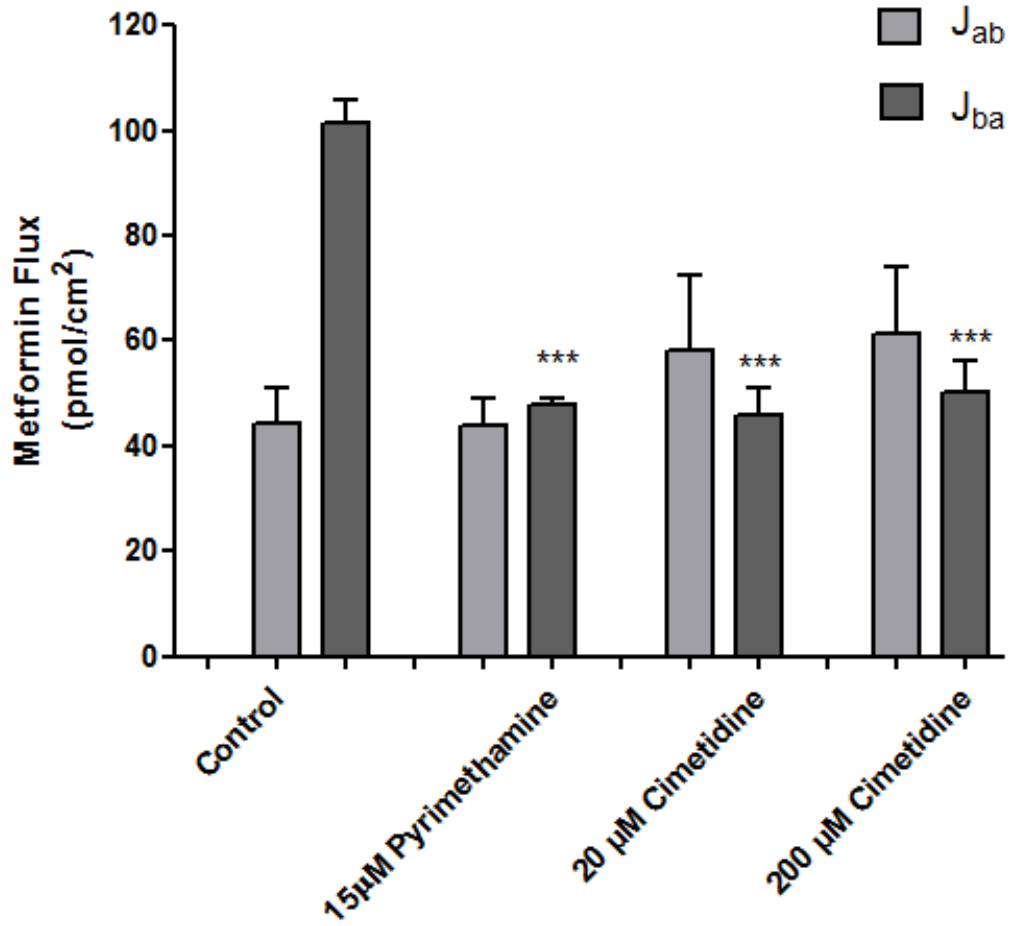
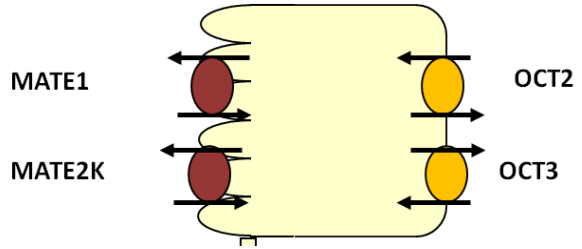
Case Study 1: aProximate™ PTCs Predict Metformin OCT/MATEs Interactions





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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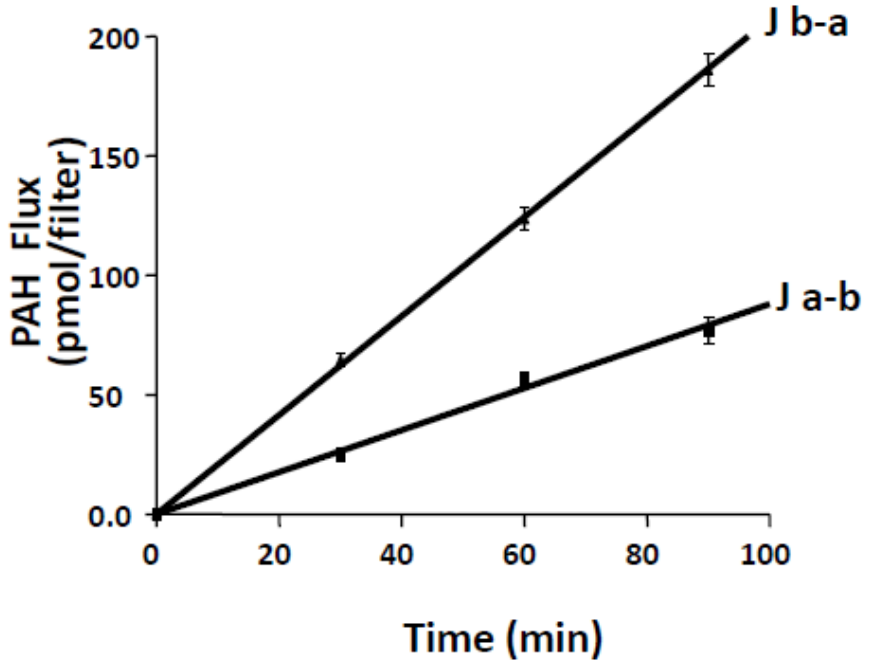
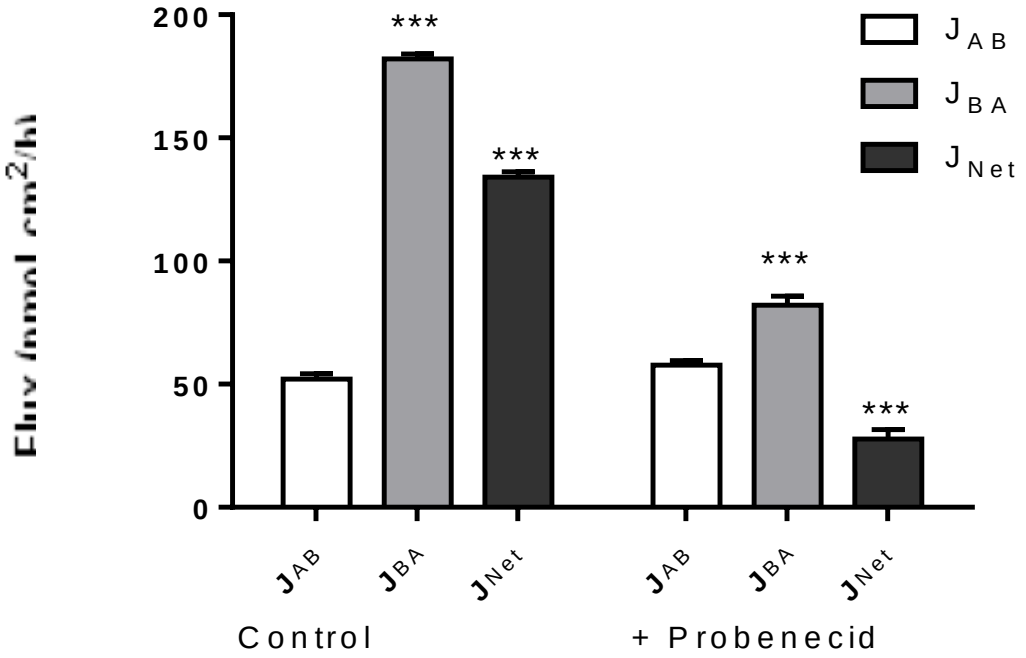
Case Study 1: aProximate™ PTCs Predict OATs /Probenicid Interactions





hOAT1 and hOAT3	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

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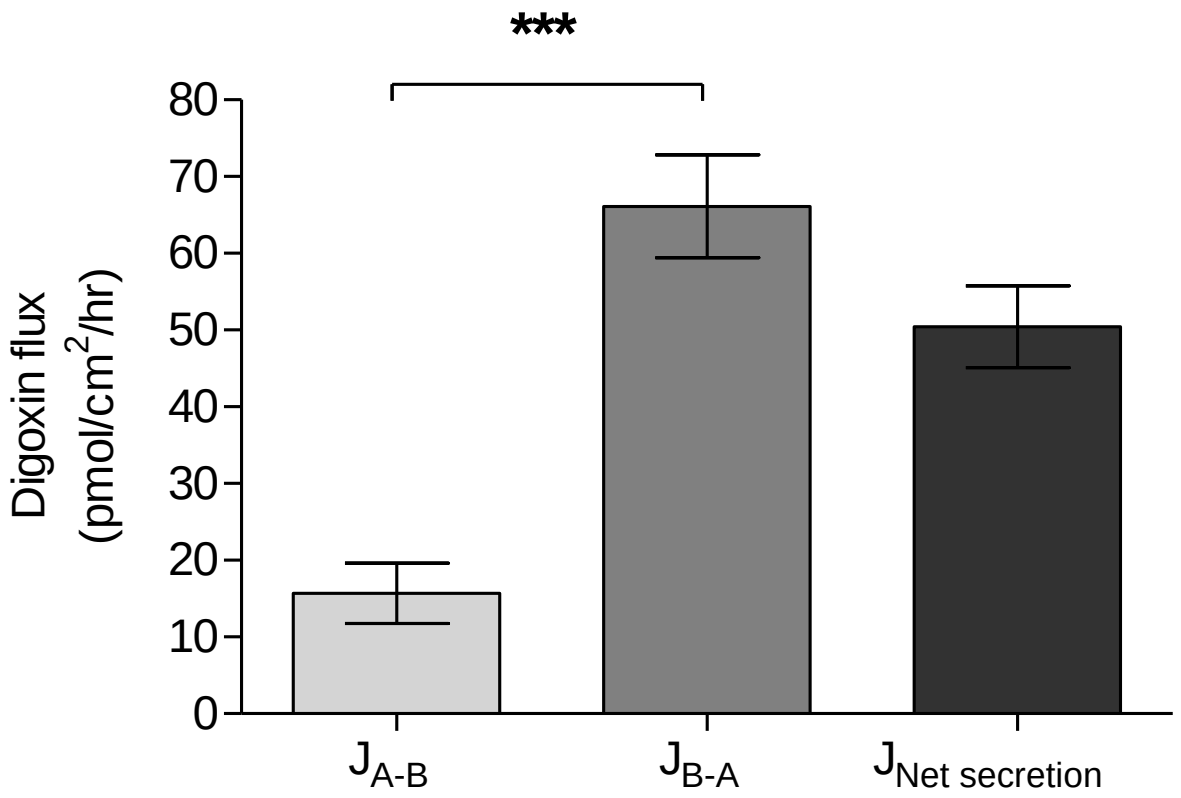
Case Study 1: aProximate™ PTCs Predict Digoxin /Quinidine Interactions



Inhibitor	IC_{50} µM
GF120918	0.8
Carvedilol	0.13
Nicarpidine	0.91
Verapamil	1.23
Miberadil	2.53
Quinidine	5.00
Ranolazine	34.8
Ketaconazole	53.1
Diltiazem	102.9
Isradapine	160.1

P-gp	**Digoxin**	**Quinidine**	**N.D.**	**56**
	Digoxin	**Quinidine**	**N.D.**	**33**
	Digoxin	**Quinidine**	**N.D.**	**34**

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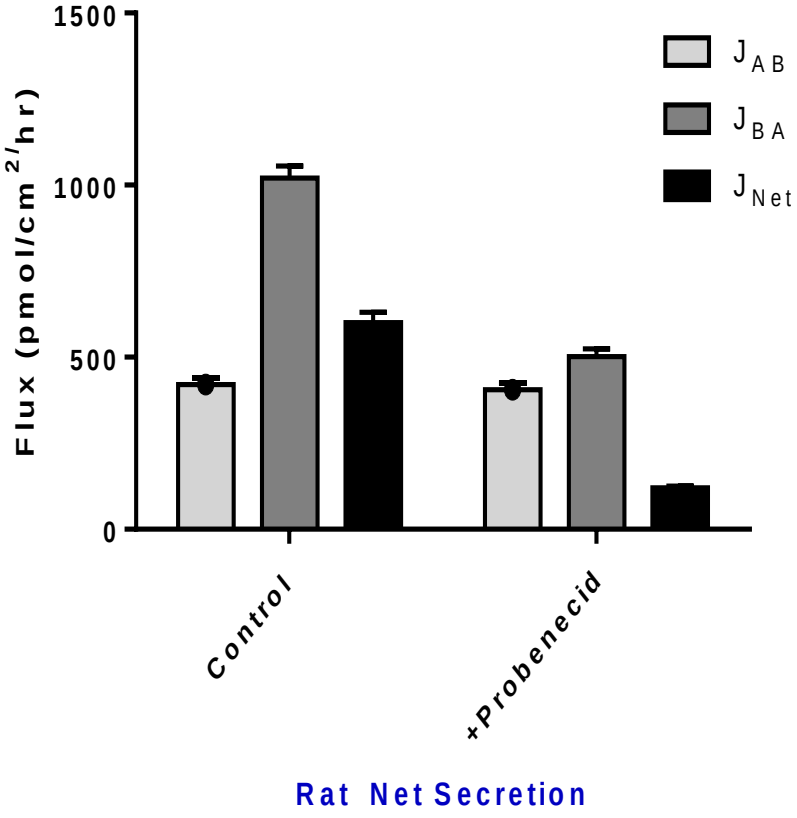
Case Study 2 - Problems with the Regulators

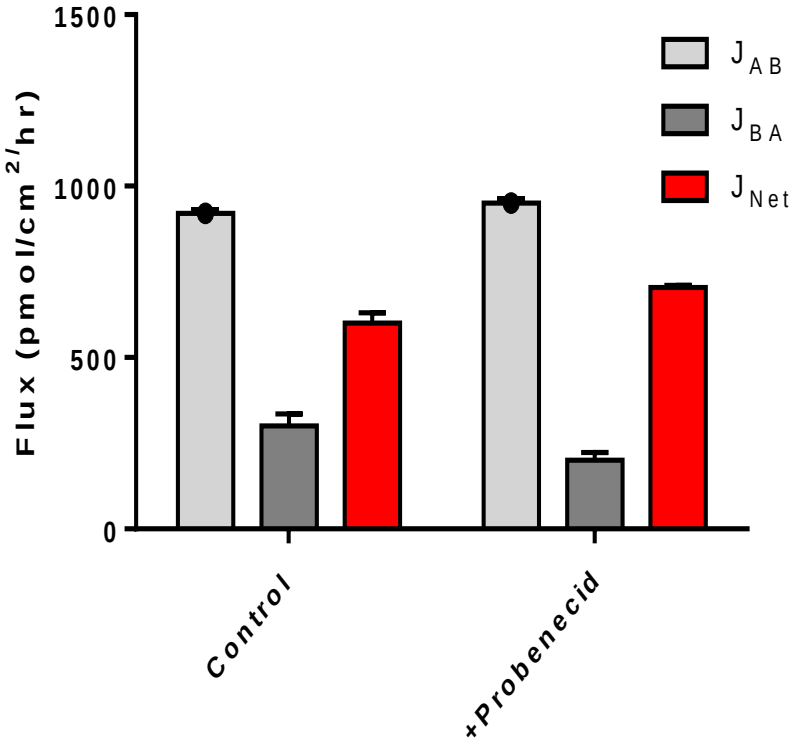
- Anionic Drug Molecule
- Substrate for OATs and MRPs
- Rat in vivo - good renal clearance – evidence of tubular secretion
- Dog in vivo - poor clearance -evidence of absorption
- Dog in vivo - systemic toxicity apparent

Identification of clinically important transporter-mediated Drug-Drug interactions during drug development and post market in clinic (drug induced AKI)

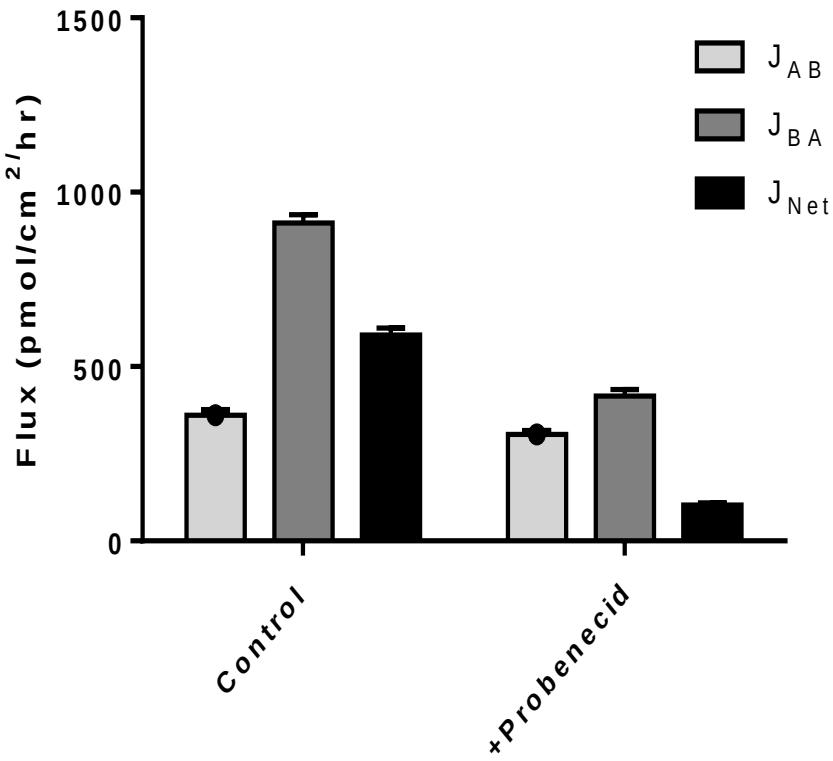
Case Study 2: aProximate™ PTCs Predict Species Differences in Drug Handling



Rat Net Secretion



Dog Net Absorption



Human Net Secretion

Data matches in vivo data

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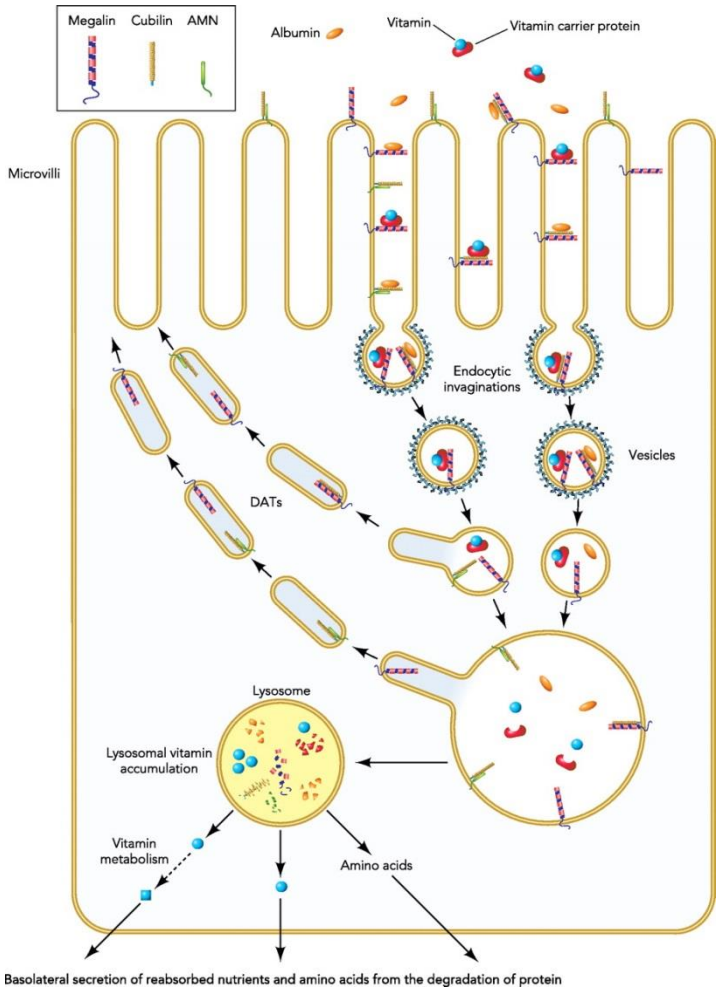
Megalin and Cubulin are key Uptake Transporters of Large Molecules in the Proximal Tubule



Endogenous Substrates

Albumin
α2-microalbumin
β-microalbumin
Apolipoproteins
Cytochrome c
Intrinsic factor-vitamin B12
Insulin
Lysozyme
Prolactin
RAP
Retinol binding protein
Thyroglobulin
Transcobalamin-vitamin B12
Transferrin
Transthyretin
Vitamin D binding protein

Xenobiotics

Aminoglycosides
Polymyxins
Rifamycin

Oligonucleotides
siRNA
Peptides
Antibodies

New Therapeutic Areas
60% of Large Pharma Pipeline is Biologics
Few RENAL DATA

KO -/- Mouse models suggest that Megalin and Cubulin mediated endocytosis is ONLY mechanism for small proteins large peptide uptake

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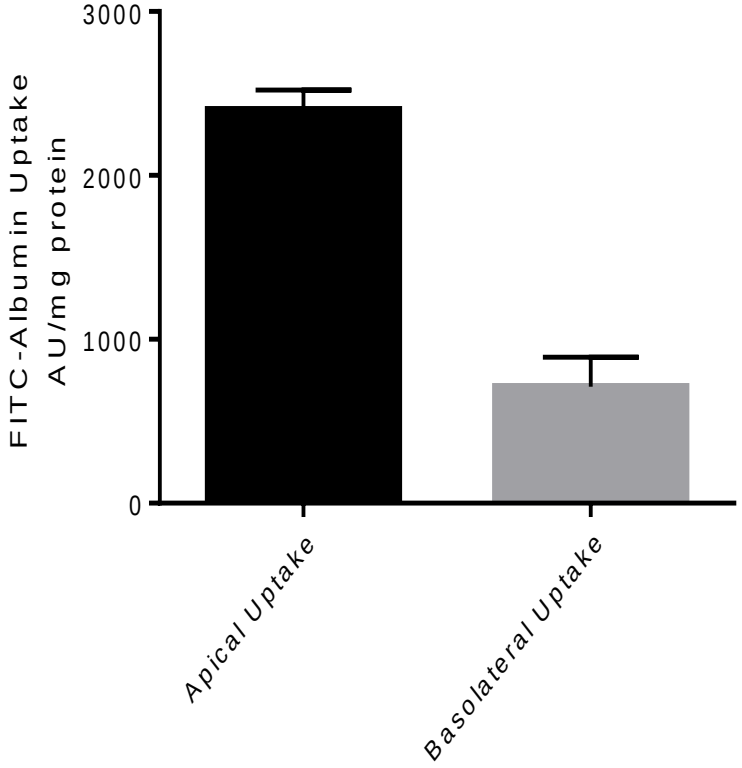
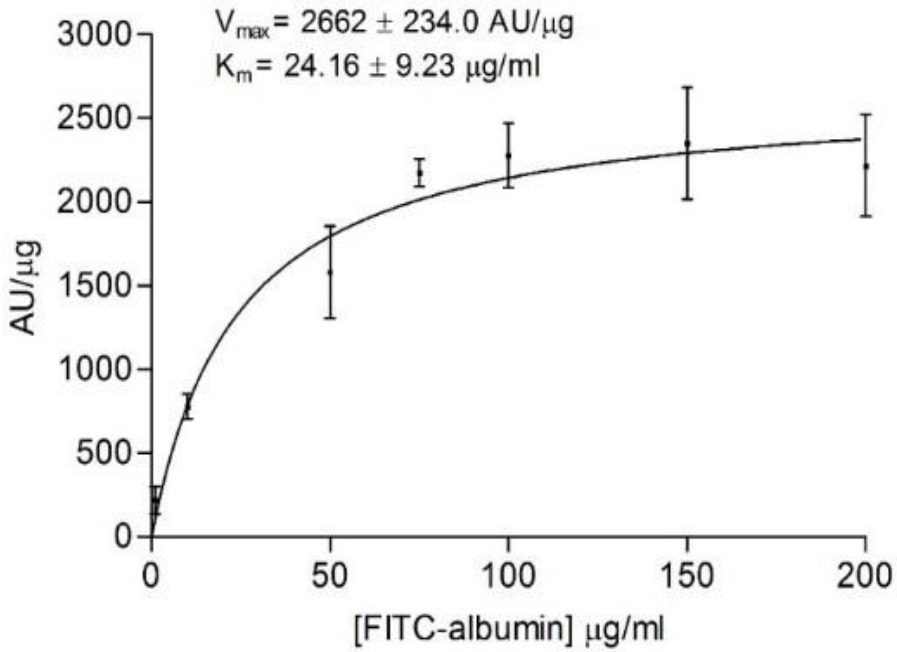
Functional Expression of Megalin and Cubulin





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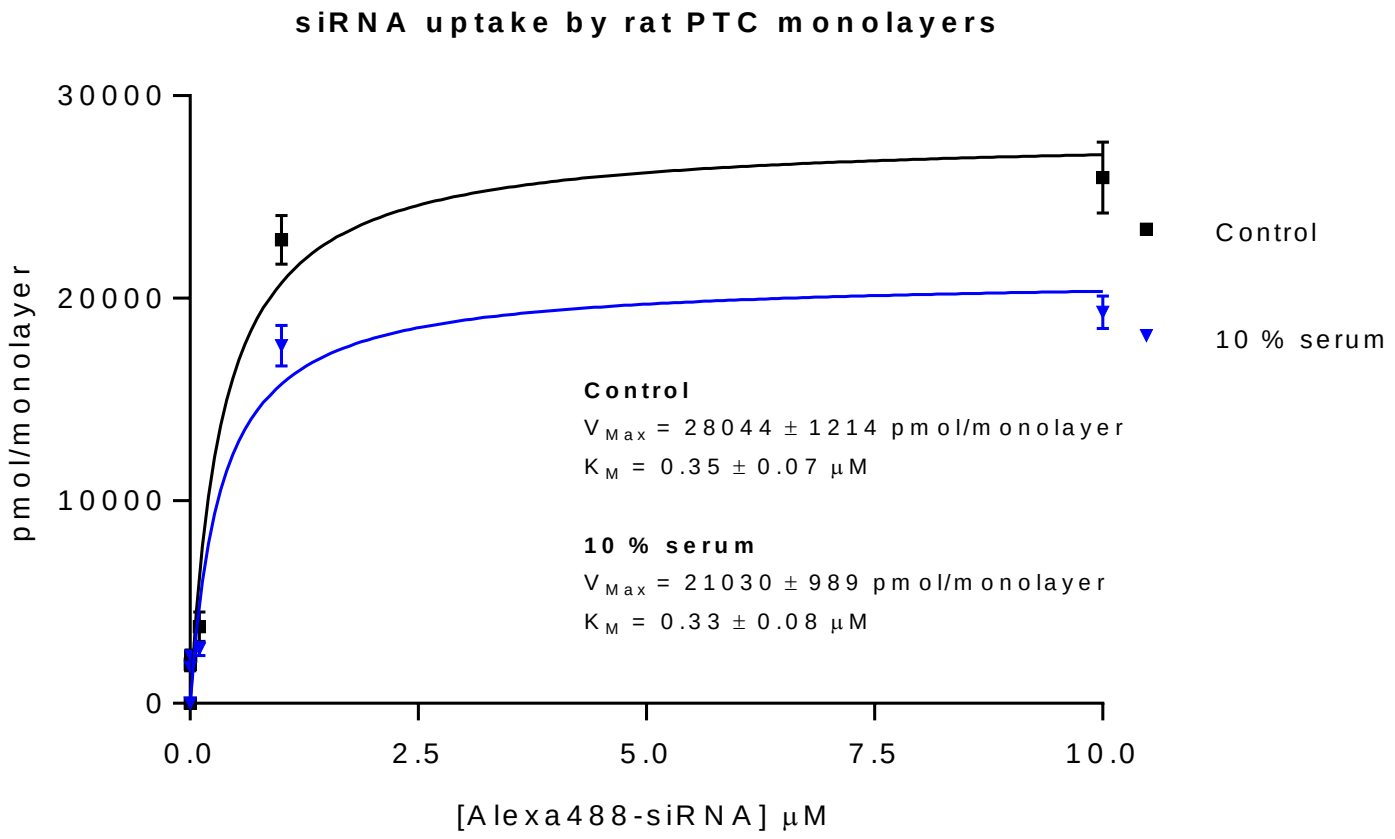
Demonstration of uptake of a siRNA Construct into rat aProximate cells



ONLY kidney model that has demonstrated siRNA or AON uptake

Renal Biomarker Strategy

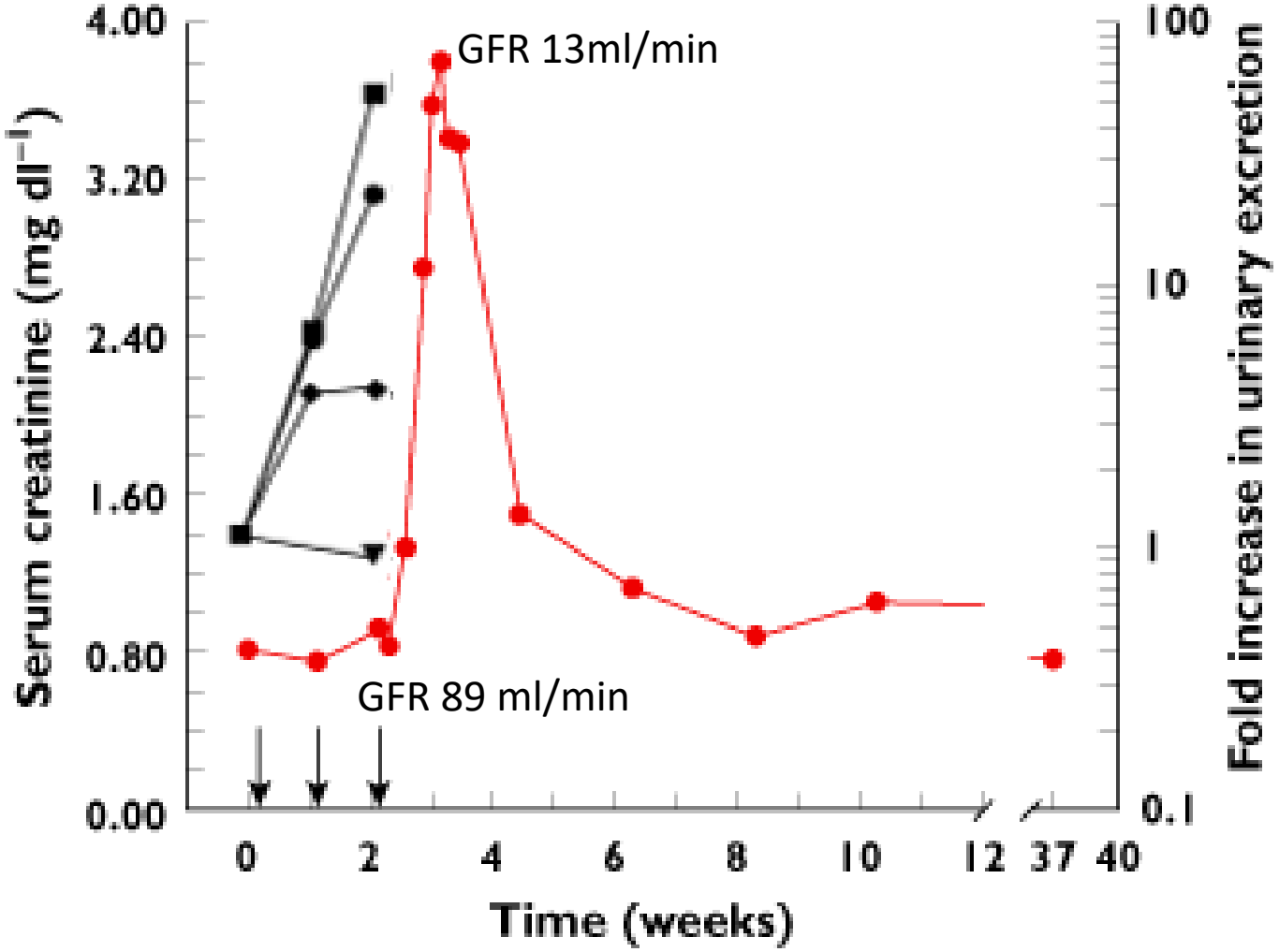
Glomerulus	Total protein Cystatin C Albumin B2M	FDA/EMEA Guideline Biomarkers of Nephrotoxicity in Preclinical Studies
Proximal Tubule	KIM 1 Clusterin TF3*	FDA/EMEA Guideline Biomarkers of Nephrotoxicity in Preclinical Studies
	NGAL NAG IL 18 ά GST**	Emerging New Biomarkers of Nephrotoxicity in Preclinical Studies
Distal Tubule	TF3* αGST FABP	• TF3 is not well validated in vivo • ά GST** is not very stable in urine

Drivers: FDA White Paper 2008 and EMEA White paper 2014 Directs Pharma to develop Better and Earlier Detection of Toxicity

August 2018 Long Awaited Guidance on Biomarkers in clinical studies published. - We have validated 4/5 in aProximate™Platform

Case study 3 - Renal Toxicity hits phase 1 Trial of Biologic SPC5001

56 year old female
SPC5001
5mg/kg



van Poelgeest et al Am.J.Kid. Dis 62 (2013)

■ **KIM 1** ● **αGST** ▼ **B2M** ▲ **NAG**

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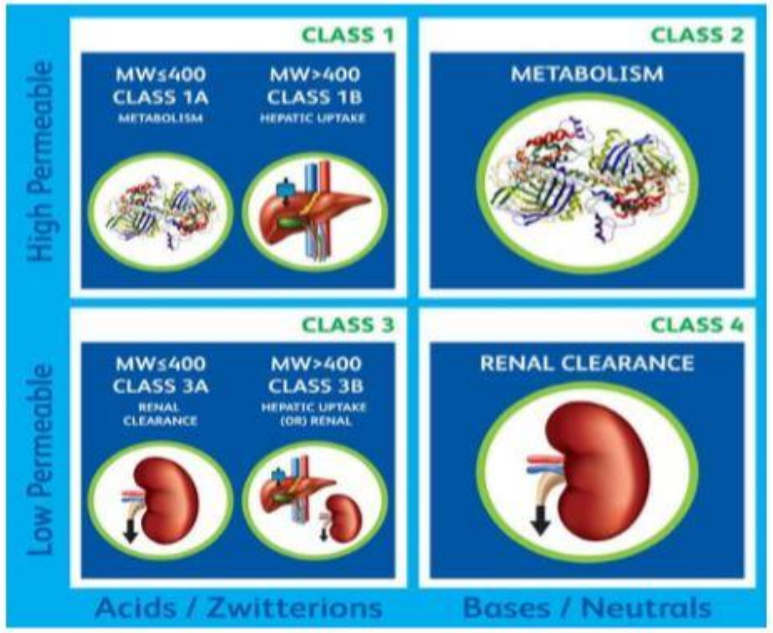
Validation of Assay in high throughput 96 well Transwell Format

Piyush Bajaj
Matt Wagoner

- BIOMARKERS KIM-1 NGAL Clusterin (*Osteopontin HO-1)*
- Live in cell Real-Time Assay of ATP-depletion
- Live off cell Real Time Assay of Cell death (LDH release)
- TEER –measurement of Monolayer integrity

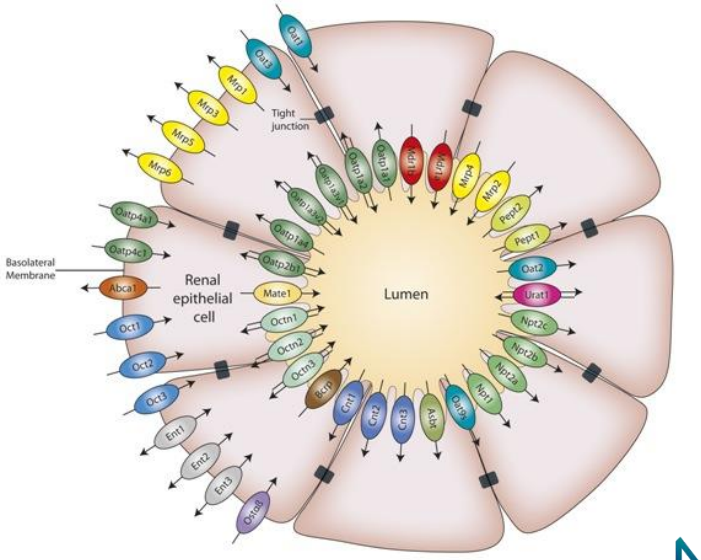
Toolkit of 36 compounds:

Diverse Chemical Structures



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

Range of Mechanistic exposure routes



ROC 36 compounds Demonstrating Strength of aProximate Model

Selection of the 36 compound (19 positives, 17 negatives) set

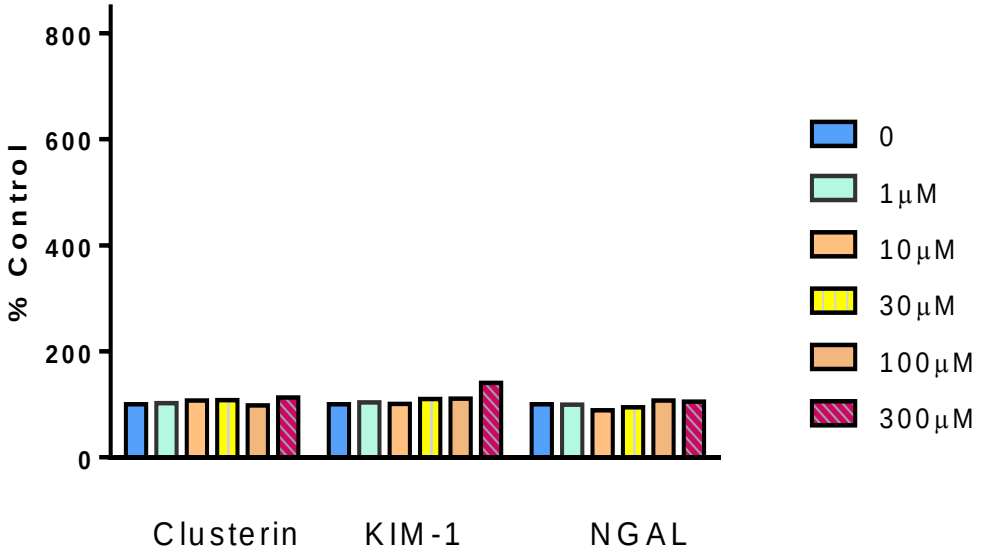
Compound selection was done using literature and Pharmapendium database

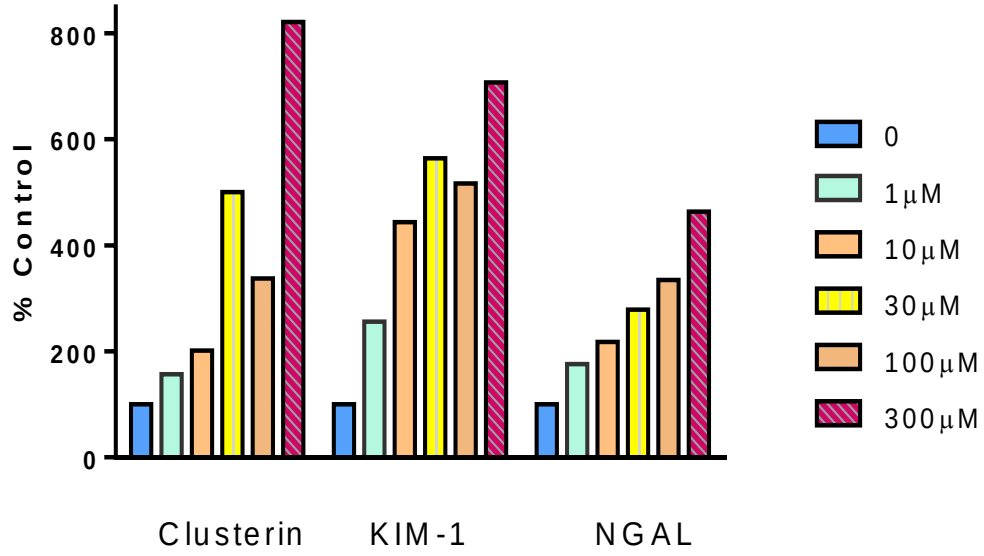
Compounds	Preclinical | Clinical |Post-marketing								
	Renal tubular acidosis	Renal tubular atrophy	Renal tubular disorder	Renal tubular dysfunction	Renal tubular necrosis	Acute kidney injury	Renal failure	Renal/tubular specific AEs	Total AEs
Adefovir	-|-|34	-|1|5	2|6|102	-|-|21	16|-|14	-|2|19	-|10|55	18|19|250	347|545|2168
Amphotericin B	-|7|32	3|-|-	11|1|44	-|-|-	8|-|13	-|5|208	-|5|163	22|18|460	482|495|5047
Carbamazepine	-|-|3	-|-|1	1|-|8	-|-|-	-|-|19	1|-|162	-|14|133	2|14|326	439|2744|21920
Cyclophosphamide	-|-|3	-|-|1	4|1|11	-|-|-	-|1|13	-|-|308	-|1|260	4|3|596	840|442|23749
Cyclosporine A	-|-|15	2|-|90	22|-|88	-|-|-	-|6|221	-|-|1063	-|1|900	24|7|2377	723|1538|39191
Foscarnet	-|-|-	-|-|-	1|1|15	-|-|-	-|-|14	-|1|71	-|2|65	1|4|165	111|232|860
Cimetidine	-|-|-	-|-|2	-|-|1	-|-|-	-|-|1	-|1|32	-|-|9	0|1|45	14|449|1251
Cisapride monohydrate	-|-|2	-|-|-	-|-|-	-|-|-	-|-|-	-|-|14	-|-|285	0|0|301	348|580|6737
Crizotinib	-|-|-	-|-|-	2|-|-	-|-|-	-|-|1	-|3|97	-|1|67	2|4|165	571|1504|8020
4-Aminopyridine	-|-|1	-|-|-	-|-|-	-|-|-	-|-|-	-|-|50	-|-|79	0|0|130	292|1185|47227
Digoxin	-|-|4	-|-|-	-|-|1	-|-|-	1|-|43	-|-|891	-|-|883	1|0|1822	51|364|14106
Quinidine	-|-|-	-|-|-	-|-|-	-|-|-	-|-|-	-|-|-	-|-|1	0|0|1	25|163|115

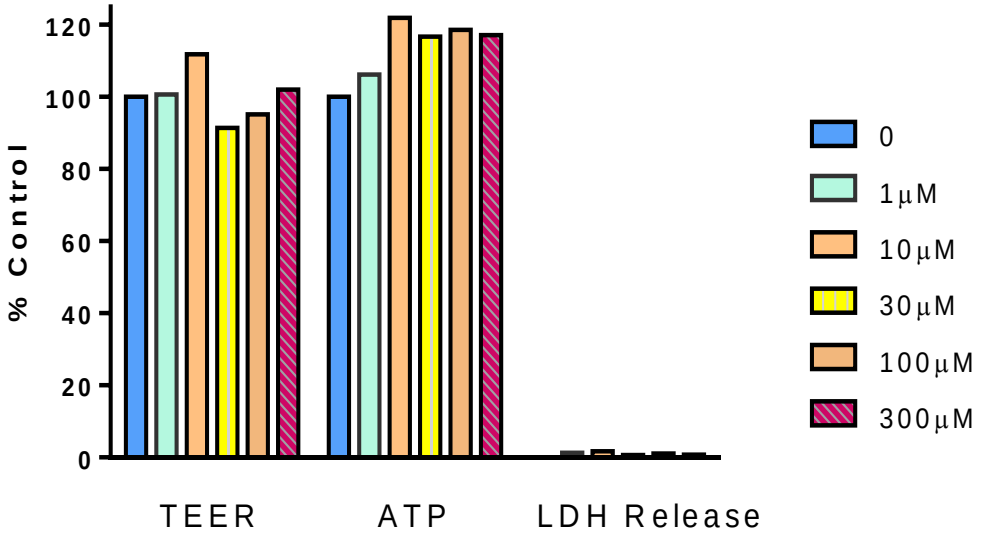
Negatives were selected to have urine excretion and strong in vivo activity

Compound	Reason for picking	Urine excretion (%)
Cisapride	Cardiotoxicant	0.2-53
Digoxin	Cardiotoxicant;P-gp substrate	53
Cimetidine	OCT2 substrate	48
Ranitidine	Cardiotoxicant, hepatotoxicant	27
Ximelagatran	Hepatotoxicant	80
Trovafloxacin	Hepatotoxicant	6
4-Aminopyridine	CNS toxicant	60
Crizotinib	OCT2 substrate, P-gp Inhibitor; cardiotoxicant	1.3-22
Quinidine	Class I antiarrhythmic agent	20
Verapamil	L-type Calcium channel blocker	70
Furosemide	Secondary PT-toxicant	39

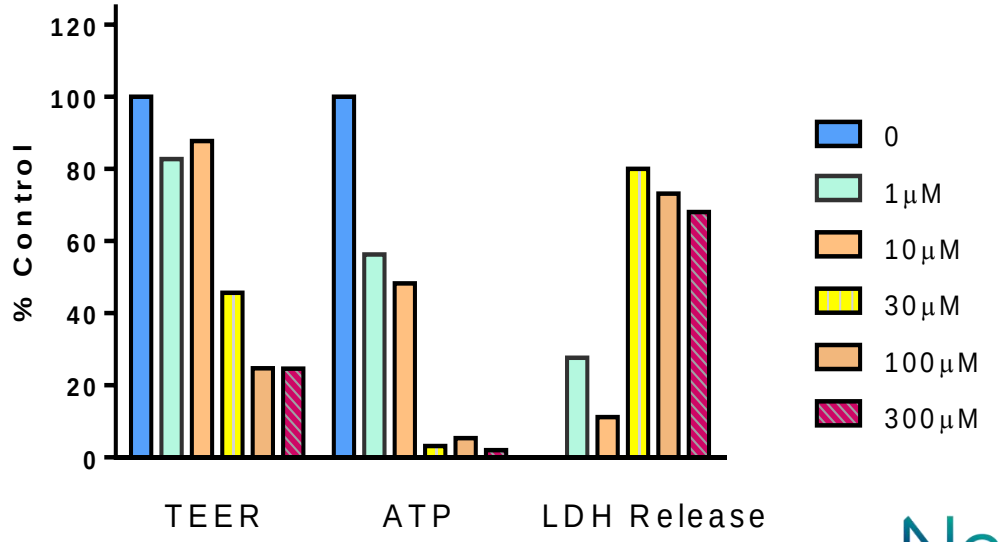
High Content comparison of Compound 6 and 24 on PTC Health







Compound 6



Compound 24

ROC Scorecard of 36 compounds Demonstrating Strength of aProximate Model

Compound scorecard using in vitro safety margin

Takeda

Compound	Clusterin	KIM-1	NGAL	ATP	LDH	TEER	Total
Primary PT-toxicant							
	1	1	1	1	1	1	6
	1	0	1	1	1	1	5
	1	1	1	1	1	0	5
	1	1	1	1	1	0	5
	1	1	1	1	1	0	5
	1	1	1	1	0	0	4
	1	1	1	1	0	0	4
	1	1	1	0	1	0	4
	1	1	1	1	0	0	4
	1	1	0	0	0	1	3
	1	1	1	0	0	0	3
	0	1	1	0	0	0	2
	0	1	1	0	0	0	2
	0	0	0	0	0	0	0
	0	0	0	0	0	0	0
	0	0	0	0	0	0	0
	0	0	0	0	0	0	0
	0	0	0	0	0	0	0
	0	0	0	0	0	0	0
Secondary PT-toxicant or Non-nephrotoxicant							
	1	1	1	1	0	1	5
	0	0	0	1	0	1	2
	0	1	1	0	0	0	2
	0	0	0	0	1	0	1
	1	0	0	0	0	0	1
	0	0	0	0	0	0	0
	0	0	0	0	0	0	0
	0	0	0	0	0	0	0
	0	0	0	0	0	0	0
	0	0	0	0	0	0	0
	0	0	0	0	0	0	0
	0	0	0	0	0	0	0
	0	0	0	0	0	0	0
	0	0	0	0	0	0	0
	0	0	0	0	0	0	0
	0	0	0	0	0	0	0
	0	0	0	0	0	0	0

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

	TP	FN	TN	FP	Sensitivity (%)	Specificity (%)	Accuracy (%)
All 6 end-points	13	6	12	5	68.4	70.6	69.4
Biomarkers + ATP	13	6	13	4	68.4	76.5	72.2
Any biomarkers	13	6	14	3	68.4	82.4	75.0
At least 2 biomarkers	**13**	**6**	**15**	**2**	**68.4**	**88.2**	**77.8**

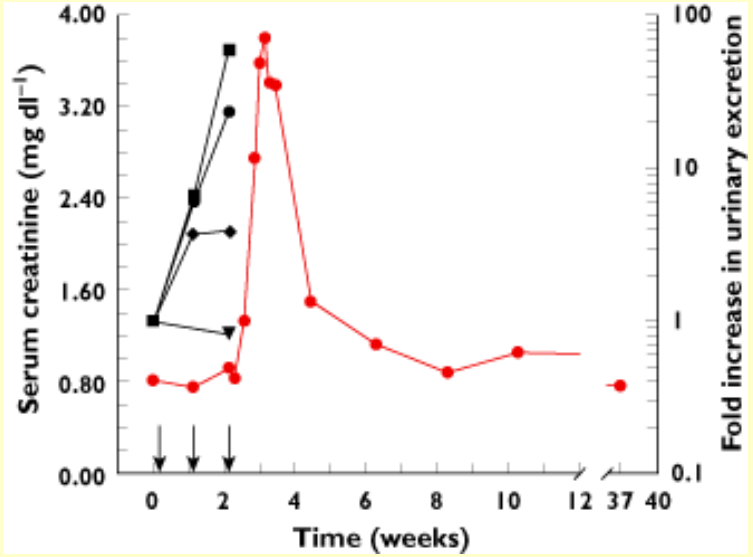
INDUSTRY BEST MODEL

1 – model predicted to be toxic

0 – model predicted to be non-toxic

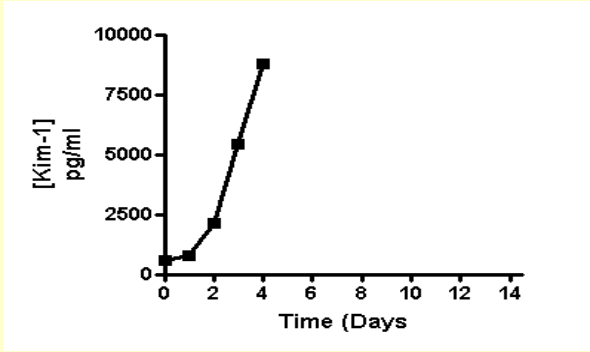
Case Study 3- aProximate Human PTC cells PREDICT SP5001 toxicity

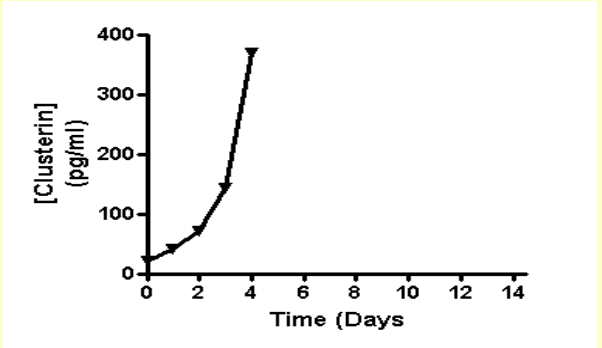
In vivo data human kidney

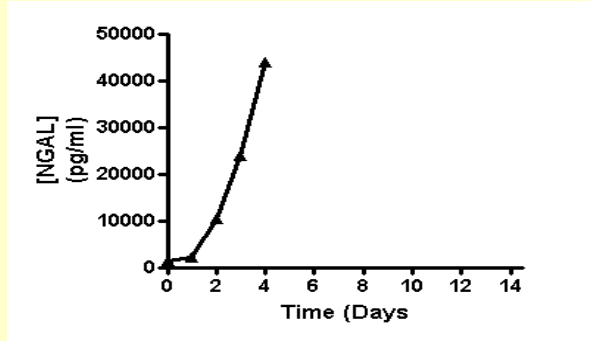


- Creatinine
- KIM 1
- αGST
- B2M
- NAG

In vitro data Human aProximate PTC







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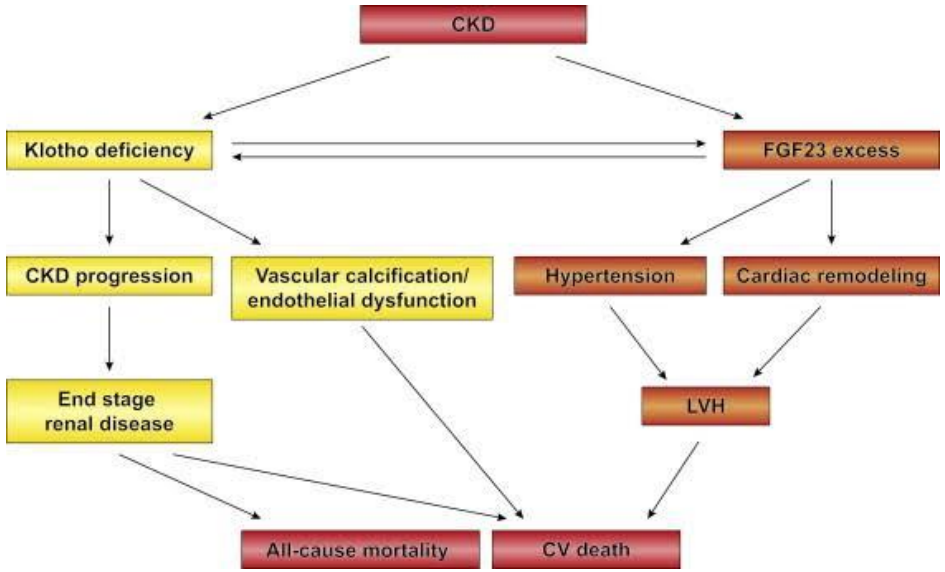
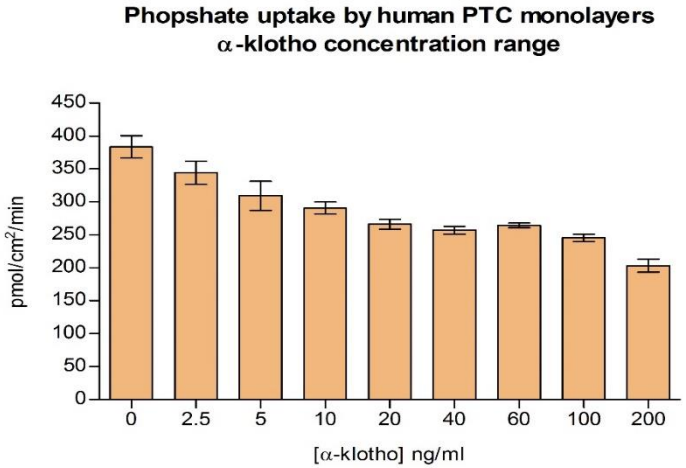
aProximate™ Platform is Unique Platform for Transporter, DDI and Nephrotoxicity Studies

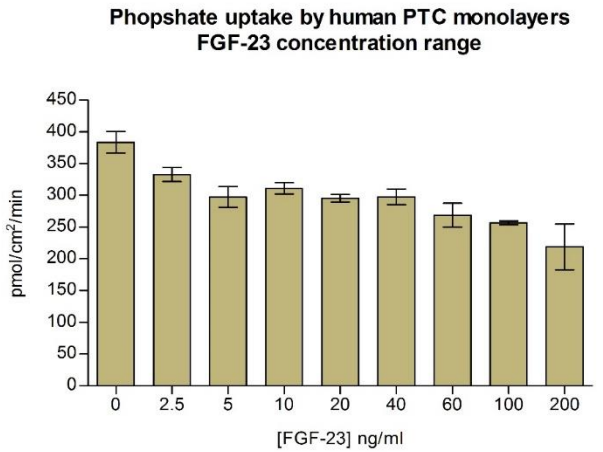
- Ability to generate Cross-species Human Dog, NHP , rat and mouse platforms is unique
- In vitro transporter and DDI data is predictive of clinical outcome
- Maps on to FDA Guidance for Transporter and DDI studies
- Outperforms animal studies in predictiveness and utility
- Biomarker data validates models as predictive of clinical outcome NO other model has such strong validation data
- Biomarkers validated map directly onto August 2018 FDA Qualification of Biomarkers for clinical studies
- High throughput low cost solution for Industry
- *Development of Complex Kidney construct*
- *Development of 3D microfluidic kidney models*
- *Development of an iPSC derived complex kidney model*

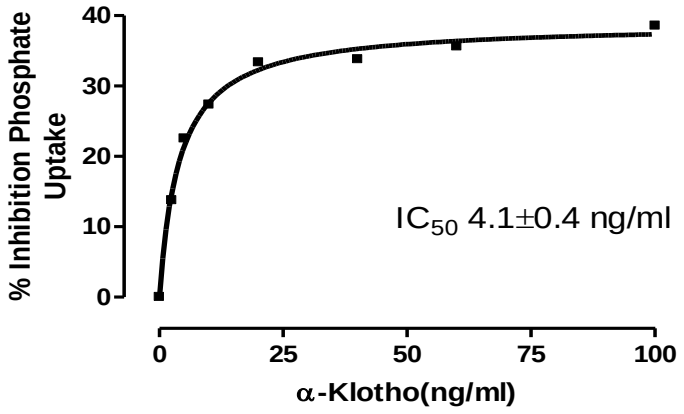
Newcells BIOTECH

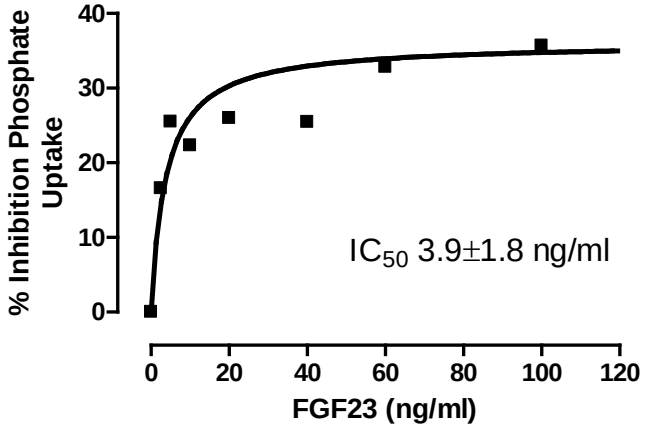
Case study 4: aProximate™ as a model to test drug efficacy and disease modelling





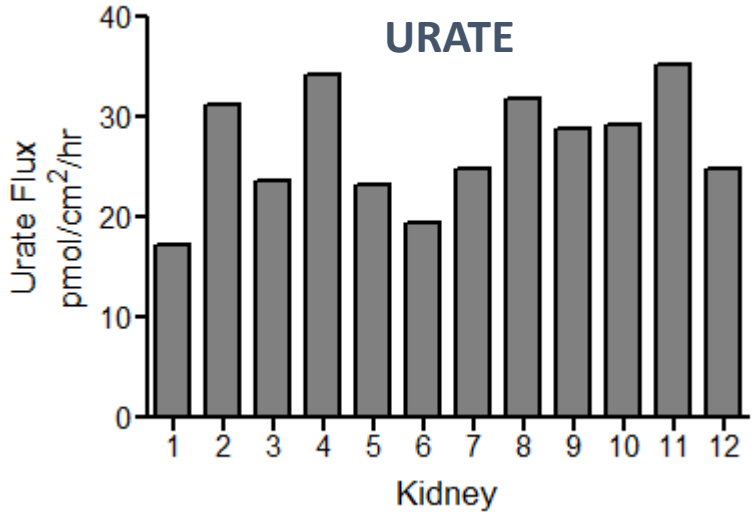


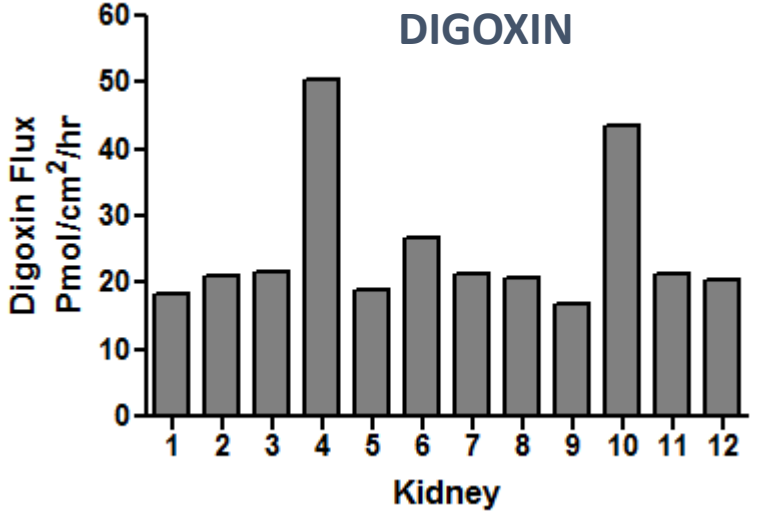


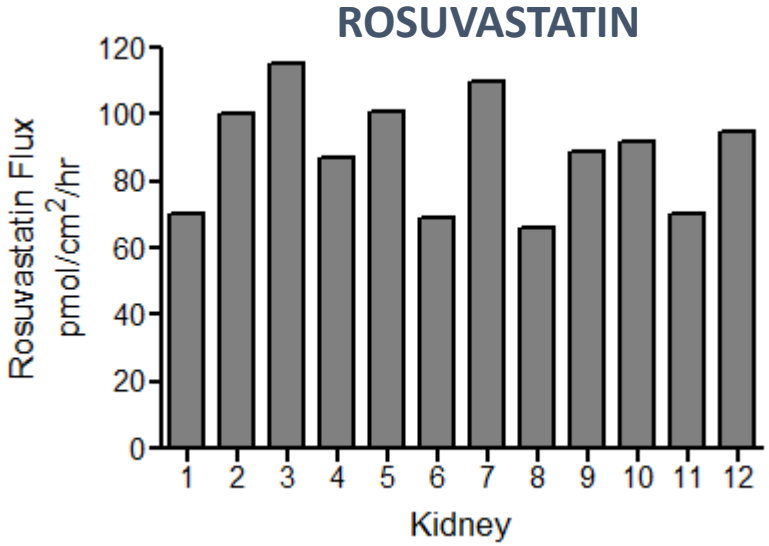


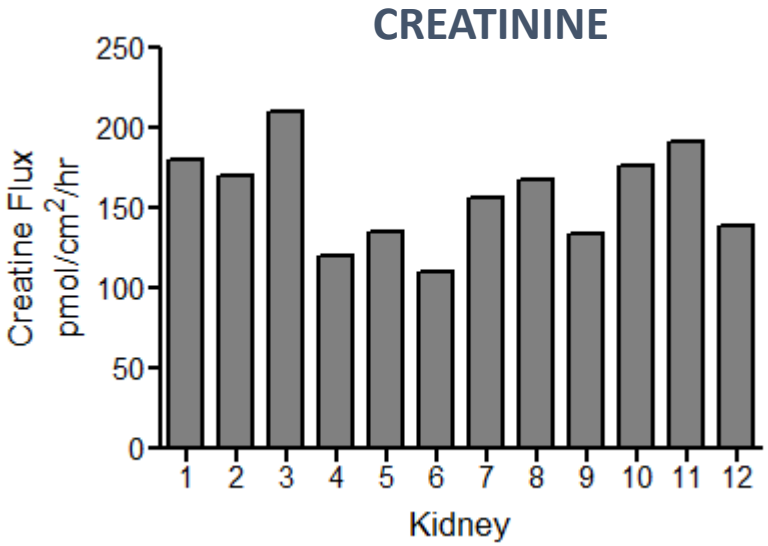
Development of α-Klotho mimetics would decrease CKD progression and CV complications

What is the Variability between Individual Kidneys?









Data from 48 Different Kidneys (12 kidneys per compound)