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%)
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

Environmental/dietary nephrotoxicants

Drugs

Infection

Surgery

Age
Development and validation of predictive biomarkers for clinical use

Progress in clinical biomarker space
Shortfall in predictive pre-clinical systems
An example of drug induced tubular toxicity in the rat

- 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...
# 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?