

Challenge 15: NephroTube

Q. Are enzyme and membrane systems in a biomimetic, microfluidic system within the scope of this Challenge or does the system need to be cell or tissue based?

A. If the suggestion is not overcomplicated and takes into account that we do not know everything about the tubular compartment of the kidney then we are open to anything that meets the criteria of the Challenge.

Q. Do you want to focus on the rat or the human?

A. If solving the Challenge by going straight to human is achievable with clearly demonstrable translation to the *in vivo* clinical situation then that is the ultimate aim. However, given the difficulty of the Challenge we wanted to make clear that if a predictive rodent model were achievable (ie cells cheaper and more easily available, more known nephrotoxic (positive) compounds in rats to test the system) there would be a business need and a 3Rs benefit of such a system in addition to a human system.

Q. Would part of the Challenge be to identify the mechanism of action of the compounds?

A. No, the platform is intended to be used as a screen. The mechanism of action is a secondary question not the main reason for the Challenge.

Q. You mention that ultimately you would like a 12-well format, is this needed in Phase 1?

A. The ultimate goal would be to have a medium to high throughput assay. However, in Phase 1, one well that is predictive would be better than high throughput. We could envisage a staged process that builds up to 12 wells. The reason for wanting a multi-well format is that we want to look at different concentrations to work out what concentration of drug is important. To get IC50 curve data we would need multiple points and would like an assay to generate that in a single setting i.e. multiple wells.

Q. How essential is co-culture of proximal tubule cells with other cell types?

A. This is open and we would like to leave that up to the applicants to make an expert judgement. However, the limitations of a single cell system should be considered, certain cells are essential to the function of the kidney and therefore co-culture may be the best approach.

Q. Do you have a preference for the culture system? Ex-vivo or *in vitro*?

A. From a 3Rs perspective, a culture system is preferred. However, if an ex-vivo system works it would still have a large 3Rs impact. This would need to be described clearly in the proposal. If meaningful, predictive data can be shown from an ex-vivo approach it would be completely acceptable.

Q. In cardiotoxicity, we can look at the number of ion channel targets and then perform mathematical modelling to predict human toxicity. Has anyone tried this for nephrotoxicity and would it be a suitable approach?

A. This would be very challenging because we have less understanding of the mechanisms of nephrotoxicity. We are not aware of any channel or receptor that gives rise to a phenotype as

extreme as hERG for instance. Conceptually, this would be a great approach however we are not sure our scientific understanding is advanced enough to support this yet.

Q. Do you want an assay where the cell can be stressed with drug multiple times as would be the case with a patient (e.g. over a period of a month) or used once and thrown away?

A. If we are studying early phases of nephrotoxicity, we are not sure that we will need to culture for long periods in order to detect toxicity. Ideally, we could have a system that might show regeneration or evidence of recovery, but it seems too complicated for this stage in the development of this Challenge.

Q. Are you expecting a fully functioning model in Phase 1?

A. No, Phase 1 is a Proof of Concept study. Clear evidence that the system works is critical but a fully functioning model is not essential.

Q. Will access to human tissue be part of the in-kind contributions?

A. No, we do not have human tissue readily available. This may be one reason to focus on the rodent system.

Q. Do you have a negative compound dataset that you are confident do not show nephrotoxicity with which to test the model?

A. Yes, we cannot share information on compounds that are currently still in clinical development, but we have other examples with the potential to share in Phase 2 of the CRACK IT competition.

Q. What annotation will be provided with the compounds?

A. In principle, if a compound is made available it will be with the related data, including associated human toxicity, dose of toxicity, pathology, metabolite information (if known) and the number of animals with observed nephrotoxicity.

Q. Do you know the precise mechanism of toxicity or primary targets of the compounds that will form part of the in-kind contributions?

A. Not always. We would concentrate on those compounds causing toxicity in the proximal tubule however we don't always know the mechanism of action.

Q. Will you be able to provide the metabolite that is causing the nephrotoxicity?

A. We do not always have the metabolite but the compound can be provided and the method to activate via an S9 preparation.

Q. Are you thinking about the system measuring mixtures and drug:drug interactions? For example, cyclosporins and statins.

A. If it's necessary to test the system properly then yes. However, this may be too complicated and is not a specific deliverable for the Challenge.

Q. Can you name one or two specific compounds that you want data on to demonstrate the system in Phase 1?

A. We would want to have confidence that the system works with known nephrotoxicants e.g. aminoglycosides. The choice remains with the applicants, but choice of nephrotoxicant will not impact the application. The application will be judged on the quality of the science and if the sponsors feel the compounds suggested are not ideal, they will discuss this with the applicant.

Q. Will the panel of compounds available as in-kind contributions for Phase 2 include a diverse range of mechanisms of action?

A. It will be difficult to test lots of mechanisms of action in this assay as most of the time we do not know the detail of the mechanisms of toxicity, but there is the potential for a range of compounds to be provided.

Q. What are the best endpoints with which to validate to the assay?

A. We are open to what the applicant thinks are the best endpoints for their assay from their experience. Biomarkers are often not fit for purpose or validated for acute kidney injury. Previously, various endpoints have been considered but there is no comprehensive list. The sponsors are available to advise on specific suggestions that the applicant thinks may be important.

Q. Do biomarkers have to be translatable to the clinic?

A. This is a 'nice to have' rather than essential. It is much more important that the assay is fit for purpose.

Q. Does the model also need to show recovery from the biomarker up or down-regulation?

A. This would be the ideal, gold-standard model but is probably too difficult for this Challenge. It is not a requirement.

Q. Who should we contact with questions in order to get the sponsors' consensus view?

A. Applicants can contact individual sponsors, however they must be aware that the answers they receive are that of the individual sponsor. To get a consensus view from all three sponsors, please contact Kathryn.chapman@nc3rs.org.uk or cathy.vickers@nc3rs.org.uk who will obtain a consensus reply from the sponsors. Please be aware that this may take a few days when planning for your application.

Q. How would the regulators view these assays?

A. Firstly, they would be used to screen out nephrotoxic drugs to the proximal tubule so that animals are not being used for this purpose. It would only be after much further validation that we would think about regulatory acceptance of an alternative model.

For further information and to be put in contact with the sponsors, please email

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