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ProtoTrials®

Enhancing human *ex vivo* translational outcomes

Global leaders in specialist biomarker and bioanalysis research services

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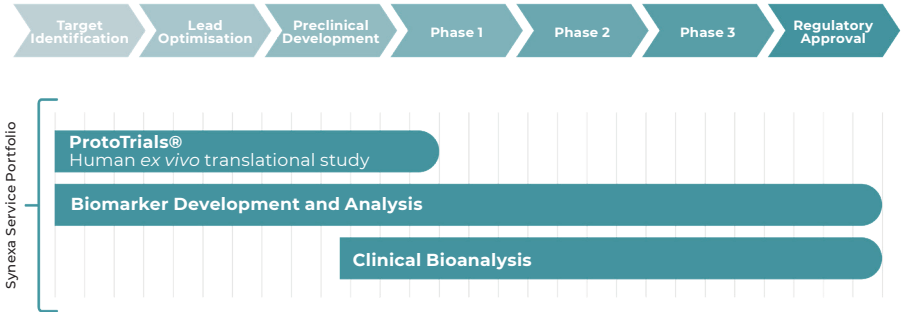
What is ProtoTrials®?

ProtoTrials® is a proprietary human *in vitro* translational immunology platform. ProtoTrials® studies are adaptive and collaborative in nature and draw together decades of scientific expertise, an extensive selection of technologies and customisable assays, along with access to a broad portfolio of samples from both healthy and diseased states.



ProtoTrials® Applications:

- Allow early insight into the efficacy, safety and mechanism of action of a therapy where the immune system is a target and/or a therapeutic modality.
- Prioritise and refine therapeutic indications by assessing the efficacy of a candidate therapy across multiple immunologically driven diseases.
- Focus the biomarker panels and analytical techniques to those which will generate the most significant clinical and biological insights.
- Benchmark the candidate drug against other drug leads and commercially available competitor products.
- Generate serendipitous discoveries of interesting aspects of therapeutic performance.

Case Studies

ELECTION OF LEAD CANDIDATE: JAK3 Inhibitor

Background

Client developed a JAK3 inhibitor and wanted to select a lead candidate to take into a first in man study. In addition, they wished to obtain a provisional indication of efficacy in a panel of autoimmune diseases.

Strategy

Synexa utilised a series of functional flow cytometry assays to assess the impact of the JAK3 inhibitor on the activation of Th1, Th2, Th17, Treg, DC and NK cell subsets. Initial studies utilised samples from healthy volunteers which were stimulated with a range of cytokines, mitogens and antibodies specific for the immune cell subset of interest. Assay readouts included cell activation markers and intracytoplasmic cytokines. The efficacy of the JAK3 inhibitor was benchmarked against Tofacitinib.

Outcomes

We identified the lead candidate with greatest anti-inflammatory efficacy and confirmed its biological activity in samples taken from patients with rheumatoid arthritis and SLE. The work also identified anti-inflammatory effects.

RECEPTOR ACTIVATION QUANTIFICATION: Microbial TLR Activation

Background

The intestinal microbiota is not only involved in digestion but has a dynamic relationship with the host's immune systems as well. The complex interplay between the microbiota and the immune system is currently the focus of much research. This interplay starts early in life, where the steadily evolving microbiota trains the intestinal immune system to recognise and tolerate the commensal organisms, while at the same time keeping them from escaping the gut. The immune system is also taught to differentiate between commensal and potentially pathogenic bacteria, directing secretory IgA preferentially against the pathogens.

There is a growing requirement to assess the Total Inflammatory Milieu (TIM) of the gastrointestinal tract and how this is influenced by the relative balance of pathogens versus symbiotic microbiota.

Analytical Strategy

Synexa developed and validated a NF-kappaB (controls transcription of cytokines) reporter cell line assay to robustly quantify TLR activation in stool supernatants. The cell line expresses the following TLR receptors:

- TLR2 – recognises peptidoglycan, lipoteichoic acid and lipoprotein from gram-positive bacteria, lipoarabinomannan from mycobacteria, and zymosan from yeast cell wall
- TLR4 – recognizes and is activated by the major constituent of the outer membrane of Gram - negative bacteria, lipopolysaccharide (LPS)
- TLR5 – recognises flagellin from both Gram-positive and Gram-negative bacteria.
- TLR6 – specific for diacylated lipopeptides such as lipoteichoic acid, found on the cell wall of gram-positive bacteria
- TLR8 – recognizes ssRNA in the endosomal compartment and leads to the secretion of cytokines.

Outcomes

Assay has been used in a clinical trial setting to monitor the TIM of therapies that modulate gut microbiota. The assay can be used in a translational research setting to study the impact of probiotic and/or prebiotic on TIM and aid in determining through which specific TLRs a therapeutic effect is exerted.

TARGET ENGAGEMENT EVALUATION: Modulators of Treg Polarization

Background

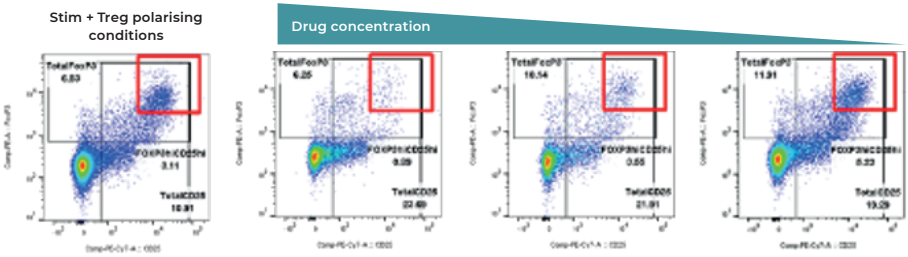
Regulatory T-cells (Treg) are a key mediator of immune tolerance. Tregs are an important therapeutic target both in autoimmune disease (objective is to increase Treg activity) and in immune oncology (objective is to decrease Treg activity). A client developed a small molecule to inhibit Treg polarization which demonstrated promise in an animal model but needed to confirm efficacy in the human immune system.

ProtoTrial Strategy

Peripheral blood mononuclear cells were isolated from healthy volunteers and samples were enriched for naïve CD4 T-cells using magnetic bead separation. The naïve CD4 T-cells were polarized to differentiate into Tregs and the dose-dependent impact of the molecule on Treg polarization was quantified by flow cytometry.

Outcomes

The study demonstrated the ability of the molecule to inhibit Treg polarization in a dose-dependent manner.



CYTOKINE RESPONSE ASSESSMENT: Ex Vivo LPS Stimulation Assay

Background

Ex vivo LPS stimulation and cytokine release assays are an important analytical tool.

Analytical Strategy

The principal of the assay is well established and requires blood (whole blood or diluted) to stimulated with LPS and the measure the levels of key cytokines (e.g. IL-1B, IL-6, IL-18, TNF-a) in culture supernatants. To ensure the model could be used robustly in a clinical trial we studied a broad range of conditions to assess their impact on analytical variability.

Outcomes

Findings from the study included: A high degree of inter-subject variability in the level cytokine response. Intra-subject diurnal variability in cytokine response. Significant impact from fasting status on cytokine response. Certain cytokines were more robust predictors of a given therapeutics' efficacy.

Learn more from our biomarker experts

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