In vitro TDAR: A human immune response assay for the assessment of immune modulation

Overall aim

The aim of this Challenge is to develop a human *in vitro* T-cell dependent antibody response (TDAR) assay to assess the immune enhancement properties of immunomodulatory therapeutics during development.

Duration

Phase 1: six months, Phase 2: Up to three years

Budget

Phase 1: Up to £100k, Phase 2: Up to £1 million

Sponsor(s)

Bayer AG, GSK, Novartis Pharma AG and Merck Healthcare KGaA

Background

Immune modulators

Immune modulators – defined as therapeutics that modify the immune response – are one of the fastest growing drug classes in preclinical drug development. They are being developed across multiple indications including viral diseases, autoimmunity and cancer immunotherapy. The cancer immunotherapy market is expected to grow to $106.6 billion in 2022 and reach $193 billion in 2027 (Vision Gain report, 2018). Currently, there are around 2,720 immune modulating biologics and 1,179 immune modulating small molecules in development across all indications, and they are increasingly being used as combination therapies, for example, with immune checkpoint inhibitors in oncology indications (Cortellis databank analysis, Feb 2020).

Regulatory guidelines require that any potential adverse effects of new pharmaceutical agents on the immune system are evaluated prior to use. Immunotoxicological effects can occur either independent of the target of the drug (off-target) or in the case of immune modulators, immunotoxicity can occur as exaggerated pharmacology (on-target). The impact of exaggerated pharmacology needs to be investigated in preclinical programmes, as excessive immune stimulation can lead to hypersensitivity reactions, increased propensity to develop autoimmunity or cytokine release syndrome (Brennan *et al.*, 2018).

The TDAR assay

One of the gold standards recommended by regulatory guidelines to assess the immunotoxic effects of pharmaceuticals on immune function is the *in vivo* TDAR assay (European Medicines Agency, 2006 and 2011; Food and Drugs Administration, 2012 and 2020). It is a holistic assay that assesses the function and interplay of several cellular and humoral components of the immune system involved in mounting immune responses. The model antigen used to evoke an immune response in the assay, such as Keyhole Limpet Hemocyanin or Tetanus Toxin, is taken up by antigen presenting cells (APCs) such as dendritic cells, processed and presented in the context of major histocompatibility complex (MHC) molecules. Subsequently, T-cells recognise this specific MHC:peptide complex, become activated and in turn activate B-cells via both secreted cytokines and receptor interactions. Activated B-cells then differentiate into plasma cells that produce the appropriate antigen-specific antibodies. Any alteration to the antigen-specific responses detected in the *in vivo* TDAR assay can indicate potential effects of the therapeutic candidate on any of the involved cell types, including APCs, T-cells and B-cells.

Assessing immune enhancement

The TDAR assay is well-established for detecting immunosuppression due to off-target toxicity and on-target pharmacology in rodents and non-human primates (NHPs) for small molecules and biologics respectively. New immunomodulatory therapeutics can cause toxicity through on-target enhancement of the immune response (exaggerated pharmacology). For the assessment of immune enhancement, adaptations to the method, such as optimising the antigen concentration to induce a sub-maximal immune response, are required.

For biologics

The testing of biologics can usually only be conducted in NHPs due to the lack of target cross-reactivity in other species. When assessing immune suppression, the initiation of a robust immune response is required, however, to detect immune enhancement, a sub-maximal response is needed to look for increases to the immune response. NHPs show a high individual variability in their immune response and as a result, it is difficult to define a standardised antigen concentration to be used in the immunisation protocol of the TDAR assay for immune enhancement. If the evoked immune response is either too high or too low, the assay is insensitive in detecting an immune enhancing effect. Data obtained in such cases is difficult to interpret and could lead to an underestimation of the potential risk. Due to this variability, large numbers of NHPs are often required to assess immune enhancement in TDAR assays to ensure reliable results.

For small molecules

For small molecules, the TDAR assay is usually performed in rodents, typically rats. Immune modulating small molecule therapeutics are currently in early development but there is no established method to measure immune enhancement using the TDAR assay in rodents. As immune modulating

small molecules progress in development, this will lead to an increased use of rodents to evaluate the immune enhancement properties of these therapeutics.

In vitro models

In vitro systems to detect immunosuppression have been reported but are not applicable for the assessment of immune enhancement (Fischer *et al.*, 2011; Collinge *et al.*, 2020), which is dependent on the proper matrices and spatial organisations of relevant immunological compartments. The system described by Fischer *et al.*, 2011 uses rodent cells and may not translate to humans. A human lymphocyte activation assay that is similar to the *in vivo* TDAR assay has been reported (Collinge *et al.*, 2020). However, the assay has only been evaluated using immunosuppressive compounds and uses cells from influenza-immunised donors, which means that only the secondary immune response can be measured.

With growing evidence that 3D *in vitro* immune microphysiological systems (MPS) such as the lymph node can be assembled (Shanti *et al.*, 2018 and 2020; Sun *et al.*, 2019), in principle there is a sound basis to adapt and validate these systems for the assessment of immune enhancement of immunomodulatory therapeutics. Immune responses initiated at different locations throughout the body are coordinated by secondary lymphoid organs, mainly the lymph nodes. The lymph nodes provide an optimal environment for the lymphocytes to effectively perform their function. A number of lymph-node-on-a-chip models have been reported, mostly in relation to chemotaxis, and the cellular response to chemokines (Shanti *et al.*, 2018 and 2020). The use of such MPS models in pharmaceutical testing has however not been reported, especially in relation to T-cell responses.

The aim of this Challenge is to develop an *in vitro* human immune response assay to investigate the T-cell dependent antibody response for the preclinical assessment of immune enhancement of immunomodulatory therapeutics. As no species mimics the human immune response in all aspects a human *in vitro* immune response assay could better predict clinical outcomes (Bjornson-Hooper *et al.*, 2019). Additionally, a system allowing for multiple replicate testing and benchmarking to already marketed therapeutics with known clinical effects would be advantageous, especially for poly-specific modalities (bi- or tri-specific antibodies) where identifying a relevant species for immunotoxicity and efficacy testing is problematic as these modalities may not cross react with any preclinical species.

3Rs benefits

A predictive human *in vitro* model developed through this Challenge could reduce the number of NHPs used to assess the immune enhancement properties of preclinical immunomodulatory biologics. Testing biologics usually requires 20 to 40 NHPs per study (typically cynomolgus macaques). Due to the high inter-animal variability in NHPs in their immune response in the *in vivo* TDAR assay studies, large animal numbers (n=10 per group) compared to standard toxicology studies (n=4 to 6 per group) are required to draw robust conclusions (Lebrec *et al.*, 2011, 2014). Study designs are not standard and are defined by the mechanism-of-action and pharmacokinetics of the therapeutic under investigation. For example, a study may include up to three dose groups plus a vehicle control, with up to ten

animals per group. The immunisation schemes, sampling and analytical methods used are specific to the therapeutic under investigation. Commonly used antigens include Keyhole Limpet Hemocyanin, Tetanus Toxin or Hepatitis B antigen and read-outs of the immune response include measuring antigen-specific IgM and IgG antibody titres, numbers of antigen-specific B and T-cells.

There is currently no established method to assess immune enhancement in the rodent TDAR assay. As immune modulating small molecules progress in development, a human *in vitro* model could prevent the development of rodent models for assessing immune enhancement.

Once a robust and reliable model has been developed to detect immune enhancement it also has the potential to be adapted to detect *immunosuppressive* effects of pharmaceuticals and environmental chemicals (Boverhof *et al.*, 2014), and could further replace the regulatory requirement for the use of rodents and reduce the number of NHPs used for immune function safety testing.

Key deliverables

- The aim of this Challenge is to develop a human *in vitro* TDAR assay combining all the relevant cell types to assess the immune enhancement properties of preclinical immunomodulatory therapeutics. The assay should model the complex nature of the immune response, which involves the interaction of several key cell types and mediators in the morphologically coordinated architecture of lymphoid organs, such as the lymph node.

- A component of Phase 2 of this Challenge is to interact with regulatory authorities early to discuss what is required to qualify and validate the assay for regulatory decision making. This could help future regulatory acceptance if a successful *in vitro* assay is developed.

- While the focus of this Challenge is to develop an *in vitro* human TDAR assay to assess immune enhancement of immunomodulatory therapeutics, the ability to also assess immunosuppression as part of this Challenge would be considered advantageous.

Phase 1 Deliverables

Essential:

- Establish a proof-of-concept/prototype human *in vitro* immune cell culture system which includes professional APCs, T-cells and B-cells to enable efficient antigen processing, presentation, priming and immune response, in a 'lymph-node-like' physiology/environment to facilitate the proper spatial organisation and interaction of cells. For example, the inclusion of other cell types such as stromal cells to promote physiology could be of benefit.

- Demonstrate stable cell culture (e.g. viability, cell phenotype, functionality, reproducibility) for up to 14 days, as indicated by relevant markers/read-outs. For example, consistent expression of functional receptors on each cell subset (e.g. MHC on APCs, co-stimulatory

molecules etc); functionality of each cell type (e.g. efficient antigen uptake in APCs, activation/proliferation of T-cells, activation of B-cells etc).

- Demonstrate a dose-response relationship of the immune response to a model antigen (e.g. Keyhole Limpet Hemocyanin or Tetanus Toxin, preferably without use of an adjuvant), to show it is feasible to detect immune modulation in the *in vitro* system.

- Demonstrate read-outs for viability and humoral antibody responses. For example, antigen-specific IgM/IgG or antigen-specific B-cells/ plasma cells.

- Allow repeated sampling to enable evaluation of the kinetics of the immune response.

- Robust plans to deliver Phase 2 of the Challenge, including commercialisation and dissemination.

Desirable:

- Up to three weeks stable culture to evaluate the effect of a therapeutic on the secondary immune response.

- Initial demonstration that upregulation of immune response can be detected.

Phase 2 Deliverables

Essential:

- Refinement and extensive optimisation and assessment of the *in vitro* human TDAR assay. The assay must:

  - Provide robust immune response read-outs (e.g. antibody or plasma cells, T-cell activation).

  - Use a defined source and supply of cells.

  - Use a defined model antigen(s) that is available at consistent quality, stability and immunogenicity, and from several suppliers.

  - Use several positive controls/benchmarking immunomodulatory therapeutics to assess predictivity to the clinic.

  - Have the ability to test multiple therapeutics at several concentrations in parallel (e.g. ten or more in a single run).

  - Provide evidence of, and a protocol for, the donor numbers required for each run based on an assessment of the donor variation and subsequent definition of the associated power required to establish robust responses in the assay with benchmarking immunomodulatory therapeutics.

- Be easy to implement and use, demonstrate reproducible and robust read-outs and be easily transferable between laboratories

- Assessment of inter/intra-laboratory reproducibility of the model concerning relative ranking of benchmarking compounds.

- Evidence of early interaction with regulatory agencies:

  - Discussion concerning design, proof of predictability and validation package required for regulatory acceptance of an *in vitro* approach.

Desirable:

- Potential for the model to be linked to other MPS organ systems.

- *In vitro* cross-species comparison (rat/cynomolgus monkey/human).

- Additional CD8+ T-cell antigen and read-out.

Sponsor in-kind contributions

The Sponsors will provide:

- Scientific advice and support.

- Experience of regulatory requirements in the development of immunomodulatory therapeutics.

- In-house testing of the model.

- Provision of immunomodulatory therapeutics with known effects.

- Comparison with existing in-house preclinical and clinical *in vivo* data from particular benchmarking therapeutics.

- Support with regulatory interaction.

- Engagement with experts in the field to disseminate the outcomes of the Challenge.

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