Complex Bioassays: Bridging Analytics and Clinical Relevance in Biosimilar Development

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Introduction

Establishing similarity for complex biologics requires more than physicochemical comparability, it demands functional evidence reflecting the mechanism of action in a biologically relevant context. Regulators place greater weight on analytical and functional data over comparative clinical efficacy studies. Complex primary cell-based assays are integral to this framework, complementing physicochemical and simpler functional methods within an orthogonal approach. Integrated with high-resolution analytics, these assays strengthen confidence in biosimilarity and support robust regulatory submissions. This poster illustrates how complex primary cell-based assays contribute to biosimilar comparability by addressing key mechanisms of action and fulfilling roles central to regulatory expectations integrating biological activity assessment, indication extrapolation and the linkage of analytical data with clinical relevance.

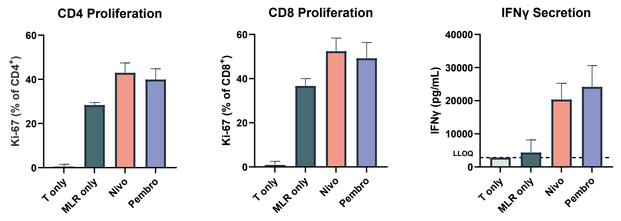
Key mechanisms of action for monoclonal antibodies

Mechanism of Action	Originator Molecules with Approved or Pipeline Biosimilars
Neutralisation	Adalimumab, Infliximab, Etanercept, Golimumab, Ustekinumab, Tocilizumab, Risankizumab, Secukinumab, Guselkumab, Omalizumab, Bevacizumab, Aflibercept, Denosumab.
Checkpoint Modulation	Pembrolizumab, Nivolumab, Abatacept, Ipilimumab, Atezolizumab, Durvalumab, Avelumab
Receptor Blockade	Dupilumab, Cetuximab, Tocilizumab,
Cell Depletion	Rituximab, Ocrelizumab, Daratumumab, Trastuzumab, Pertuzumab.

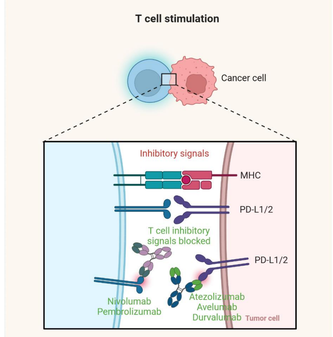
Complex cell-based assays serve multiple roles, measuring critical quality attributes, supporting indication extrapolation, probing physiological relevance and confirming or dismissing potential biological activities. These functions go beyond simple binding or receptor activation, reflecting the **full biological activity** relevant to clinical performance.

Checkpoint Modulation

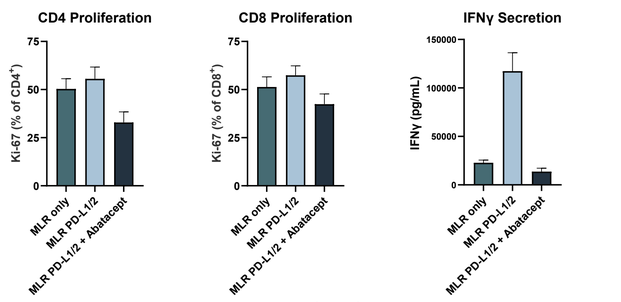
Mixed Lymphocyte Reaction: Nivolumab and pembrolizumab



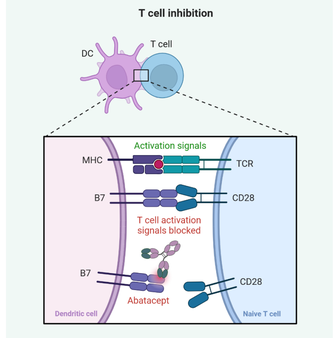
A one-way MLR that combines T cells, collected from one donor, with dendritic cells, derived from monocytes of a second (allogeneic) donor (moDCs). The results show activation of T cells measured through the secretion of IFNγ and proliferation measured by Ki-67 with enhanced T cell activation in the presence of nivolumab and pembrolizumab.



Mixed Lymphocyte Reaction: Abatacept



One-way mixed lymphocyte reaction (MLR) shows T-cell activation was further enhanced by PD-1/PD-L1 blockade. This synergistic effect was inhibited by abatacept resulting in reduced IFNγ secretion and proliferation, quantified using Ki-67 expression in $CD4^+$ and $CD8^+$ subsets.



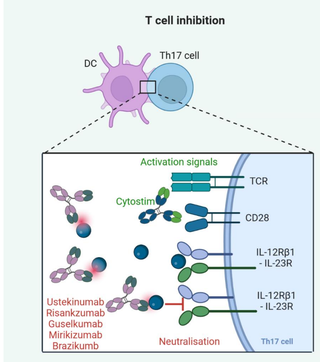
There is an **unmet need** for robust, quantitative assays to assess immunomodulatory function. While current approaches rely on reporter assays, these lack physiological relevance. Our work demonstrates the potential of MLR as an orthogonal, clinically meaningful tool, and we are **actively developing quantitative solutions** in this area.

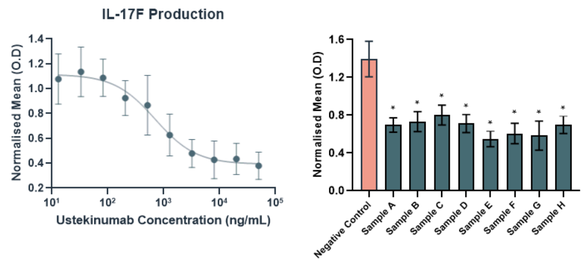
Benefits of RoukenBio's approach

- Platform methods with standard assay designs greatly reduces time spent in optimisation
- Access to physiologically relevant primary human cells (healthy and disease)
- Expert development for challenging assays
- In-house Cell Engineering capabilities to complement your project
- Proven track record across 20+ biosimilar therapeutics

Neutralisation

Neutralisation of IL-23 mediated IL-17 secretion by ustekinumab



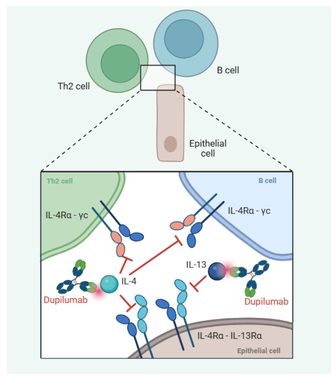


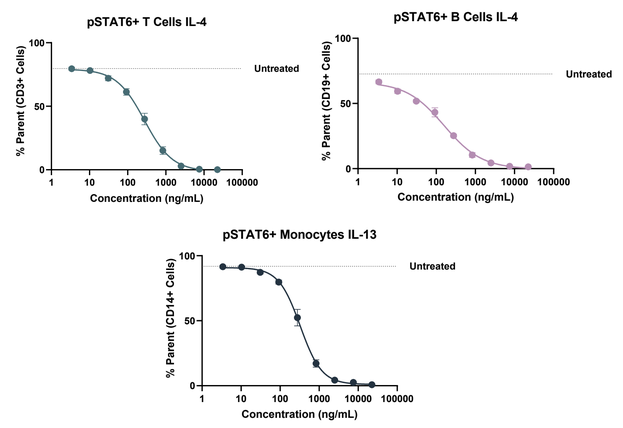
Peripheral blood mononuclear cells (PBMC) were stimulated with IL-23 alongside a low level of Cytostim to enhance activation of Th17 responses. Ustekinumab was added at increasing concentrations and demonstrated a clear dose-dependent reduction in IL-17F, measured by ELISA, compared to positive controls, confirming effective inhibition of IL-23-driven pathways.

Although the assay is **semi-quantitative**, it adds value by enabling functional assessment in a pathway central to Crohn's disease and psoriasis. This approach supports **indication extrapolation** by demonstrating comparable activity in a physiologically relevant context, even when quantitative data are not possible.

Receptor Blockade

Inhibition of pSTAT6 signalling in PBMC by dupilumab

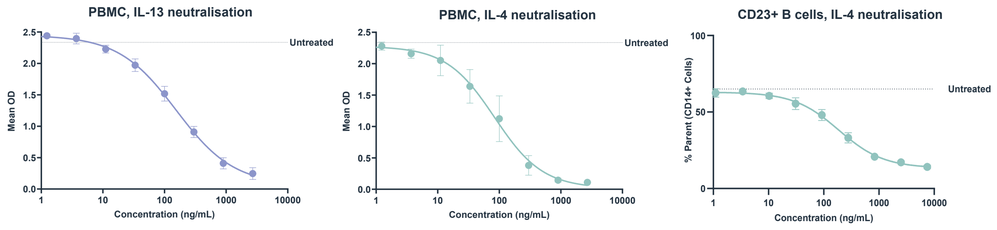




Peripheral blood mononuclear cells (PBMC) were stimulated with IL-4 or IL-13 to activate downstream STAT6 signalling as measured by flow cytometry. Dupilumab mediated dose-dependent inhibition of STAT6 phosphorylation in T cells, B cells and monocytes following cytokine stimulation, effectively reducing the proportion of pSTAT6+ cells across all subsets and confirming robust blockade of IL-4/IL-13 receptor signalling.

Inhibition of TARC/CCL17 secretion

Inhibition of CD23 expression



Beyond STAT6 signalling the associated functional responses were assessed, dupilumab suppressed secretion of TARC/CCL17 from PBMC and reduced CD23 surface expression on B cells. These phenotypic and secretory changes demonstrate downstream impact on inflammatory pathways, reinforcing the physiological relevance of the assay.

Quantitative and sensitive assays that capture the **full spectrum of biological activities** relevant to the clinical performance of the product are the gold standard for biosimilar analytical comparability. Here we present receptor blockade assays, exemplifying the ideal tools for biosimilar functional assessment and regulatory confidence.

SUMMARY

Complex primary cell-based assays offer distinct advantages in biosimilar comparability by providing functional insights that complement traditional binding and reporter assays. Our poster showcases three key examples.

Integrating complex primary cell-based assays into biosimilar comparability workflows enhances the clinical relevance and robustness of functional testing. Even qualitative assays, such as the ustekinumab example, provide important evidence for functional similarity in disease relevant pathways and can inform regulatory strategy, including indication extrapolation. We invite discussion on emerging quantitative approaches, particularly in areas of unmet need such as checkpoint modulators.

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