In Vitro Models for the Prediction of Drug-Induced Nephrotoxicity

Yao Li, Karthikeyan Kandasamy, Jacqueline Kai Chin Chuah
Sijing Xiong, Peng Huang, Kim Guan Eng and Daniele Zink

Institute of Bioengineering and Nanotechnology

In Vitro Nephrotoxicology

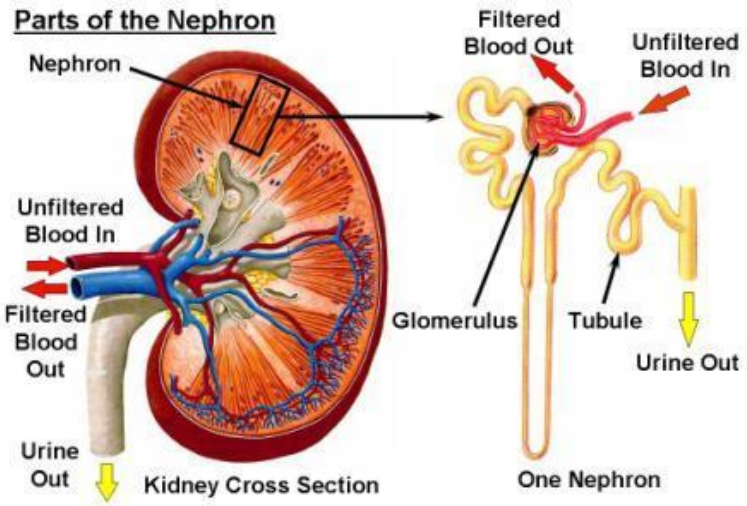
Problems

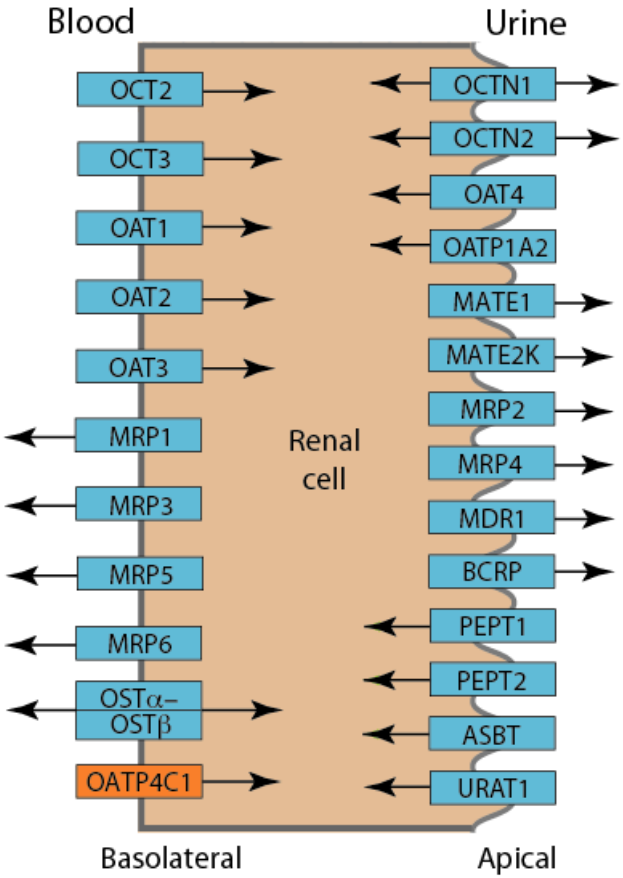
- Nephrotoxicity can induce acute kidney injury (AKI) and chronic kidney disease/ end stage renal disease.
- ~ 5% of all hospitalized patients develop AKI (~ 30%-60% of ICU patients; 40%-70% mortality).
- ~ 20–25% of hospital and community-acquired AKI due to drug-induced nephrotoxicity.
- Kidney toxicity accounts for 2% of drug attrition during pre-clinical studies and 19% in phase 3 (Redfern et al., 2010).
- The nephrotoxicity of alternative and new drugs is often underestimated (e.g. COX2 inhibitors, tenofovir).

***In Vitro* Models**

- Altered legislation (REACH, Cosmetics Directive) and new initiatives (Tox21 and ToxCast).
- Kidney: no accepted or validated *in vitro* models available.
- No activities of regulatory authorities on *in vitro* nephrotoxicology.
  (ECVAM: 1 pre-validation study: Duff et al., 2002)

The Renal Proximal Tubule (PT)





- **Glomerulus**
  - Filtration (kidney receives 25% of cardiac output)
- **Proximal Tubule**
  - Reabsorption of water and solutes
  - Transport, metabolism and excretion of drugs

http://bts.ucsf.edu/fdatransportal/organs/kidney/

- Phase I and Phase II enzymes (CYPs, UGTs, GSTs, SULTs etc.)
- Major differences between humans and rodents

In Vitro Nephrotoxicology: Problems

- **Cells:** Most studies were performed with immortalized cell lines. Widely used cell lines are often insensitive to well-characterized nephrotoxicants (e.g. gentamycin).

- **Endpoints:** Well-established endpoints (cell viability, metabolic activity etc.) and more specific endpoints (transepithelial resistance, lactate production and potential novel AKI biomarkers) did not give useful results.

– Problems reviewed in Tiong et al., Mol. Pharm., 2014

- PREDICT-IV (EU-funded project, 21 academic and industrial partners): Focus on novel potential AKI biomarkers. Results: potential AKI biomarkers were not up-regulated in human primary renal proximal tubular cells or a newly developed cell line derived from this cell type after treatment with typical nephrotoxicants (PREDICT-IV, 5th Periodic Report, 30.06.2013).

➢ <u>IBN, ETC, BII (A*STAR) and National University Health System:</u>
JCO Development Program on "Predictive *In Vitro* Models for Liver- and Kidney-Specific Toxicity" (Program Director: D. Zink)

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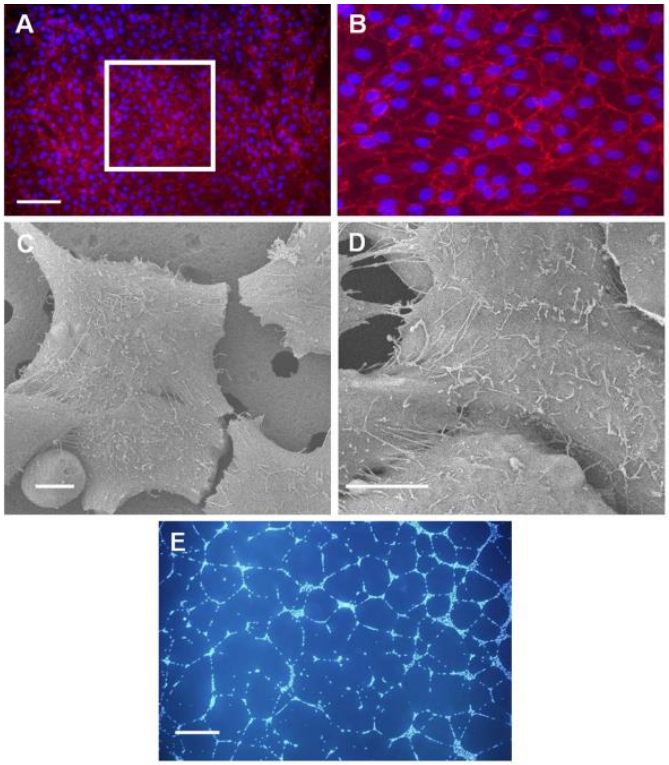
Cell Model 1: Human Primary Renal Proximal Tubular Cells (HPTC)

- Each variable was systematically characterized and engineered in our lab.

Cell Source	• Li et al., Nanotoxicology; 2012; Farah Tasnim, Doctoral Thesis
Substrates	• Zhang et al., J Cell Mol Med, 2011; Oo et al., Biomaterials, 2011; Ni et al., Biomaterials, 2012
Coatings	• Zhang et al., Biomaterials, 2009; Ni et al., Biomaterials, 2011
Media/Growth Factors	• Farah Tasnim, Doctoral Thesis; Tasnim et al., Tissue Eng Part A., 2012; Tasnim et al., Am J Physiol Renal Physiol, 2012
Microfluidics / Bioreactors	• Ni et al., Biomaterials, 2011; Oo et al., Biomaterials, 2011; Oo et al., J Cell Mol Med, 2013
Co-Cultures/ 3D Models	• Zhang et al., J Cell Mol Med, 2011; Li et al., Nanotoxicology, 2011; Tasnim et al., Am J Physiol Renal Physiol., 2012
Genetic Engineering	• Tasnim et al., Tissue Eng Part A., 2012
Quality Control and SOPs	Assays for monitoring purity and functional state are routinely applied (qPCR with > 30 markers, FACS, immunostaining, functional assays).

Disadvantages: Limited cell source, inter-donor variability, changes during passaging

Cell Model 2: 1st Protocol for Differentiating Human Pluripotent Stem Cells into HPTC-Like Cells



Epithelium with tight junctions
Polarization with brush border
Tubulogenesis on Matrigel

Narayanan et al., Kidney Int., 2013

hESC (Matrigel) (Day 0) → + BMP2 (10 ng/ml) + BMP7 (2.5 ng/ml) Renal epithelial growth medium (LONZA) → HPTC-like cells (Day 20)

Functional Features

- Increase cAMP levels in response to parathyroid hormone
- pH-dependent ammoniagenesis
- γGlutamyl transferase activity (brush border enzyme)
- Water channel activity
- Functionality is maintained in bioreactors
  - important for applications in microfluidic devices

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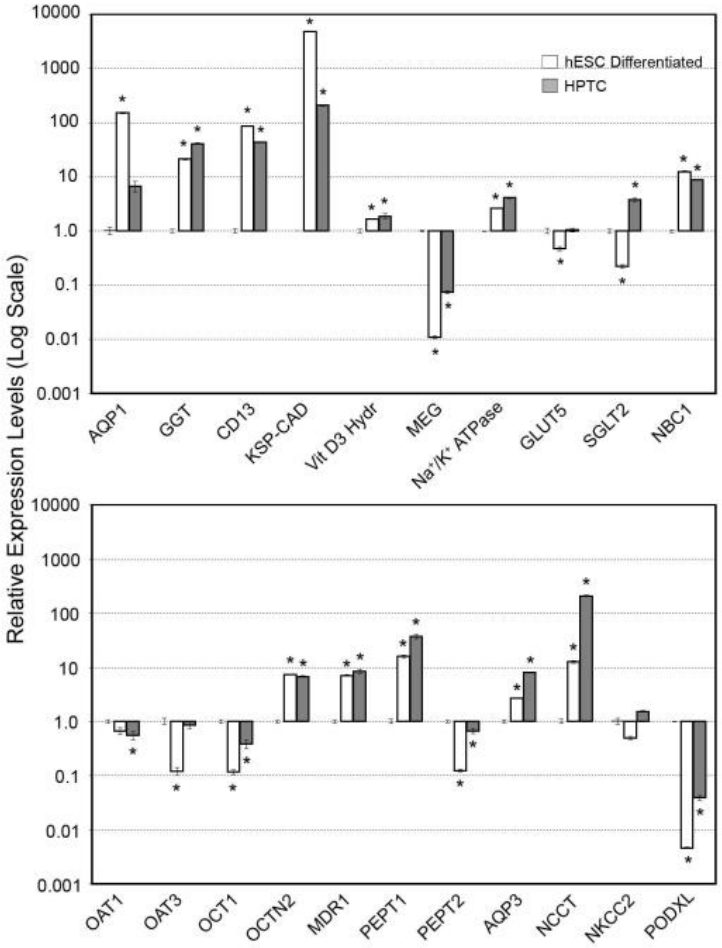
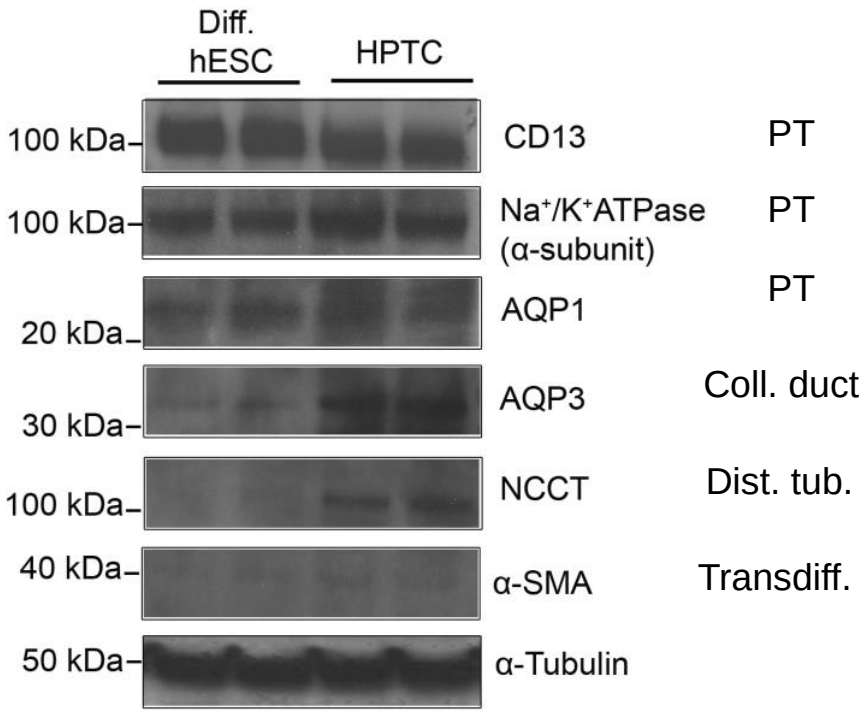
Human Embryonic Stem Cell-Derived HPTC-Like Cells



qPCR
Undifferentiated hESC = 1



Immunoblot

- Protein expression and localization was also characterized by immunostaining and FACS

Narayanan et al., Kidney Int., 2013

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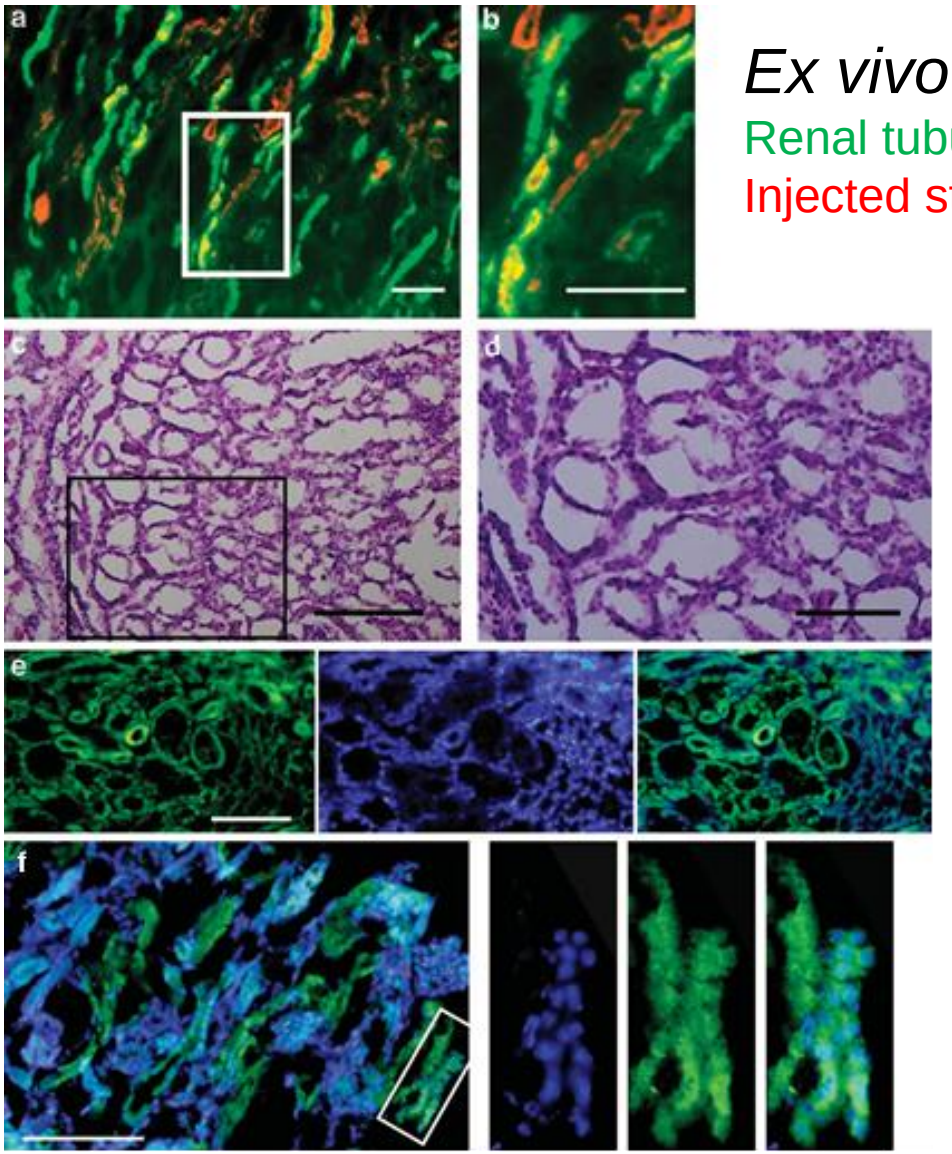
Human Embryonic Stem Cell-Derived HPTC-Like Cells



Ex vivo organ culture

Renal tubular epithelia (CK18)

Injected stem cell-derived cells

In vivo implants (SCID mice)

- AQP1-expressing tubules

DAPI

AQP1

Narayanan et al., Kidney Int., 2013

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Race for Pluripotent Stem Cell-Derived Renal Cells and Precursors

- *Narayanan et al., Kidney Int., Feb 6, 2013*: Human embryonic stem cell (hESC)-derived renal proximal tubular-like cells

- 1st sucessful application of stem cell-derived renal cells: *In vitro* toxicology
  Li et al., Mol. Pharm., 2014

- Other Recent Protocols:

- Recapitulation of embryonic development
- Stepwise differentiation of PSCs into renal precursor cells and structures
- Complex multi-step protocols
- Result: Mix of different precursor structures and cell types

- Mae et al., Nat. Commun., 2013: Intermediate mesoderm
- Xia et al., Nat. Cell Biol., Epub Nov 17, 2013: ureteric bud progenitor cells
- Taguchi et al., Cell Stem Cell, Epub Dec 12, 2013: metanephric mesenchyme
- Takasato et al., Nat. Cell Biol., Epub Dec 15, 2013: metanephric mesenchyme and ureteric buds that self-organize into renal precursor structures *in vitro*
- Lam et al., J Am Soc Nephrol, Epub Dec 19, 2013: intermediate mesoderm, further differentiates into renal tubules *in vitro*
- Kang & Han., PLOS ONE, Epub April 11, 2014: nephron progenitor cells; can be further differentiated; 40 - > 50 days

- Main potential application: regenerative medicine and disease models

In Vitro Nephrotoxicology: Problems

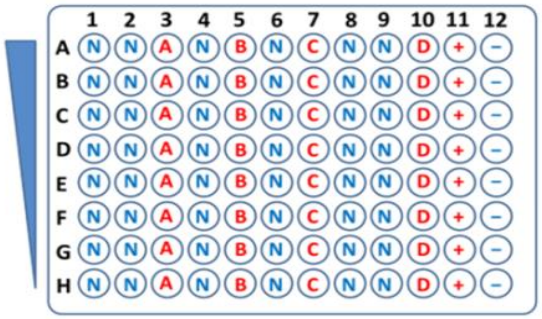
- **Cells:** Most studies were performed with immortalized cell lines. Widely used cell lines are often insensitive to well-characterized nephrotoxicants (e.g. gentamycin).

- **Endpoints:** Well-established endpoints (cell viability, metabolic activity etc.) and more specific endpoints (transepithelial resistance, lactate production and potential novel AKI biomarkers) did not give useful results.

The Injury Mechanism-Specificity of Endpoints Inversely Correlates with Predictivity

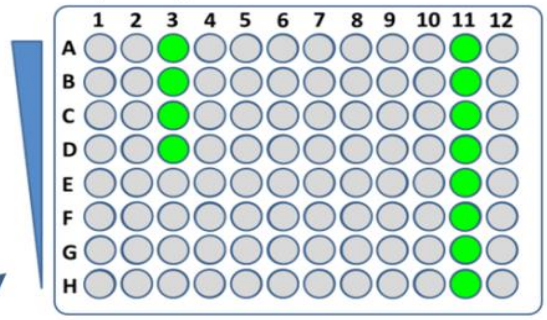
- Inflammation and pro-inflammatory factors play important roles in the pathophysiology acute kidney injury (AKI).
- Pro-inflammatory markers are activated by a wide range of stressors and injury mechanisms.

Injury mechanisms often unknown in pre-clinical screens



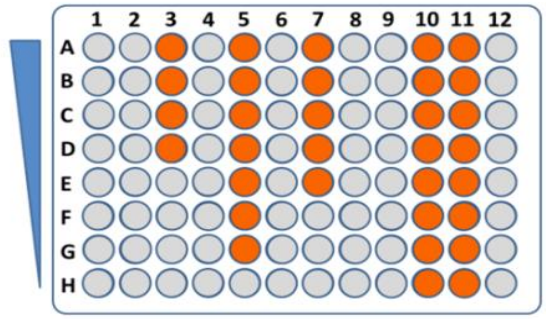
- A, B, C, D: Nephrotoxic compounds that injure PTC by mechanism a, b, c, d
- N: Not toxic for PTC
- +: Positive control
- −: Negative control

Endpoint specific for mechanism 'a'



- Information on injury mechanism
- 25% Sensitivity

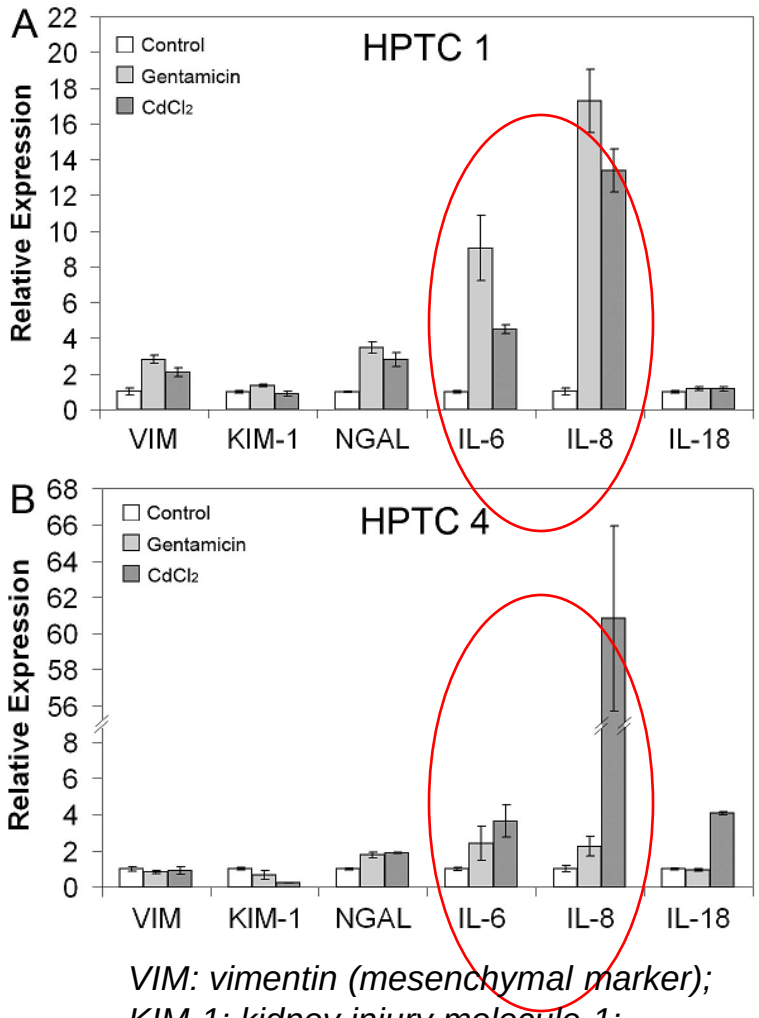
Endpoint triggered by cell stress or injury



- No information on injury mechanism
- 100% Sensitivity

Tiong et al., Mol. Pharm., 2014

Marker Gene Expression in Response to Nephrotoxicants



VIM: vimentin (mesenchymal marker); KIM-1: kidney injury molecule-1; NGAL: neutrophil gelatinase-associated lipocalin;

Li et al., Toxicol. Res., 2013

- Two batches HPTC treated overnight with high doses of PT-specific nephrotoxicants
  - Gentamicin: 2.5 mg/ml
  - $CdCl_2$: 10 μg/ml

- Nephrotoxic drugs induce interleukin (IL)-6 and IL-8 expression in proximal tubular cells (PTC) *in vivo*
- Only IL-6 and IL-8 showed consistently significantly increased expression levels

- VIM is expressed *in vivo* after PT injury
- KIM-1, NGAL and IL-18 are promising potential novel biomarkers for acute kidney injury (AKI)

- *In vitro* primary rat and human PTC always express high levels of injury markers, also in the untreated state

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Set of 41 Compounds with Well-Characterized Effects on Human Kidneys

1-22 Nephrotoxicants, toxic for PT in humans

23-33 Nephrotoxicants, not toxic for PT in humans

34-41 not nephrotoxic

No.	Compound
1	Gentamicin
2	Tobramycin
3	Rifampicin
4	Tetracycline
5	Puromycin
6	Cephalosporin C
7	5-Fluorouracil
8	Cisplatin
9	Ifosfamide
10	Paraquat
11	Arsenic(III) oxide
12	Bismuth(III) oxide
13	Cadmium(II) chloride
14	Copper(II) chloride
15	Germanium(IV) oxide
16	Gold(I) chloride
17	Lead acetate
18	Potassium dichromate
19	Tacrolimus
20	Cyclosporin A
21	Citrinin
22	Tenofovir
23	Vancomycin
24	Phenacetin
25	Acetaminophen
26	Ibuprofen
27	Furosemide
28	Lithium Chloride
29	Lindane
30	Ethylene glycol
31	Valacyclovir
32	Lincomycin
33	Ciprofloxacin
34	Ribavirin
35	Glycine
36	Dexamethasone
37	Melatonin
38	Levodopa (DOPA)
39	Triiodothyronine
40	Acarbose
41	Atorvastatin

Only 3 studies on *in vitro* nephrotox that have tested > 10 compounds:

- Duff et al., 2002
- Wu et al., 2009
- Lin & Will, 2012

These models were either not predictive or their predictivity had not been determined

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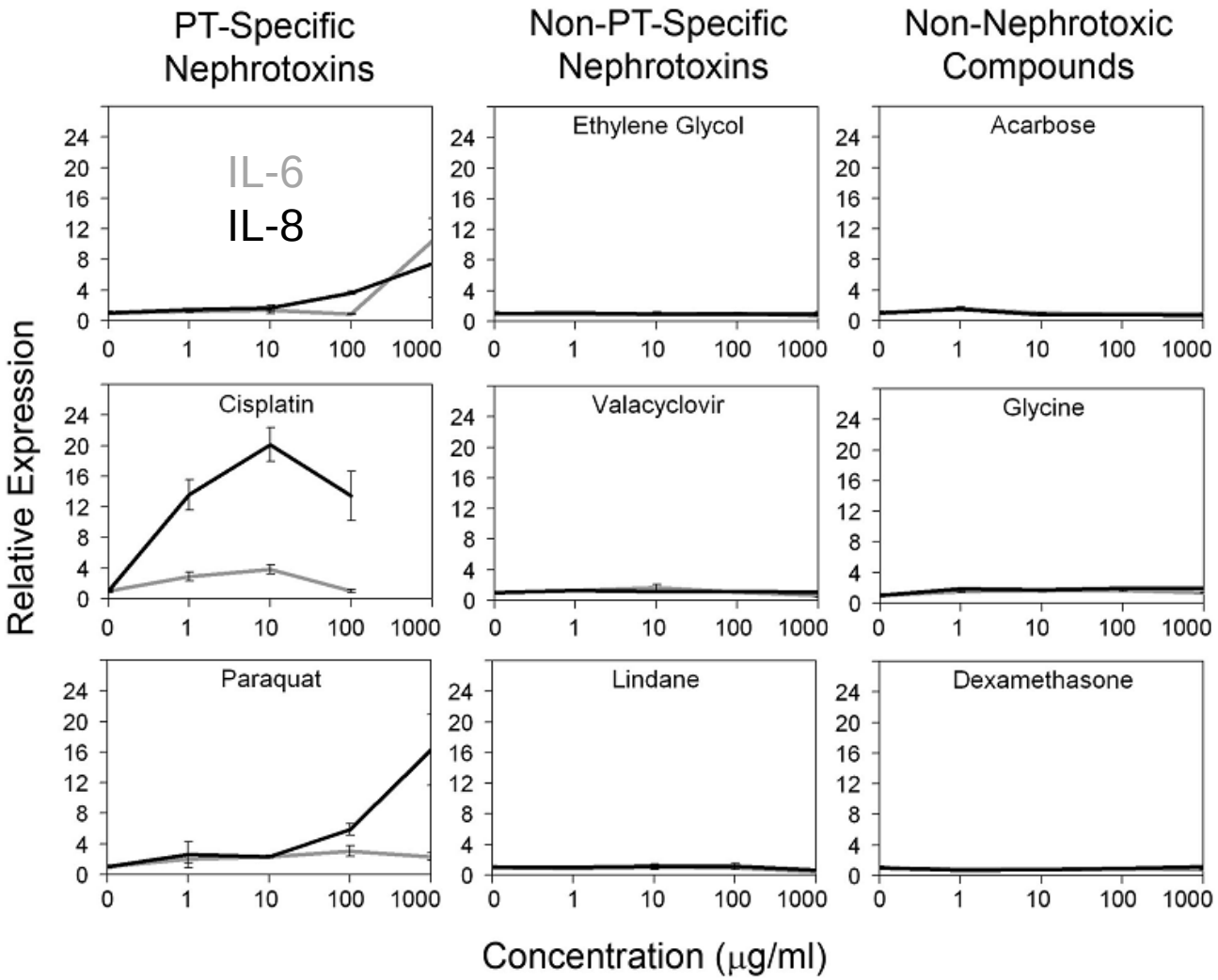
IL-6/IL-8 Expression after Exposure to Different Groups of Test Compounds



- All results were normalized to vehicle controls

Li et al., Toxicol. Res., 2013

Major Performance Metrics

Endpoints	Cell Type	Sensitivity	Specificity	Balanced Accuracy	PPV	NPV	AUC
IL-6/IL-8 Expression	HPTC 1*	0.91	0.90	0.90	0.91	0.94	0.94
	HPTC 2*	0.77	0.84	0.81	0.85	0.76	0.81
	HPTC 3*	0.64	0.79	0.71	0.78	0.68	0.82
	HPTC mean*	0.77	0.84	0.81	0.85	0.79	0.85
	HPTC median*	0.77	0.84	0.81	0.85	0.76	0.82
	HK-2*	0.50	0.79	0.65	0.73	0.60	0.71
	LLC-PK1*	0.64	0.74	0.69	0.74	0.67	0.73
	HPTC-like	0.68	0.84	0.76	0.83	0.70	0.80
ATP Depletion	HPTC-like	0.48	0.79	0.63	0.71	0.58	0.65
	HPTC 1	0.50	0.74	0.62	0.69	0.56	0.65
GSH Depletion	HPTC 1	0.45	0.74	0.60	0.67	0.54	0.60
LDH Leakage	HPTC 1	0.64	0.58	0.61	0.64	0.58	-
Cell Death	HPTC 1*	0.42	0.95	0.69	0.89	0.62	-

Balanced accuracy
= (sensitivity + specificity) / 2

PPV: positive predictive value
= TP / (TP + FP)

NPV: negative predictive value
= TN / (TN + FN)

AUC: area under the curve of receiver operating characteristic curves

Li et al., Mol. Pharm., 2014; Li et al., Toxicol. Res., 2013

Always same test set of 41 compounds and same conditions

- 1st model with proven predictivity
- The predictivity was low when the same cell types were used with well-established endpoints

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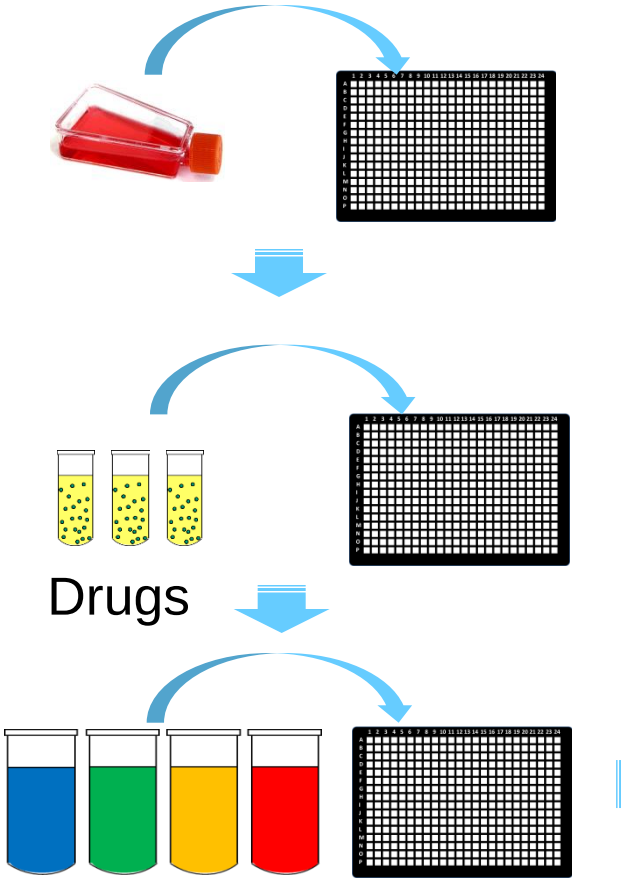
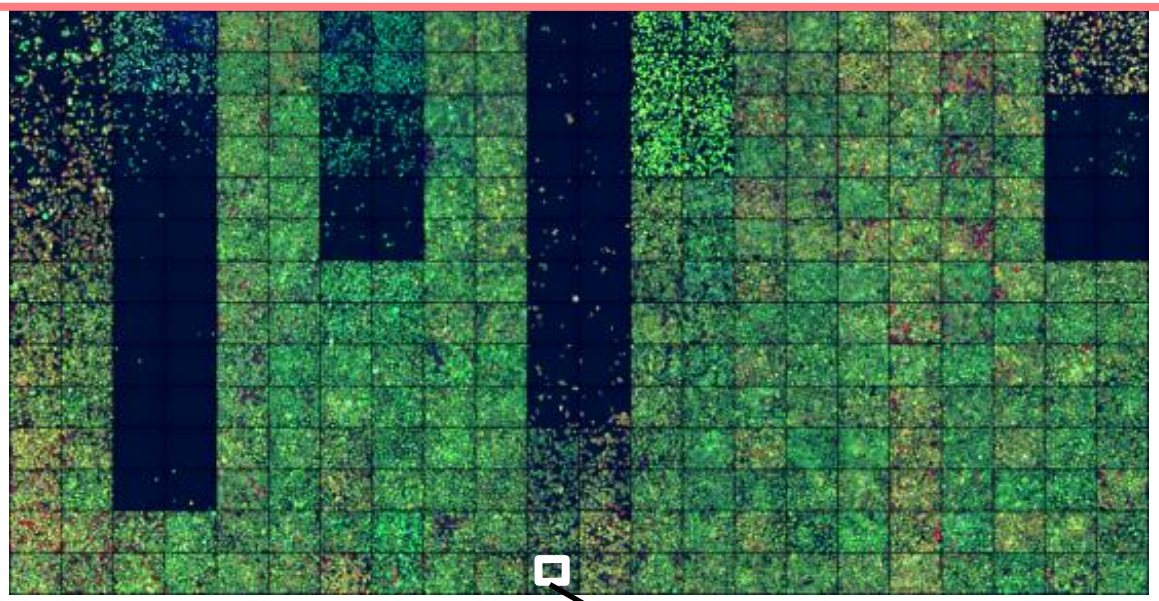
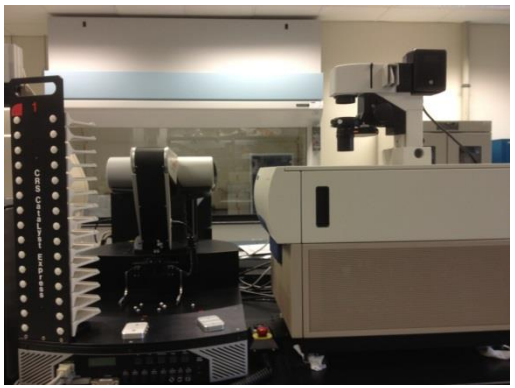
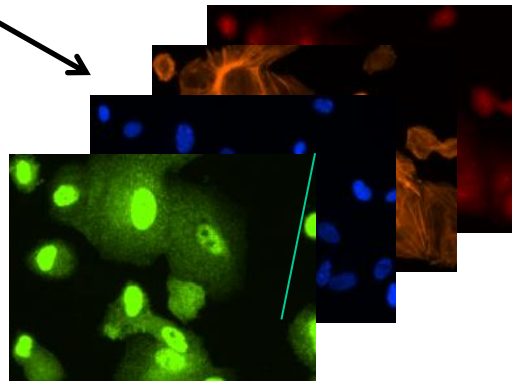
Currently Developed: Predictive Model for High Content Screening









- Now adapted to 1536-well format

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In Progress

- Development of a predictive *in vitro* model based on human induced pluripotent stem cell-derived renal cells.

Acknowledgments

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