Monoclonal antibody therapeutics (mAbs) can cause infusion-related reactions in patients, which may only become apparent in first-in-man trials. To provide more information to drug developers before drugs reach clinical trials, ProlImmune has developed ProStorm™, an *in vitro* cytokine release assay which can help to predict the likelihood of first infusion reactions.

Various types of infusion-related reactions may occur in response to biotherapeutics. First infusion reactions occur within minutes of infusion and depend on mAb interacting with their target and/or stimulating the innate immune system. Such reactions include cytokine release syndrome, and the related tumour lysis syndrome (which also leads to cytokine release). Symptoms of cytokine release syndrome range from fever, headache and skin rashes to bronchospasm, hypotension and even cardiac arrest. Severe cytokine release syndrome is described as cytokine storm, and can be fatal. Fatal cytokine storms have been observed in response to infusion with several mAb therapeutics, and the cytokine storm reactions observed in first-in-man trials of TGN1412 ignited public and industry concern relating to early-stage mAb trials. ProStorm™ is designed specifically to indicate when a cytokine storm first infusion reaction may be a risk for a therapeutic.
The ProStorm™ assay uses fresh whole blood sourced from 20-50 healthy volunteer donors recruited for your study by ProImmune. To maximise assay sensitivity, blood is used undiluted, and drugs are added in a range of concentrations to cover the peak exposure range of drugs in the body. Following incubation, cytokine release is measured using ProImmune’s high-sensitivity ProArray Ultra® platform, to give an accurate profile of cytokine release. TNFα, IFNγ, IL-2, IL-4, IL-6, IL-8 and IL-10 levels are measured. Erbitux®(Cetuximab), associated with a low incidence of first infusion reactions in the clinic, is included in the ProStorm™ assay to provide a baseline, and Campath® (Alemtuzamab), which frequently causes first infusion reactions, is included as a biologically relevant comparator. In reporting the assay data, we include analysis of the significance and dose-dependency of responses for test drugs.

To harmonize methods for prediction of cytokine release assays, the European Medicines Agency (EMA) held a workshop in 2009. They agreed that animal models are not predictive of first infusion reactions, so in vitro cytokine release assays (such as ProStorm™) are best for identifying this hazard. The EMA also advise that users of in vitro assays should consider absolute and relative changes in cytokine release profiles and use biologically relevant comparators in their analysis, exactly as ProStorm™ does.

Predictive assays such as ProStorm™ can be used by drug developers to mitigate risk when approaching first-in-man trials. ProStorm™ cannot be used to quantify risk, but if a potential hazard is indicated then trials can be designed with this in mind. For example, a lower starting dose can be chosen, and antihistamines and steroids can be administered in advance of the drug infusion to mitigate potential reactions. TGN1412 was an unusual case: first-infusion reactions do not usually spell the end for a therapeutic. Many established therapeutics, including Ofatumamab (Arzerra), Adalimumab (HUMIRA) and Infliximab (Remicade®), have high incidences of first infusion reactions. Prior information on the risks of a first infusion reaction allows this risk to patients to be managed, so that the drugs can still be applied successfully and widely adopted by clinicians. ProStorm™ can thus be a valuable tool, providing information that may help in bringing a therapeutic to market.
How does the Assay Work?

ProStorm™ is designed to aid in the prediction of first infusion-related reactions, from therapeutics such as monoclonal antibodies (mAb), and is an *in vitro* cytokine release assay.

We source blood samples from a donor cohort chosen to match the needs of your project. Most often, we will use cohorts of 20, 40 or 50 healthy volunteers, but we are able to source donors with known conditions if this is required.

Fresh whole blood is used in the assay.

We use your drug preparation at a range of concentrations designed to mimic the peak exposure during a first infusion.

We add your drug preparation to the fresh whole blood samples, and after incubation, harvest the serum to measure cytokine levels. Typically, we would measure TNFα, IFNγ, IL-2, IL-4, IL-6, IL-8 and IL-10, but in consultation with you we will select the best panel.

Cytokine levels are measured using our ProArray Ultra® technology for the most accurate quantification.

Our team analyses your data to give you results that can be used in informing the next stages of your research.
What does a ProStorm™ dataset look like?

We present two types of analysis as standard - plots showing the response from your donor cohort by cytokine for each drug/concentration, and a threshold (cut-point) analysis to indicate potential risk.

Cytokine Response Data

Figure 1: Prostorm™ results. IL-6 responses from a cohort of 29 donors, stimulated with (left to right) Remicade®, aggregated Remicade®, Erbitux®, Campath®, Herceptin® and Vectibix®, at a range of concentrations. Medians are indicated by red lines.

Figure 1 shows IL-6 responses to a range of drugs - note the high responses from Campath®, and from aggregated Remicade®.
Threshold Analysis

In addition to plots showing the cytokine responses of your whole donor cohort to each drug and dose combination tested, we also present a threshold (cut-point) analysis. In this analysis your mAb therapeutic is compared to Erbitux® (Cetuximab), as this is known to elicit a low incidence of first infusion-related reactions.

**Figure 2: Cut-point analysis of ProStorm™ datasets.**
A threshold (red line) is set based on the 95th percentile of the test cohort response to Erbitux®.

This threshold is then applied to the results from other test mAbs. Donor responses above threshold are clearly visible (filled blue points).

This analysis approach is particularly powerful when used across a range of drug concentrations and when used with a large donor cohort. The results shown are from 29 healthy donors, and show the IL-6 response to 100µg/ml drug.

We will also typically include Campath® (Alemtuzamab) as administration of this mAb is associated with a high incidence of first-infusion reactions, and can serve as a useful comparator.

**Figure 3: Erbitux® responses and 95th centile thresholds applied across increasing drug concentrations, IL-6 responses from a 29-donor cohort.**
Figure 4: Campath® responses with Erbitux® 95th centile thresholds applied across increasing drug concentrations, IL-6 responses from a 29-donor cohort.
A particularly attractive feature of the assay is the use of fully formulated therapeutics. This allows the effects of changes in formulation to be easily assessed. In one of our exploratory studies at ProImmune, we investigated the cytokine responses to Infliximab (Remicade®) and aggregated Remicade®. The results (figure 5) clearly show the increased reactivity of fresh blood to the aggregated formulation.

Figure 5: Remicade® and Aggregated Remicade® responses with Erbitux® 95th centile thresholds applied across increasing drug concentrations, IL-6 responses from a 29-donor cohort. Positive responders are indicated by filled data points.
References


2 Transplantation. 1990 Apr;49(4):697-702.


Does safety make a difference in selecting the right TNF antagonist? Fleischmann R, Yocum D. [PMID: 15228616]