Title of Challenge

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

Background

Cytokines are soluble signalling molecules which are released by cells of the immune system in response to antigen binding. Large molecule therapeutics such as antibodies can also trigger cytokine release and immune cell activation either directly by their V regions or by Fc regions binding to Fc receptors. A severe example of this was seen in the trial of TGN1412, an anti-CD28 IgG4, which activated T-cells resulting in massive cytokine generation and severe pathology in the volunteers that received it (1, 2).

The *in vivo* testing of biologics, such as monoclonal antibodies, is a regulatory requirement with the cynomolgus monkey being the most commonly used species. Whilst this monkey is used in toxicity studies for a range of drugs, it has poor predictivity for the potential of antibody-induced cytokine release in humans (3). This may be in part due to differences in the expression of antigens such as CD28, the lower affinity of the cynomolgus FcR for human Fc or the presence of regulatory signalling molecules that humans lack such as CD33-related Siglec (4).

A number of groups have developed *in vitro* assays using human leukocytes to detect antibody-induced cytokine release (for example; 5, 6). However, there is a divergence between groups in cellular preparation, antibody presentation and bioanalytical platform, which has led to poor comparability of data between groups. Whilst many groups have shown that their models produce the expected hierarchical responses to antibodies against antigens such as CD3 and CD28, there has not yet been an attempt to correlate this with clinical data to demonstrate the predictive value of these models. In addition to this, the majority of the current models neither make an attempt to measure the kinetics of the cellular cytokine response to antibody, nor cellular division, which are both important parameters in the assessment of the potential of an antibody to cause cytokine release syndrome.

The lack of a standard format for these assays in combination with the poor predictive value of the current models has highlighted the need for a new generation model that will be sensitive and predictive of clinical outcome, and will therefore inform both candidate selection and be predictive of toxicology in preclinical or clinical studies.

3Rs benefits

Biologics form approximately 30% of the global drugs pipeline. Immune responses caused by these drugs, including desired pharmacology and adverse events are assessed in preclinical studies, primarily using the cynomolgus monkey. Depending on the drug, the therapeutic area and company practices up to 144 monkeys may be used in a standard toxicology package for biologics (7). Immune responses are assessed either as part of the standard toxicology package or as standalone studies. *In vitro* assays which accurately predict immune responses, including the likelihood of a cytokine storm, would reduce the number of monkeys used by avoiding drugs which are destined to fail on safety or efficacy grounds being taken into preclinical studies.

Additional 3Rs benefits include:

- Screening and optimising molecules prior to preclinical studies to avoid unnecessary use of animals. For instance, a robust *in vitro* screen for a monoclonal antibody could show whether engineering was needed to increase potency (if cytokine release is not stimulated enough) or reduce toxicity (if cytokine release is too high or not part of the expected pharmacology) prior to animal studies;

- Integration into the testing paradigm for biosimilars or manufacturing changes to use fewer (if any) animals for approval;

- Providing information on the mechanism of action and/or potency which could be used to inform study designs allowing fewer animals to be used and avoiding doses which result in significant adverse events or mortality.

Need for collaboration

The events that control cytokine release are not well understood, yet underpin the development of predictive models for drug testing. The expertise to further understanding in this area and to develop conceptual models is probably within academia or SMEs, however, the expertise to develop these models into bioanalytical tools lies within industry.

Overall objectives

- To develop *in vitro* human cell-based models for the testing of antibody-based therapeutics that will allow the prediction of human cytokine release.

- To develop a parallel assay with cells from non-human primate (e.g. cynomolgus monkey) to predict cytokine release in preclinical safety assessment.

Key deliverables

An assay to predict *in vivo* cytokine release.

Phase I*:*
- Must generate detectable cellular cytokine release in response to antibody binding;

- Must be formatted appropriately to allow the collection of time-course data for cytokine release;

- Must be amenable to measurement of the division of responder cells. Demonstrate that the assay predicts clinical outcome e.g. through comparison of currently marketed drugs with historical preclinical and clinical data.

Phase 2*:*
- Should be able to demonstrate the use of both fresh and frozen material as a source of responding cells;

- Should be amenable to medium to high throughput screening and allow for multiplex analysis;

- Must contain suitable controls and replicates to allow validation as described in FDA guidance document (http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070107.pdf).

Industry sponsor

Huntingdon Life Sciences (HLS)

In-kind contributions

Projects of this nature require extensive analytical and instrumentation support, especially in the latter stages during validation and conversation to higher throughput platforms. In-kind contributions from HLS will be the provision of clinical antibodies where appropriate, multiplex analysis platforms (Cytometric Bead array, MSD platforms), automation (liquid handling robots) and tissue banking facilities. HLS has extensive experience in high throughput *in vitro* screening strategies and will provide this expertise for the project. There may be also the potential for the academic partners to participate in bulk ordering of reagents with the other partners to reduce reagent costs.

Industry sponsor access to foreground Intellectual Property

There will be no restriction on IP exploitation. Applicants will be free to publish or commercialise where appropriate and no preferential access will be required by Huntingdon Life Sciences.

Duration

Up to three years

Budget

Up to £500,000 in total, inclusive of VAT where applicable

Funding model

Although success in this project will require a multi-disciplinary approach, there are various ways in which this could be managed. It is unlikely that an applicant from a single organisation would be able to access all the required expertise, and applications are therefore welcomed from consortia, in which one organisation takes the lead (the Contractor) on behalf of the others (the Subcontractors). More than one such consortium could be funded, particularly if the proposed technologies take substantially different routes.

References

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Keywords

Biologics, cytokine release, *in vitro*, non-human primate, human, toxicology.