Title of Challenge: NephroTube

Background

Drug-induced organ toxicity accounts for 30% of all drugs that fail prior to reaching the market. Within this, nephrotoxicity accounts for 2% of failures in the preclinical stages and 19% of all failures in Phase III. There is a significant translational gap between the preclinical models of nephrotoxicity and their predictive value through the clinical stages of drug development. Current cell-based models can provide valuable information but do not accurately predict toxicity in humans. A more complex, human tissue-based, in vitro model that can accurately measure toxic effects would reduce the failure rates from preclinical to clinical stages of development by providing a more relevant model.

A Challenge to develop predictive in vitro screens for nephrotoxicity was featured in the CRACK IT 2011 Competition. No awards were made and the Challenge has now been amended to capitalise on novel microfluidic technologies that have recently become available and focus on human tissue systems rather than multiple preclinical species.

3Rs benefits

- A typical investigative animal study to assess nephrotoxicity would use at least 26 rodents (and substantially more animals if both sexes were necessary). New in vitro technologies that replace the need for animal use in toxicity testing could significantly reduce these numbers
- Use of predictive human systems in preclinical toxicology testing will reduce the drug attrition that results from poor translation from preclinical studies into humans
- Where animals are still used, information on the underlying mechanisms of toxicity will help refine study designs including dosing regimes and species selection

Need for collaboration

Renal damage from toxicity can occur in a number of kidney compartments, but most toxic and drug induced injuries affect the proximal tubules either as a primary or secondary effect. Recent advances in cell and tissue culture, 3D- and bioprinting, microfluidics and silicon chip technology offer the potential to replicate the cellular architecture of the kidney tubule in 3D along with physiologically relevant fluid flow rates both in healthy and injured states.

The technical components of this Challenge require collaborative expertise in cell and tissue culture, microelectronics, microfluidics and engineering.

Overall aim

The aim of the Challenge is to develop a multi-compartmental, microfluidic tissue assay that models the renal tubular injury observed in nephrotoxicity. The assay should model the 3D architecture of the kidney tubules with microfluidics and chip arrays and possess the ability to reproduce the tubular response to known nephrotoxicants.

Key deliverable

The platform should be useable in the pharmaceutical industry setting and have the following characteristics.

General Criteria:

The platform needs to:

- Be transferrable between laboratories
- Not be cost-prohibitive (e.g. suggested range, <£1000 - 4000 per compound at the commercial stage).They need to be quicker and cost less than conducting an in vivo evaluation
- Show evidence of translation to the preclinical models (e.g. commonly used rat and mouse endpoints)
- Show evidence of translation to the clinic (e.g. human biomarkers)
- Demonstrate reproducibility (e.g. adequate AVR performance characteristics)
- Be suitable for drug testing (e.g. to allow for the determination of an IC50 value)

Phase 1

Objectives:

- To identify a scaffold with performance characteristics that closely mimic the native setting (e.g. proximity to endothelial cells, flow rates)
- To develop a cell-based system that has the key physiological and functional features that define it as stable
- To demonstrate proof-of-concept that reproducible results can be obtained from the system

Device criteria and cell culture:

- Chip or plate as long as device is robust and cost-efficient
- Re-useable or disposable
- Mimics tubular structures (forming a lumen) with polar cells attached to a semipermeable membrane
- Mimics transition of compounds via blood to epithelial cells to urine (e.g. urine and blood flow)
- Demonstrates tubular injury markers (e.g. KIM-1, NGAL, etc.)
- Multi-well system (e.g. a plate of below 12 wells is not compatible with industry screening)
- Facility for multiple cell lineages
- The biomaterial characteristics must allow for cell growth and drug compatibility e.g. low ability for drug adsorption
- Essential that drugs do not bind to the plastic, this needs to be demonstrated with reference drugs
- Scaffold should be compatible with evaluation of physiological/functional endpoints (e.g. TEER, solute/protein uptake, pathology examination) and longitudinal sampling

- Comparison to conventional 2D cell culture models
- Evaluation and standardisation of chosen platform e.g. if a cell line, understand batch-to-batch variation in cell phenotype, receptor or mRNA expression
- Cell-line could be an engineered reporter line if the principal requirements for phenotypic proximity to the native setting are met
- Defined set of rodent transporters (e.g. S1, S2, S3 component of proximal tubule)
- Presence or absence of PgP, OAT, OCT, MATE (all four essential and as a minimum)
- Ideally characterization of other transporters (e.g. megalin/cubilin, peptide OATP1,2)
- Validate rationale for use of material (e.g. PDMS) in a microfluidic device

Phase 2

Required:

A predictive rodent tissue based model of nephrotoxicity able to:

- Model the proximal (and/or distal) kidney tubule
- Reproduce known nephrotoxic events and have measurable predictability with in vivo models
- Provide reproducible data
- Mimic the flow of filtrate through the tubules in normal and injured states
- Have functional renal transport capacity (anions, cations and peptides)
- Provide an accurate read out of tubular necrosis and toxicity
- Identify / measure current biomarkers of renal tubular injury
- Provide a definite phenotype of tubular injury
- Provide a roadmap of how to move from the rodent model into a system that is directly translatable to human nephrotoxicity

Desirable:

- The development of the assay into using human cells, providing increased predictability and translation

Objectives:

- To have assessed a wide panel of different markers and functional endpoints with the same set of compounds (the tubular toxicity panel)
- That the temporal relationship and window of effect on a specific assay endpoint is understood, can be quantified and where there are opportunities for multiplexing, this opportunity is assessed
- To evaluate a tubular toxicity panel for concentration responses against and determine the correlation between in vitro assay endpoints (biomarker, injury, apoptosis, necrosis, etc) and in vivo experimental/clinical situations
- To define a standard operating protocol for the NephroTube assay capturing a detailed methodology of the assay, reagents, preparation, endpoint robustness QC and validation, etc
- To independently replicate the system and observations

It is anticipated that in the development of the assay a range of parameters will be assessed, including, but not limited to

- Temporal induction of current biomarker standards (Kim-1, NGAL, etc) as well as innate immune pathway triggers (inflammasome, TLRs, etc) in response to nephrotoxicants, hypoxia, and/or oxidative stress challenge

- Injury and repair mechanisms: ‘epithelial sloughing/shedding’ and regeneration, epithelial barrier function (TER) ,description of changes in pathology by EM
- Mitochondrial toxicity, and cell apoptosis or necrosis, changes in tubular transport, membrane potential, ROS/ cytokine production, protection of macromolecules (e.g. DNA/RNA) against oxidative stress challenge
- Efficacy of drugs against AKI endpoints as well as capacity to inform metabolism
  - The selection of endpoint(s) in the assay will be determined on the basis of observed correlations between the performance of compounds in the tubular toxicity panel in the NephroTube assay and observed effects in *in vivo*.
  - Cells in the fluidic cultures should be characterised. It is known that primary cell cultures often contain a mixture of cell types including’ unwanted’ cell types that can outgrow the key cells of interest. An understanding of the percentage of epithelial cell versus fibroblast and other cells would be required as well as clarity on overall activity of tissue over time

Commercialisation/ uptake strategy:

- Commercial availability of cell lineages should be considered as a critical element
- A commercial strategy to ensure broad uptake of the platform should also be outlined

Sponsor contributions

Phase 1

Sponsors will provide expertise in nephrotoxicity assays, including the specific requirements for the assay for it to be used in a commercial research and development setting

Phase 2

The industry Sponsors are able to:

- Provide data on reference tool compounds and terminated candidates with particular relevance to their nephrotoxicity where available
- Identify compounds causing nephrotoxicity in preclinical models with the intention of providing relevant compounds to validate the microfluidic tissue assay where possible
- Provide expert input into identifying translatable biomarkers, i.e. those known to signal rodent and human nephrotoxicity e.g. Kim-1
- Examine the potential for in-house data replication as part of the assay validation

Duration

Phase 1: six months. Phase 2: up to three years

Budget

Phase 1: up to £100K. Phase 2: up to £1 million

Sponsors

Pfizer, GlaxoSmithKline, Roche