NC3Rs Challenge:
"A predictive in vitro screen for nephrotoxicity; from mice to men and back again"

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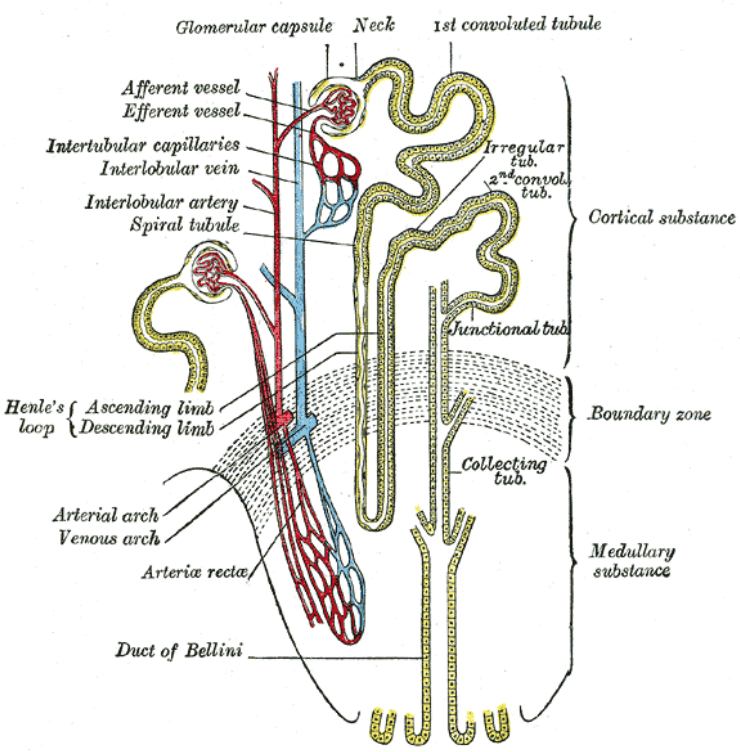
Industry sponsors: Roche, Astra Zeneca & UCB

AstraZeneca ucb Roche

Background

- The kidney is one of the main target organs for toxicity
- Kidney toxicity accounts for 2% of drug attrition during preclinical studies and 19% in phase 3
- The kidney has a complex anatomy and functional units, difficult to mimic *in vitro* and to diagnose *in vivo* (histopathology)
- Impressive recent advances in the investigation of translational biomarkers for nephrotoxicity
- **There is a clear need for in vitro experimental models to both predict and investigate drug-induced toxicities in the kidney**

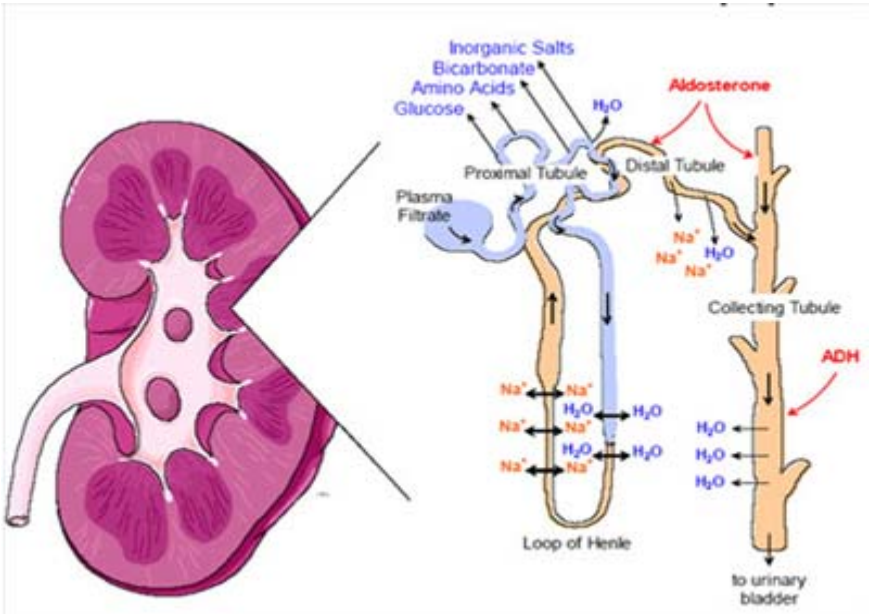


Redfern W S et al. (2010) Impact and frequency of different toxicities throughout the pharmaceutical life cycle. The Toxicologist 114(S1): 1081

Dieterle, F., F. Sistare, et al. (2010). "Renal biomarker qualification submission: a dialog between the FDA-EMEA and Predictive Safety Testing Consortium." Nat Biotechnol 28(5): 455-62

Kidney tubular injury

- Kidney tubule is the most common site of chemical-induced renal injury
  - Selective accumulation of compounds into this segment (urine concentration)
  - Leaky epithelium favoring flux of compounds into proximal tubule cells
  - Tubular transport of organic anions and cations, low molecular weight proteins GSH conjugates
  - CYP P450s & cysteine conjugate beta-lyase
  - Susceptibility to ischemic injury (compounds interfering with renal blood flow, cellular energetics, mitochondrial functions)



Mechanisms of kidney tubular toxicity

- Plethora of potential causative agents
  - Parent compound itself – exposure data in the kidney tubule
  - Metabolite(s) species-specific one(s) or activated via a species-specific mechanism
- Plethora of possible mechanisms, including
  - Intrinsic reactivity towards specific kidney tubule organelle(s) or macromolecule(s)
    - eg amphotericinB/membrane, fumonisinB1/enzyme inhibition, Hg++/sulfhydryl group binding
  - Toxification via biotransformation (incl. reactive metabolite)
  - ROS production
  - Lowering of tubular cell cytoprotective capabilities
    - eg HO-1/Bach1 pathway reported for tubular toxicants
  - Downregulation of specific transporters located in the tubule
  - Alteration of renal blood flow
  - Ionic imbalance

Aim of the challenge

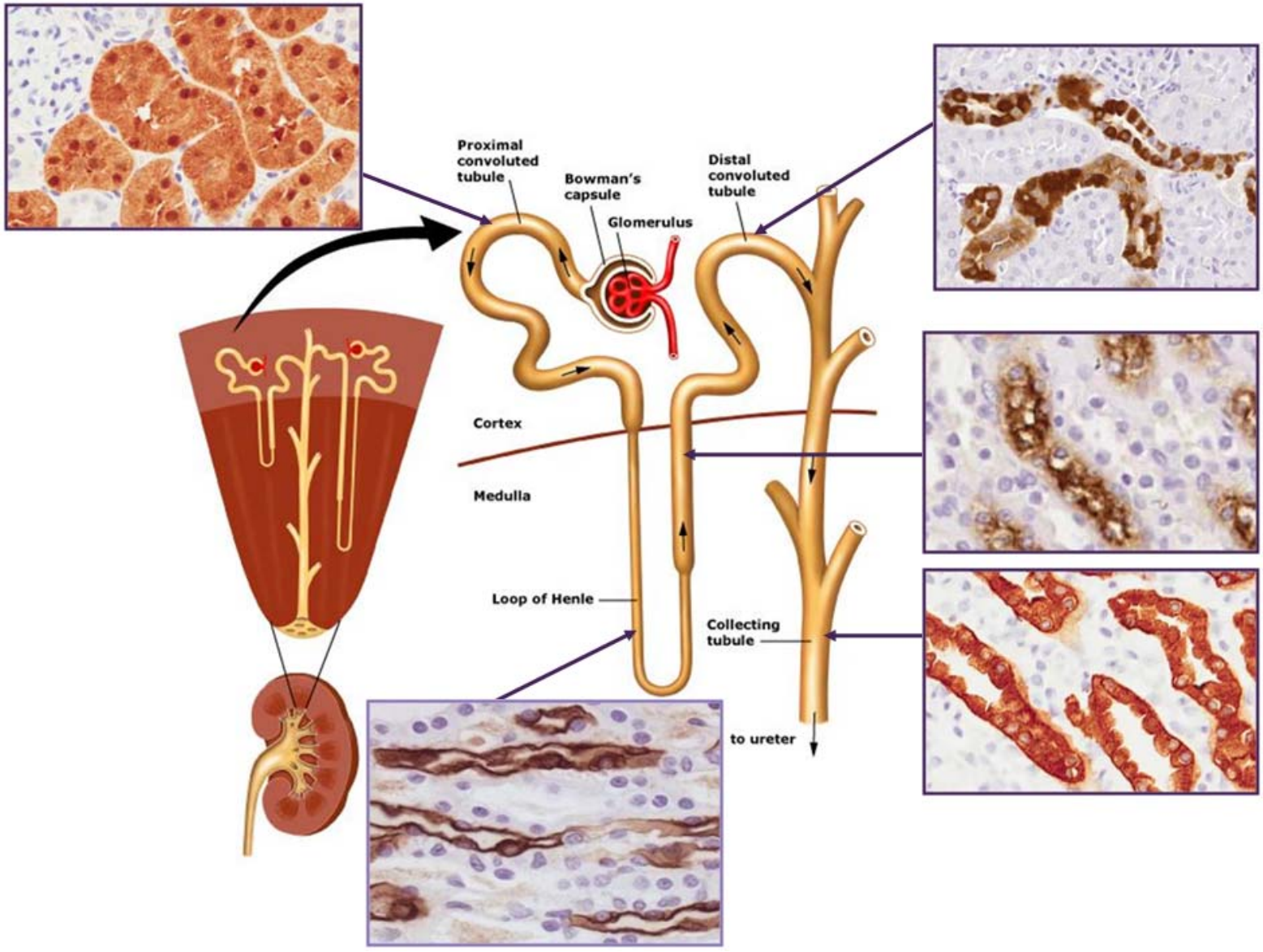
- The aim of this challenge is to establish ***in vitro* predictive assays** that can provide **reliable nephrotoxicity assessment**
  - Identify/develop in vitro models of sufficient relevance to non-clinical species in the context of drug development
    - mouse, rat and dog and man
  - Predict nephrotoxic liabilities *in vitro* and assess the relevance to man
  - Address the mechanistic basis of nephrotoxicity
    - interplay of several cell types
    - Compare effects in rodent, non-rodent (e.g. dog) and human-derived cellular systems enabling translation to man

Kidney cultures for safety assessment

- There are several published examples of cell culture of proximal tubular cells from rat (e.g. Primary cells, NRK-52E) and human (e.g. Primary cells, HK-2) for the assessment of (tubular) nephrotoxicity, however
  - There is a need to implement standardized assays
  - There is a need for a sustainable resource for cells
  - There is a need for standardized characterisation of the different cell populations
    - Specific markers (e.g. IHC, gene expression)
    - Functional assays (e.g. Albumin uptake, enzymatic activity)
  - There is a need to compare across species
    - Human, rat, dog, mouse
  - There is a need to validate the systems

Lash, L. H., D. A. Putt, et al. (2008). Toxicology 244(1): 56-65.
Suzuki, H., T. Inoue, et al. (2008). J Appl Toxicol 28(2): 237-48.
Zhang, X. F., C. L. Ding, et al. (2011). Toxicology 286(1-3): 75-84.
Fuchs T and Hewitt P (2011): A Toxicogenomics approach for the establishment of an in-vitro nephrotoxicity screening system, Poster at DGPT, 2011

Main focus: Kidney tubular cell types



Complex culture systems may be needed to to recapitulate kidney function *in vitro*

Subramanian, B., D. Rudym, et al. (2010). "Tissue-engineered three-dimensional in vitro models for normal and diseased kidney." Tissue Eng Part A 16(9): 2821-31

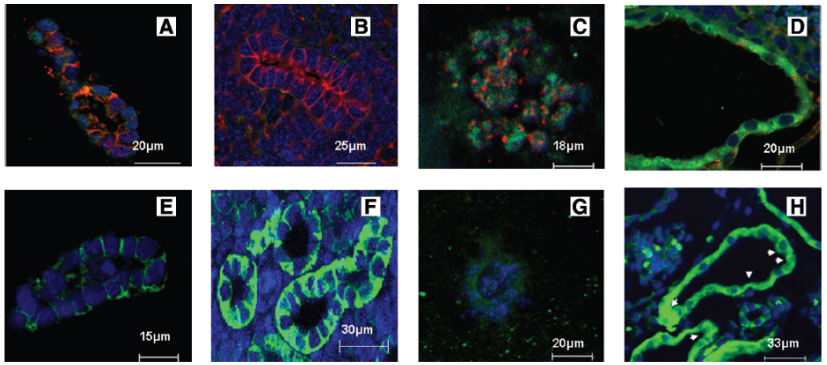
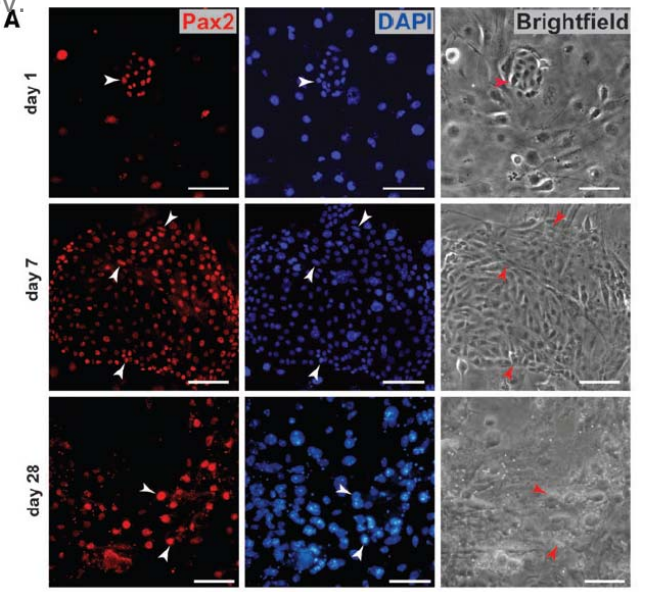
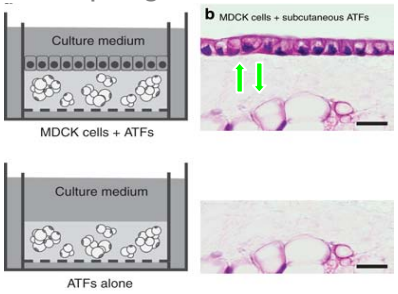


FIG. 4. Evaluation of marker proteins in structures developed in scaffolds. (**A–H**) Immunofluorescence of cells stained for specific markers from paraffin sections. (**A–D**) Cells were stained for E-cadherin (red), N-cadherin (green), and nucleus (blue). (**A**) Normal cells. (**B**) Normal kidney. (**C**) Disease cells. (**D**) Disease kidney. (**E–H**) Stained for Na^+ K^+ ATPase pump (green) and nucleus (blue). (**E**) Normal cells. (**F**) Normal kidney. (**G**) Disease cells. (**H**) Disease kidney. Arrowheads highlight mislocalization. Scale bar in μm. Color images available online at www.liebertonline.com/ten.

Fuente Mora, C., E. Ranghini, et al. (2011). "Differentiation of Podocyte and Proximal Tubule-Like Cells from a Mouse Kidney-Derived Stem Cell Line." Stem Cells Dev.



***Udo et al., Kidney Int* (2010) 78, 60–68**
"Adipose tissue explants and MDCK cells reciproca..
regulate their morphogenesis in co-culture"



Isolation, Characterization, and Expansion Methods for Defined Primary Renal Cell Populations from Rodent, Canine, and Human Normal and Diseased Kidneys

Sharon C. Presnell, Ph.D., Andrew T. Bruce, B.S., Shay M. Wallace, B.A., Sumana Choudhury, M.S., Christopher W. Genheimer, B.S., Bryan Cox, B.S., Kelly Guthrie, B.S., Eric S. Werdin, B.S., Patricia Tatsumi-Ficht, B.S., Roger M. Ilagan, Ph.D., Russell W. Kelley, Ph.D., Elias A. Rivera, M.H.S., John W. Ludlow, Ph.D., Belinda J. Wagner, Ph.D., Manuel J. Jayo, D.V.M., Ph.D., and Timothy A. Bertram, D.V.M., Ph.D.

3Rs Benefits

- 10-20% of the animals used in R&D are employed for safety assessment
- Improved *in vitro* assays for pre-screen of common toxicities will move attrition earlier in the development pipeline by means of implementing appropriate screens. Thus:
  - Drugs destined to fail in development will not need to be tested in animals
    - **Reduction** of animal use
  - Animal experimentation can be design optimally using experimental information on the underlying mechanisms of toxicity
    - **Refinement** of study designs, including dosing regimes, endpoints and species selection
  - **Replacement** of animal experimentation is the ultimate long term goal for all *in vitro* toxicology assays

Key Deliverables

- Identification and characterisation of appropriate **cell types** (cell lines and primary cells) to address kidney function
- Establishment of appropriate **endpoints** for the detection of nephrotoxicity
- **Validation** of the predictive performance of the assay by assessing a sufficient number of compounds and generating predictive statistical models with the obtained data
- Demonstration that the model can provide **mechanistic information** on the underlying toxicological processes
- Transfer of the assay(s) to industry standard platforms and initiating the process for **formal validation** (e.g. via ECVAM)
  - **Interlaboratory transferability** (e.g. In the labs from the industrial partners)
  - Discussions on formal validation (e.g. ECVAM) to be followed up outside the scope of the collaboration

Need for a collaboration

- Academic partners
  - High scientific interest kidney function and its recapitulation in vitro
    - «Simple» cell cultures
    - Co-cultures
    - Organotypical cultures
  - Expertise in generation and characterisation of cell lines (including different species)
- Industry
  - Know how on nephrotoxicity in rodent/non-rodent (& man), based on experience in
    R & D
  - Compounds (proprietary and/or commercially available) that can be used as model compounds during the development of the assay
  - Access to technology platforms and industry standard laboratories to
    - Aid the assay development through access to technology platforms, e.g. HCI, gene expression platforms, impedance-based assays, etc.
    - Provide a first basis for transferability and validation of the methodology

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ucb

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NC3Rs

National Centre for the Replacement, Refinement
and Reduction of Animals in Research