Challenge 29: ImmuLiver Surgery Q&As

Q. Will you be providing patient samples?

A. No. We want to characterise the vaccine strain not the patients. We would like to compare metabolic dysregulation between wild type virus and Yellow Fever 17D attenuated strain.

Q. Why do you require kinetic measurements at 12 days, and is this the longest timepoint required?

A. We need to maintain the cells for this amount of time as a minimum, to cover the duration of the virus replication while keeping some flexibility on the start of the infection. We need to analyse the infection at multiple time points, by harvesting the cells and the supernatants and replicates samples are needed at each time point. For this reason, a 96-well plate format is preferred.

Q. Are you interested in understanding the metabolic dysregulation in cells in the model generally, or particularly in hepatocytes?

A. We would like to do both. We believe that the presence of other liver cells, like the Kupffer cells could modulate the hepatocyte response, and reveal differences between wild type and attenuated virus.

Q. What level of throughput does the final product/assay need to be able to deliver?

A. As high throughput as possible, with medium throughput as a minimum. Testing Yellow Fever Virus uses only relatively small number of samples but high throughput could be amenable to other viruses. Increased throughput also reduces manual operation, and has the potential to be automated.

Q. Is there a preference in terms of the scale of analysis required for the final product/assay? For example, would microscale analyses be problematic?

A. The need for microscale analyses could be problematic due to the sensitivity. If the samples are too small you must pool the samples and that means extra manipulation and lower reproducibility. Based on our experience with hepatocyte cells, we can perform routine analysis from an individual culture that contains no less than 10- 30,000 cells. Changing the analysis scale to use less material can be considered, with a validated, minimally qualified, assay.

Q. Do you see differences in hepatocytes from iPCS from different sources?

A. Yes, we have analysed hepatic markers in iPSC-derived hepatocytes from different commercial sources, provided as frozen, pre-differentiated cells, and seen some differences. These differences might be linked to the source material (different donors), but also to the control of the last step of differentiation conducted internally: we observed

variations in the optimal differentiation time using several batches of the same lot (same provider).

Q. Is it possible to provide liver tissue from infected and wild type macaques for benchmarking Yellow Fever virus pathogenesis?

A. No, we do not have samples available for this.

Q. The Phase 1 deliverables are too challenging to realise in six months. What is the minimum you would consider acceptable at the end of Phase 1 to determine if the applicants can meet the full Challenge brief?

A. The Phase 1 deliverables are the ideal that we would like to see, but we appreciate that it may be difficult to meet each of these fully in the time frame. We do not want to stifle applications by restricting applicants to a few of these deliverables. It is the responsibility of the applicant to demonstrate how what they deliver during Phase 1 will enable the full Challenge brief to be realised. However, we would expect the applicants to demonstrate they have considered the cell composition of their models and cell source. Models should as a minimum, contain at least hepatocytes and one immune cell type (e.g. macrophages or Kupffer cells).

Q. Is there an acceptable straightforward method to measure the level of vaccine infection?

A. Yes; qPCR can easily be used. There is no reporter or GFP-type approach for measuring the extent of vaccine infection.

Q. Will you provide the virus during Phase 1?

A. We will not provide our vaccine strain, but the YF17D strain is available here and here. The wild type YF virus can be handled only in a BSL3 laboratory.

Q. Could we combine the model developed during this Challenge with your existing mini-brain model used for neurovirulence testing?

A. It is an interesting idea to combine both models to set-up a full severe disease model, and we are open to it. This can be considered after the hepatic model has been developed.

Q. What level of microscopy do you want the model to be amenable with?

A. Simple immunofluorescence to determine the presence of virus.