Challenge 4: Improving the predictive capacity of *in vitro* cytokine release assays to reduce animal use and drug attrition

Surgery Questions and Answers

Background Biology:

Q. How long does it take for the cytokine storm to take place?
A. It takes 30 minutes *in vivo*. In the current *in vitro* test, it takes 18-24 hours. The time course will depend on the model system produced.

Q. Are the cytokines interacting together to form the storm; is it the result of a cascade?
A. Yes. There are different phases and different cytokines interacting at different times. This is a non-linear effect with a threshold at which the storm is induced. TNF and interferons are the first cytokines released but the real starting event is the binding of antibody to the target cell or Fc receptors.

Q. Why is it important to measure proliferation?
A. Proliferation is correlated to cytokine production, it is linked to the second wave of the phenomenon, when people relapse after the transient recovery. Proliferation can also increase the number of responding cells, thereby increasing the pathology.

Current model:

Q. What is the current in vitro model based on?
A. The current *in vitro* model uses diluted whole blood. Previous models have been developed based on PBMCs but the process was slower and variation was a problem. There is a concern that PBMCs might be the wrong type of cells for such a model if, for example, the antigen is not in the peripheral blood. Lymph node models or 3D cell models including several cell types might be more appropriate. The scaffold used in such models would have to be carefully chosen as some are not very diffusible and would hamper cytokine infiltration into the tissue.

What is needed:

Q. Why does the assay need to work with cryopreserved cells?
A. Frozen cells represent a gene pool that can be used to address variability in the general population.

Q. The response to a particular antibody varies from donor to donor, is that difference consistent or does it vary from day to day? How can the assay encompass the variability seen in the general population?
A. For an individual antibody, the rank order of the response between individual donors stays the same. However, the rank order of the response to various antibodies varies from individuals to individuals. Part of the challenge is to be able to phenotype individuals before they receive the drug and assess the risk in each phase 1 individual.

Q. *If the assay is based on normal individuals, is there a risk that it will not be relevant in the diseased population?*
Yes, this is certainly a risk, but only if the target antigen was either only expressed in the diseased population or if it was significantly over-expressed. The assay can use cells from any population, normal or diseased so developing a disease-specific model could be a consideration.

Q. Is it realistic to consider a 'one size fits all' assay?
A. A 'one size fits all' solution is probably not feasible. The solution to this challenge is likely to be a platform technology adapted to different drug types. We are equally interested in predicting cytokine response in the general population, using pooled donors, and in individual patients. One could envisage tailored therapies, with patients rationalised based on, for

example, blood content. It could also be used as a safety test tailored to each individual used in phase 1 trials.

Q. Which is more important, the scalability or the predictability? Are you interested in the underlying biology?
A. Understanding the underlying biology will likely contribute to the resolution of this challenge but the ultimate objective is an assay that can predict whether it is likely dangerous to humans. The most important thing is for the assay to be predictive.

Validation:

Q. Will animal experiments be required to validate the newly developed in vitro model?
A. Animal models currently used are not predictive of the cytokine response, the newly developed model would be expected to be validated using drugs for which clinical data is available.

Q. What are the current requirements of regulatory bodies? Can we envisage regulatory bodies to approve biologicals without non-human primate (NHP) work?
A. Currently there is no requirement regarding cytokines specifically but this is expected to change and include investigation of the cytokine response in the future. NHPs may still be needed to calculate the safe starting dose but the new assay will enable the number of animals to be reduced and prevent a drug going into an inappropriate model.

In-kind contributions:

Q. What in-kind contributions will HLS provide?
A. In-kind contribution from HLS will include access to excellent bioanalytical tools such as multiplex analysis platform (e.g. MSD, luminex), automation (liquid handling robots) and cell banking facilities. HLS also has access to clinical grade antibodies that could be made available to other partners. Partners will also have the opportunity to join an ILSI-HESI consortium of pharmaceutical companies whose goal is to work together and share expertise in prediction of cytokine release for biotherapeutics.