In vitro assays for pre-clinical safety assessment of mAbs

Pre-clinical toxicological safety testing of novel human pharmaceuticals is a prerequisite before clinical testing. The objective is to identify target organs and reversibility of toxicity in order to determine a safe starting dose for clinical trials and identify parameters for safety monitoring in humans. This article introduces in vitro immunotoxicological assays that can be used when assessing pre-clinical safety of monoclonal antibodies (mAbs), including methods for prediction of immunogenicity, cytokine release and effector functions related to the biological properties of the antibody.

Immunotoxicology

Immunotoxicology is the specialized area of toxicology that addresses potential adverse effects of xenobiotics on the immune system. Inappropriate immune activation can lead to hypersensitivity reactions and autoimmunity, whereas inappropriate inhibition may lead to increased susceptibility to infections and risk of cancer (Figure 1).
In vitro assays using primary human immune cells possess a number of advantages over the animal models classically performed as part of the pre-clinical immunotoxicology assessment. Species differences are avoided, the human drug-product can be used and the use of animals can be minimized. Importantly, because less compound is needed, it provides the opportunity to screen for adverse effects earlier in the research program.

Biologics (including mAbs) present a challenge for immunotoxicological assessment because of their immunogenic potential. In fact, as the majority of therapeutic mAbs are designed to bind to immune targets for treatment of immunopathological conditions, their primary toxicity is most often due to immunogenicity or exaggerated pharmacology related to blocking or enhancing the activities of the target.

Immunogenicity prediction and assessment

Development of anti-drug antibodies is a major safety concern with biologics and can lead to reduced or loss of efficacy, altered pharmacokinetics, or even cross reaction with the patient’s endogenous proteins potentially resulting in life-threatening reactions. A combination of in silico and in vitro prediction tools can be used to guide drug design towards lower immunogenicity potential. In silico methods can predict the T cell epitopes that are able to bind with high affinity to human MHCII, and in vitro assays can be used to confirm the capacity of the predicted epitopes to elicit a CD4+ T cell response (2).

Predicting cytokine storms

Cytokine release syndrome is an adverse clinical event associated with release of pro-inflammatory cytokines from immune cells. After the clinical trial in London where the super-agonistic mAb TGN1412 induced a near-fatal release of cytokines in healthy volunteers despite inconspicuous preclinical testing, immense efforts have been made to improve the sensitivity of cytokine release assays. One successful improvement has been to pre-incubate blood immune cells at high density prior to drug testing in order to mimic the conditions leading to the lower activation threshold that tissue resident T cells have compared to blood T cells. Another successful improvement is the immobilization of the mAbs to wells using wet or dry-coating techniques to mimic mAb cross-binding or addition of accessory cells instead of testing in aqueous phase. Both changes greatly increases assay sensitivity and successfully predicts the cytokine storm seen with TGN1412 (3,4).

Detecting Fc related effector functions and immunotoxicity of mAbs

Certain antibodies can activate effector functions through the Fc part (the non-antigen binding part) of the antibody and induce antibody-dependent processes whereby the Ab-opsonized cells are targeted for lysis or phagocytosis by immune cells or the complement system. While these effector functions are exploited for mAbs targeting cancers, they are an undesired effect when targeting inflammatory disorders.

In vitro assays using primary human cells and serum can be used to test and select the desired mAb Fc profile for best safety of lead candidates. These include macrophage phagocytosis assays and cell-based cytotoxicity assays for detection of target cell lysis (5). Fc related mechanisms that lead to alterations in immune cell composition can be identified using flow cytometry by looking at absolute counts and percentages of different leukocyte subsets of whole blood after exposure to mAb.

Designing the safety study

Although guidelines for pre-clinical safety assessment of human pharmaceuticals are published, pre-clinical safety studies for mAbs must be designed on a case to case basis founded on the immunopharmacology. This requires an in-depth understanding of the target expression and mode of action, and a full functional characterization of the biological properties of the mAb in order to assess the immunotoxicological potential and ensure minimal risk for the first-in-human clinical studies.

References