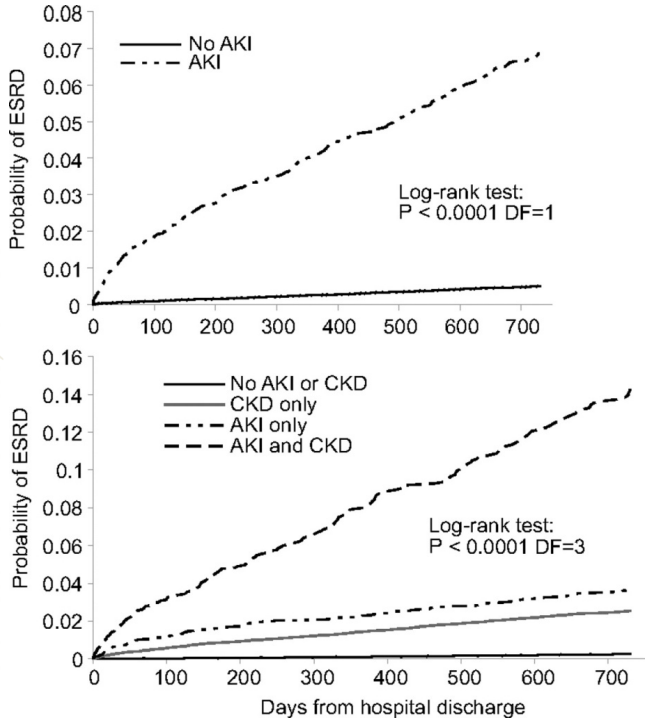
The Scale of the Problem – strong motivation for better predictors

- Nephrotoxicity resulting from drug exposure has been estimated to contribute to 20–25% of all cases AKI in critically ill patients
  - While disease prevalence in US suggests 2-5%, based on hospital admissions, the economic burden is highly disproportionate
- AKI is an independent risk factor for CKD & ESRD
- Pre-clinical nephrotoxicity is a substantial contributor to early curtailment in drug development (10-15%)

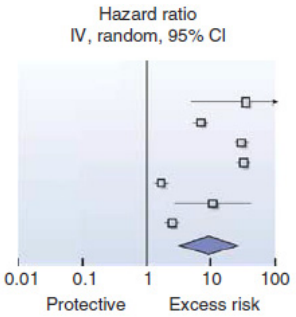


Ishani et al 2011 JASN 2009; 20:223-8

Pooled adjusted hazard ratios for CKD & ESRD after AKI.

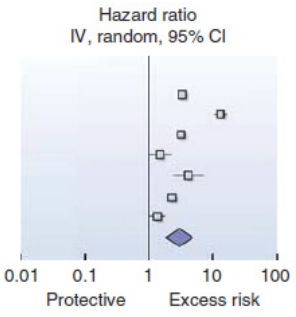
Study or subgroup	Weight (%)	Hazard ratio IV, random, 95% CI
Weiss *et al.* (13)	10.0	32.79 (4.30–249.77)
Amdur *et al.* (22)	15.5	6.64 (5.05–8.74)
Lo *et al.* (11)	15.5	28.08 (21.01–37.53)
James *et al.* (16)	15.6	29.99 (24.32–36.99)
James *et al.* (15,23)	15.5	1.60 (1.20–2.14)
Ando *et al.* (19)	12.4	9.91 (2.48–39.63)
Ishani *et al.* (21)	15.6	2.33 (1.83–2.96)
Total (95% CI)	100.0	8.82 (3.05–25.48)

Heterogeneity: $\tau^2 = 1.87$; $\chi^2 = 446.89$, d.f. = 6 ($P < 0.00001$); $I^2 = 99\%$. Test for overall effect: $Z = 4.02$ ($P < 0.0001$)



Study or subgroup	Weight (%)	Hazard ratio IV, random, 95% CI
Newsome *et al.* (14)	15.0	3.26 (2.87–3.70)
Ishani *et al.* (20)	14.8	12.99 (10.57–15.96)
Wald *et al.* (17)	14.9	3.22 (2.70–3.85)
Hsu *et al.* (10)	13.5	1.47 (0.95–2.28)
James *et al.* (15,23)	12.5	4.15 (2.32–7.41)
Lafrance *et al.* (18)	15.0	2.33 (2.08–2.61)
Choi *et al.* (12)	14.4	1.37 (1.02–1.84)
Total (95% CI)	100.0	3.10 (1.91–5.03)

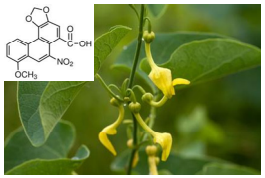
Heterogeneity: $\tau^2 = 0.40$; $\chi^2 = 252.85$, d.f. = 6 ($P < 0.00001$); $I^2 = 98\%$. Test for overall effect: $Z = 4.58$ ($P < 0.00001$)



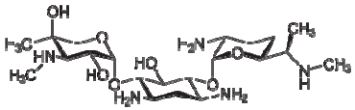
Coca et al 2012; 81, 442–448

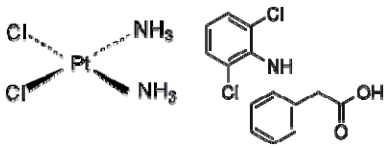
The anatomy of changes associated with tubular injury

Environmental/dietary nephrotoxicants



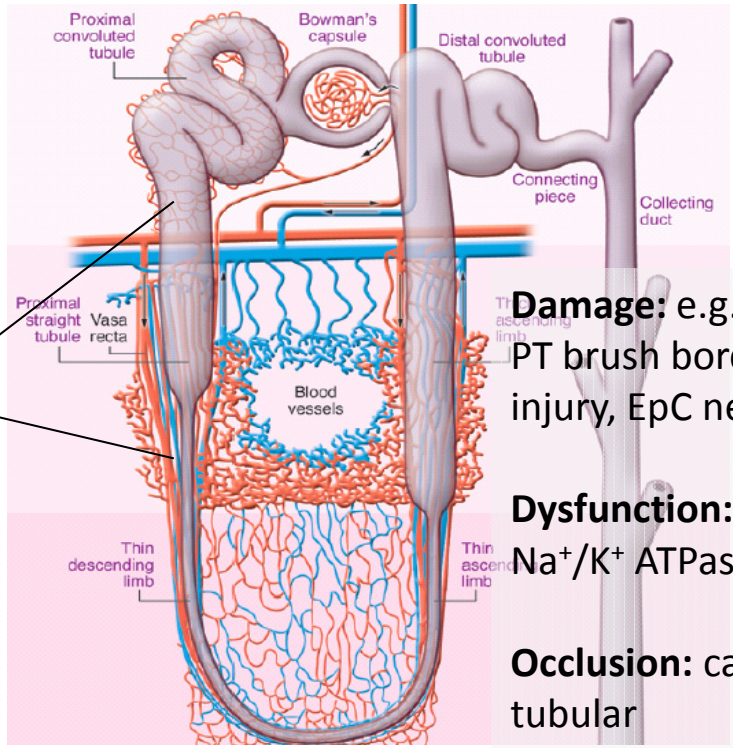
Drugs





Infection
Surgery
Age

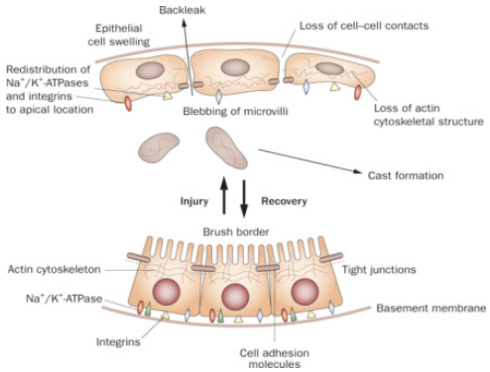
Pre-disposing factors
Existing renal impairment
Diabetes/hypertension
Liver disease
Renal ischemia
Inflammation

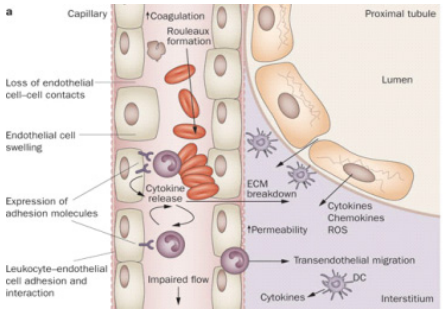


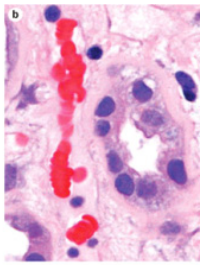
Damage: e.g. loss of PT brush border, EC injury, EpC necrosis

Dysfunction: e.g. Na^+/K^+ ATPase

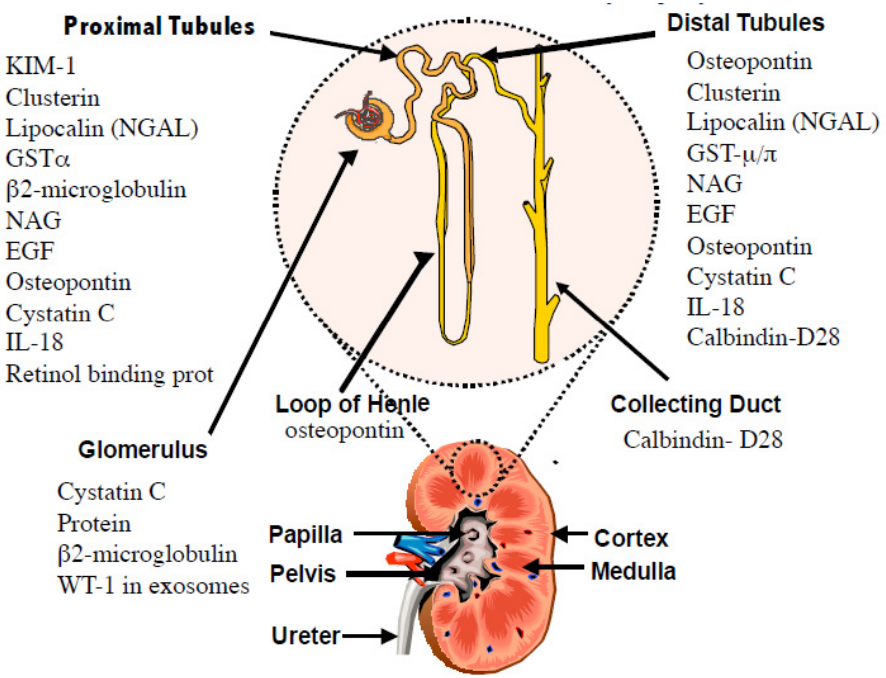
Occlusion: capillary & tubular



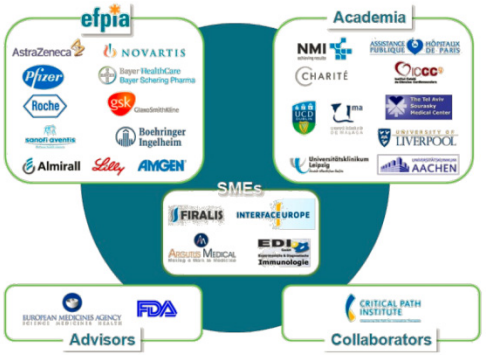




Development and validation of predictive biomarkers for clinical use



Progress in clinical biomarker space
Shortfall in predictive pre-clinical systems



nature biotechnology

Renal biomarker qualification submission: a dialog between the FDA-EMEA and Predictive Safety Testing Consortium

Frank Dieterle[1], Frank Sistare[2], Federico Goodsaid[3], Marisa Papaluca[4], Josef S Ozer[2,28], Craig P Webb[5,6], William Baer[5,7], Anthony Senagore[5,8], Matthew J Schipper[5,9], Jacky Vonderscher[10], Stefan Sultana[5], David L Gerhold[2], Jonathan A Phillips[11], Gérard Maurer[1], Kevin Carl[1], David Laurie[1], Ernie Harpur[12], Manisha Sonee[13], Daniela Ennulat[14], Dan Holder[15], Dina Andrews-Cleavenger[16], Yi-Zhong Gu[17,29], Karol L Thompson[3], Peter L Goering[3], Jean-Marc Vidal[4], Eric Abadie[4], Romaldas Maciulaitis[4,18], David Jacobson-Kram[3], Albert F Defelice[3], Elizabeth A Hausner[3], Melanie Blank[3], Aliza Thompson[3], Patricia Harlow[3], Douglas Throckmorton[3], Shen Xiao[3], Nancy Xu[3], William Taylor[3], Spiros Vamvakas[4], Bruno Flamion[4], Beatriz Silva Lima[4], Peter Kasper[4], Markku Pasanen[4,19], Krishna Prasad[4], Sean Troth[20], Denise Bounous[21], Denise Robinson-Gravatt[22], Graham Betton[23], Myrtle A Davis[24], Jackie Akunda[25], James Eric McDuffie[13], Laura Suter[10], Leslie Obert[22], Magalie Guffroy[12], Mark Pinches[23], Supriya Jayadev[11], Eric A Blomme[26], Sven A Beushausen[22], Valérie G Barlow[12], Nathaniel Collins[17,29], Jeff Waring[26], David Honor[26], Sandra Snook[13], Jinhe Lee[26], Phil Rossi[27], Elizabeth Walker[27] & William Mattes[27]

The first formal qualification of safety biomarkers for regulatory decision making marks a milestone in the application of biomarkers to drug development. Following submission of drug toxicity studies and analyses of biomarker performance to the Food and Drug Administration (FDA) and European Medicines Agency (EMEA) by the Predictive Safety Testing Consortium's (PSTC) Nephrotoxicity Working Group, seven renal safety biomarkers have been qualified for limited use in nonclinical and clinical drug development to help guide safety assessments. This was a pilot process, and the experience gained will both facilitate better understanding of how the qualification process will probably evolve and clarify the minimal requirements necessary to evaluate the performance of biomarkers of organ injury within specific contexts.

A Voluntary eXploratory Data Submission was initiated on 15 June 2007 for seven urinary renal safety biomarkers, including kidney injury molecule-1 (KIM-1), clusterin (CLU), albumin, total protein, β2-microglobulin, cystatin C and trefoil factor 3 (TFF3) in urine. The submission to the EMEA and the FDA contained data, interpretations and the proposed contexts of use for each of the biomarkers. This submission was followed by two face-to-face meetings on 12 July 2007 and 9 October 2007 between FDA, EMEA and PSTC members—the Pharmaceuticals and Medical Devices Agency of Japan also participated in an observational capacity—and several joint telephone conferences. New processes were established at the FDA and EMEA following the review process (see ref. 1 by EG. and M. Papaluca). Through these collegial communications, experts addressed data gaps in the initial submission, which were responded to by the consortium providing a large amount of additional data in the form of nine follow-up submissions.

In this article, we summarize the PSTC renal biomarker submission, analyses and conclusions, and then go on to discuss the new standards and optimal practices identified through the qualification review process at the FDA and the EMEA. We also provide details of the corresponding regulatory agency reviews and provide an overview of the dialog between the PSTC and the FDA-EMEA. By providing detailed documentation of the review process, we hope to provide guidance for future regulatory submissions by other parties, not only for kidney

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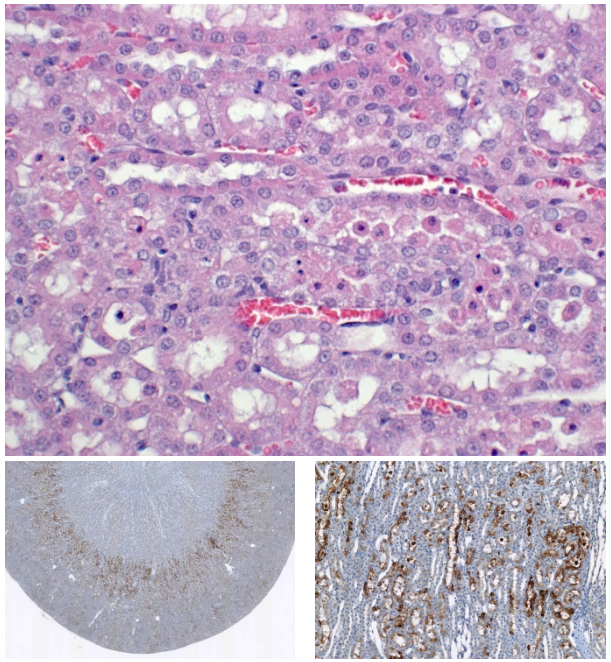
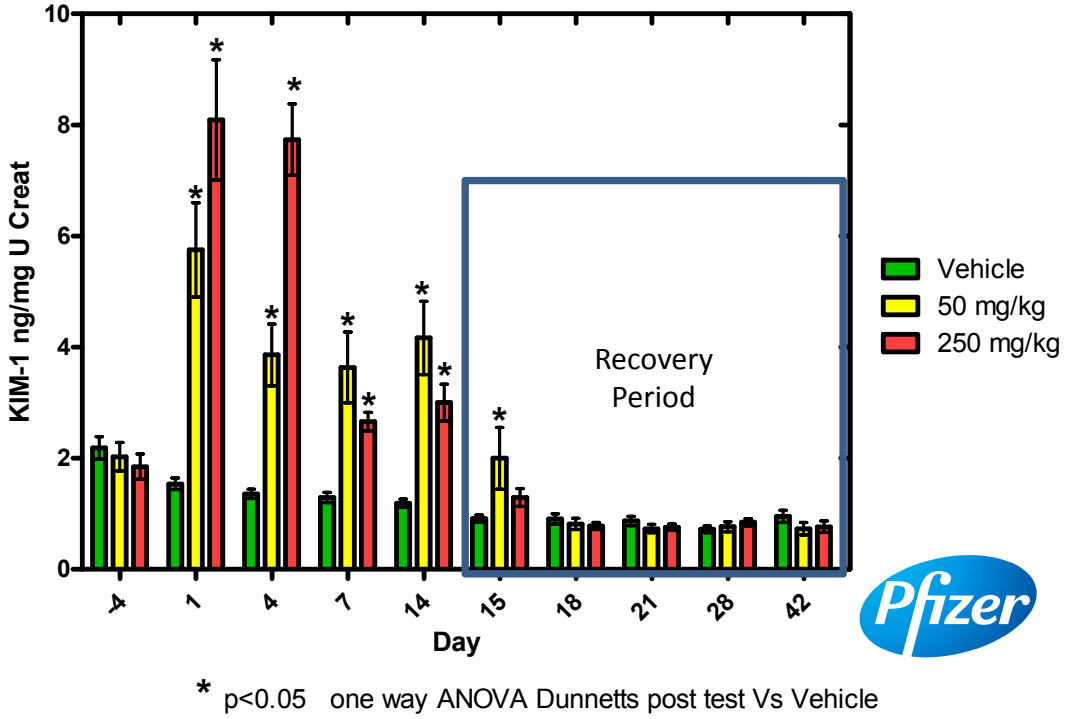
An example of drug induced tubular toxicity in the rat

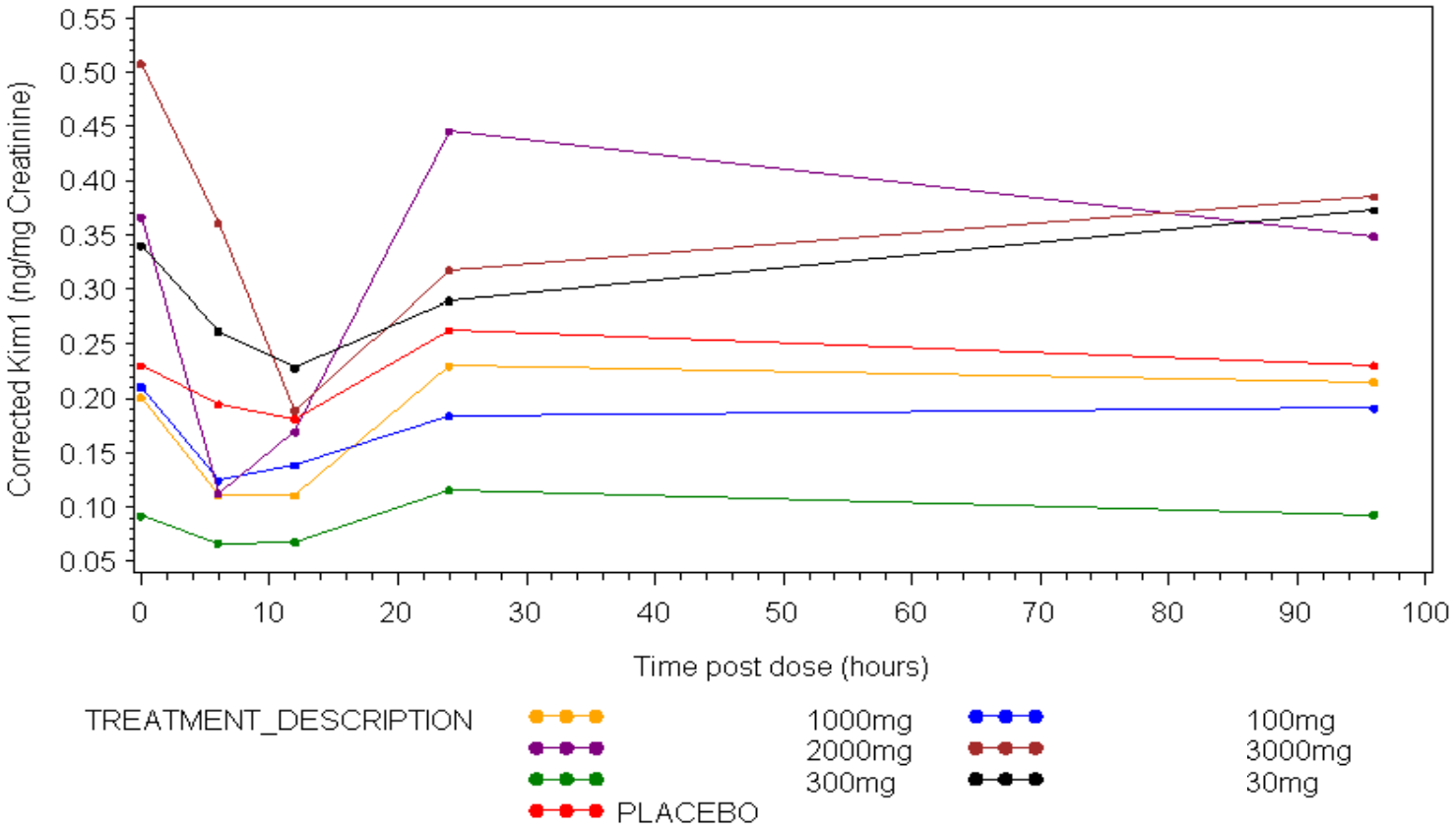




* p<0.05 one way ANOVA Dunnetts post test Vs Vehicle

- Epithelial regeneration/degeneration in pars recta of female rats
- Dose dependent pathology – reversed on cessation of dosing
  - Not observed in male rats, or in the dog
- Absence of any clinical pathology changes: No effect on CBC, electrolytes, BUN, etc
- Pathology correlated with changes in urinary Kim-1 levels

No change in urinary Kim-1 levels clinically on single dose escalation



The sorts of questions these findings raise

- Is the toxicity finding monitorable & reversible?
- Do the findings get worse with longer exposure?
- What is the 'therapeutic window' between efficacy and toxicity?
- What is the confidence the observations (in the female rat) will not translate to human?
- What is the mechanism of nephrotoxicity?
  - Is it target specific?
  - Will other similar compounds have the same effect?
- Do we have a method of screening for less harmful compounds?
  - Are these screens predictive?
  - Compartment specific?

The NC3Rs agenda as applied to Drug Development

Can we replace or reduce the requirement for animal studies to test for nephrotoxicity by access to predictive in vitro assays?

Reduce drug development costs – kill early, kill cheaply

Focus on the mechanisms that are safe

NephroTube Challenge: Summary of Key Deliverables

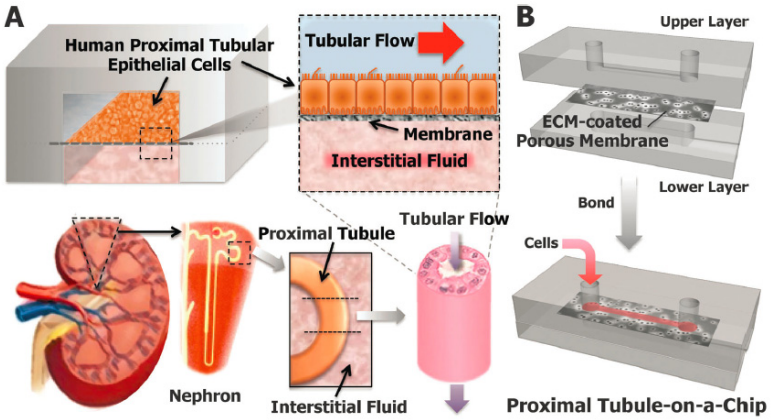
- Overall objective: An in vitro renal tubular assay whose endpoints can be used to screen for nephrotoxic potential of drugs

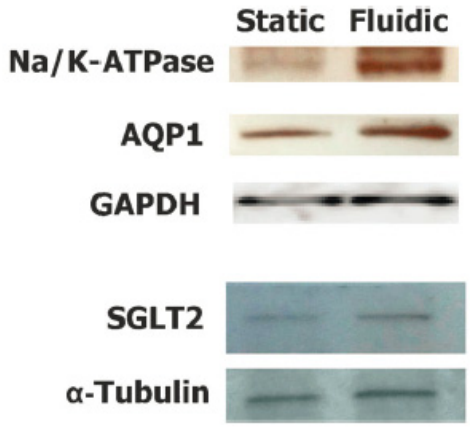
- The platform needs to:
  - Be transferrable between laboratories
  - Not be cost-prohibitive
  - Reproducible
  - Amenable to drug testing

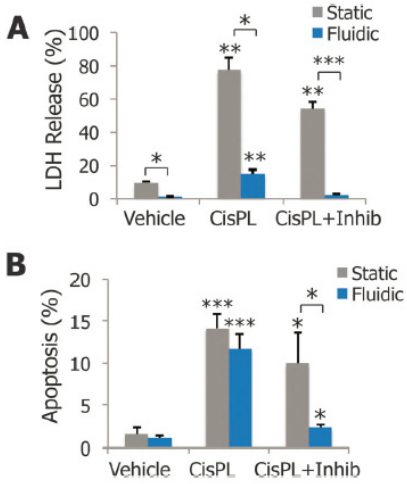
- Essential: correlation of endpoints with pre-clinical observations (rodent based).
- Highly desirable: observations correlate with/can predict clinical findings (human based)

The current state of the art

- Static 2D in vitro cell based renal models poorly predict clinical outcomes
  - Poor Kim-1 responses
  - Poor phenotypic comparison with native setting (polarity, protein expression, morphology, functional properties, etc)
  - Difficult to distinguish dose effect relationships, necrosis v apoptosis & regeneration
- Microfluidic systems may be better…..







Jang et al Integr Biol. 2013 ;5(9):1119-29

Outline of NephroTube challenge

Phase I - development

Identify a scaffold with performance characteristics that closely mimic the native setting
Identify a cell-based system that has the key physiological and functional features of the native environment
Demonstrate proof-of-principle utility against a compound test set and selected endpoints

Phase II - validation

Optimisation of scaffold, endpoints, timepoints and system
Validation with a larger compound set
Reproducibility assessment

Considerations

Endpoints and markers: TEER, Kim-1, solute/protein uptake, mitox, apoptosis, etc

Cell line availability and variability: transporter repertoire, cell performance/stability?

Facility for multiple cell lineages

Culture conditions? Static versus Dynamic?

Material sciences & drug compatibility

Rodent and/or human? Reporter line? Primary/ES derived/transformed?

Centre for reproducibility assessment

Which compound set?