APPLICATION NOTE

A Validated Immune Profiling Assay for Routine and Reproducible Immunophenotyping

Simplifying clinical trials with the Maxpar Direct Immune Profiling Assay

Introduction

Immune profiling has emerged as a vital tool in the design and execution of clinical trials, as it generates comprehensive insights into therapeutic outcomes and disease progression. Yet, generating quality reproducible data is a common challenge when working with multiple sample collection and analysis sites, larger patient cohorts and time-sensitive studies.

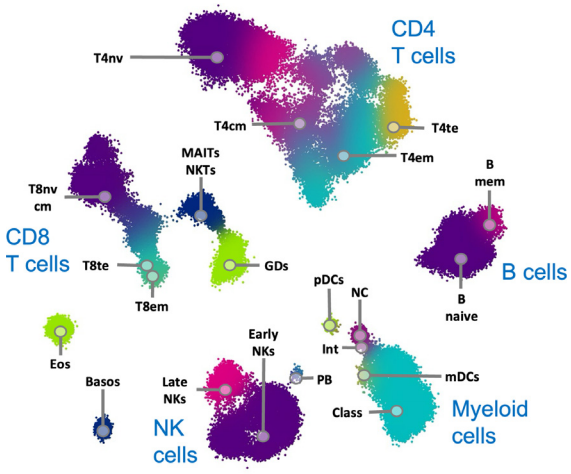
Standardized CyTOF™ assays that enable robust data generation regardless of study logistics offer significant benefits for confident immune profiling in clinical trials. This application note demonstrates the reliability and robustness of using the validated Maxpar™ Direct Immune Profiling Assay through published clinical applications, underscoring its vital role in generating predictive insights into novel biomarkers and disease mechanisms.

This application note outlines:

- Overcoming challenges with data variability using the Maxpar Direct Immune Profiling Assay across sites and assays
- The impact of validated single-tube assays in 3 clinical trial case studies:
  - Identifying mechanism of action associated with BTK inhibition
  - Revealing exhaustion markers associated with clinical response to PD-1 blockade
  - Uncovering pathway-specific correlates of response to LAG3 or TIGIT targeted immunotherapy

Routine and scalable immune profiling with CyTOF systems

CD3	CD28	CD161
CD4	CD38	CD294
CD8	CD45	CCR4
CD11c	CD45RA	CCR6
CD14	CD45RO	CCR7
CD16	CD56	CXCR3
CD19	CD57	CXCR5
CD20	CD66b	HLA-DR
CD25	CD123	IgD
CD27	CD127	TCRγδ



1 single-tube assay | **30**-plus markers | **5**-minute analysis

Figure 1. A simple single-tube workflow with automated analysis ensures high reproducibility of multiparameter data.

A validated single-tube assay eliminates tedious steps and overcomes data variability

In clinical trials, researchers must implement robust and scalable immune profiling methods to characterize the peripheral immune landscape across diverse patient cohorts while also applying rigorous attention to data quality, comparability and reproducibility. With conventional flow cytometry, it is challenging to profile diverse immune cell populations, as it requires multiple tubes and time-consuming staining protocols – increasing the likelihood for error. As a result, researchers are often limited to a small set of markers for defining each cell type and are unable to garner valuable information.

The Maxpar Direct Immune Profiling Assay, which utilizes CyTOF technology, is a 30-marker pre-optimized and lyophilized assay for deep immune profiling of human peripheral whole blood and PBMC in a single tube. Combined with an automated 5-minute data analysis template, the validated assay is an easy-to-implement solution for the routine enumeration of cell types and differentiation states.

This streamlined solution demonstrates a high degree of reproducibility across sites, with population frequencies >5% having a coefficient of variation (%CV) of less than 10% in whole blood, providing a novel approach to large study immune profiling that cannot be replicated by other technologies[1].

In addition, the Maxpar Direct Immune Profiling Assay generates reliable results when combined with sample preservation methods, a standard practice in collaboration or multi-site studies in which sample collection might not be in the same location as analysis. Figure 3 demonstrates a high correlation value between population percentages of BD Heparin, Cyto-Chex and PAXgene Blood DNA Tubes at 72 hr and BD Vacutainer Heparin Tubes at <24 hr[2] with fix-sensitive markers retained.

The Maxpar Direct Immune Profiling Assay shows proven reproducibility across sites, essential for large multi-site studies

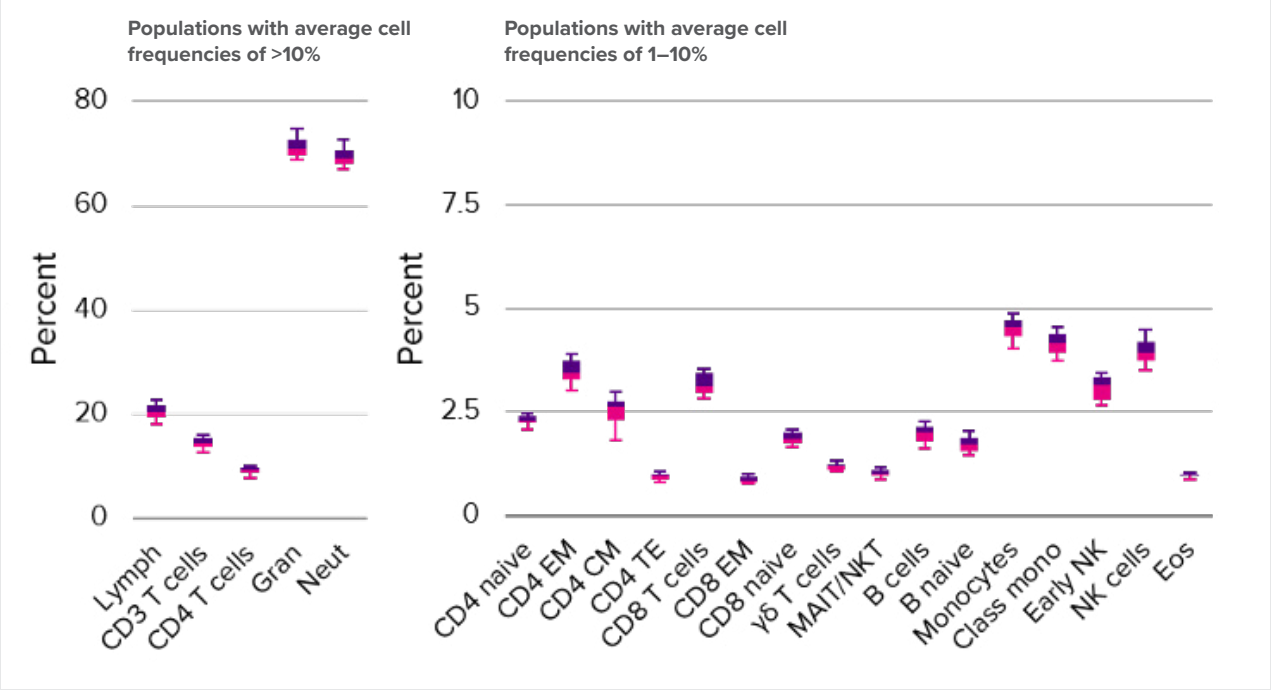


Figure 2. Performance consistency across 12 lots of Maxpar Direct Immune Profiling Assay tubes. Highly consistent results are seen for single whole-blood donor sample run at 6 sites, with 3 technicians running 4 tubes each.

The Maxpar Direct Immune Profiling Assay generates reliable results from samples stored for up to 72 hr

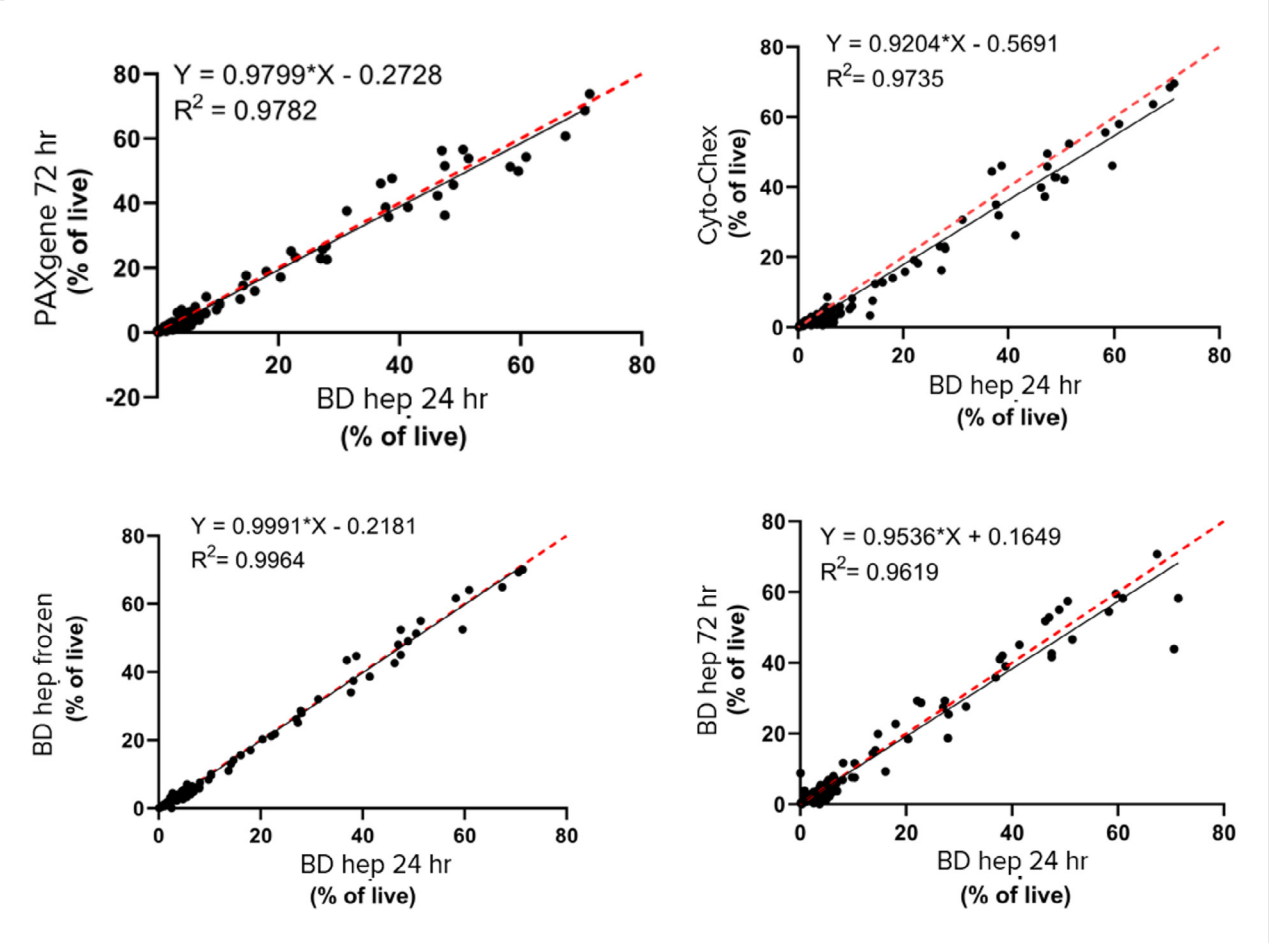


Figure 3. Correlation of cell frequencies for each of the 37 gated populations across all 6 donors for each PAXgene (72 hr), Cyto-Chex (72 hr), BD Hep (72 hr) and BD hep (frozen) vs. BD Hep (<24 hr) blood. The line of identity (slope = 1) is shown as a red dotted line, and the line of best fit for each condition is shown as a black solid line. Correlation coefficients and equations are shown on the graph.

The ability to analyze high-dimensional parameters simultaneously at the single-cell level with CyTOF technology can efficiently reveal predictive biomarkers, identify off-target effects and facilitate patient stratification – ultimately paving the way for more personalized and effective treatment strategies.

Using the CyTOF-based Maxpar Direct Immune Profiling Assay, deep immune profiling information can be performed consistently and conveniently in a single tube. Here we show cases supporting how the assay for standardized immune profiling provides the sensitivity, specificity and accuracy needed for impactful clinical trial data.

Case study

The Maxpar Direct Immune Profiling Assay identifies mechanism of action for clinical effects of Bruton's tyrosine kinase (BTK) inhibition in patients with metastatic solid tumors

In a Phase 1 clinical trial, researchers evaluated the safety and efficacy of the BTK inhibitor ibrutinib in combination with nivolumab in patients with metastatic solid tumors[3]. Blockade of BTK in chronic lymphocytic leukemia has shown promising success. However, its efficacy in solid tumor treatment remains to be fully explored.

The Maxpar Direct Immune Profiling Assay was used to assess immune changes following BTK inhibition, leveraging the broad immune population coverage of the assay and flexibility for customization. Researchers identified 35 unique immune populations and observed a significantly increased proportion of monocytic myeloid-derived suppressor cells (MDSCs), as well as a reduction in chemokine markers associated with MDSC recruitment. MDSCs are a subset of immature myeloid cells with immunosuppressive functions that directly correlate with tumor burden.

Additionally, an expansion of memory B cells was noted in response to ibrutinib monotherapy, while higher levels of total NK cells and CD8+ T cells were observed in patients with clinical benefit compared with those with progressive disease (Figure 4). The extensive coverage of 35 immune populations allowed the researchers to pinpoint alterations in MDSC levels and key immune subsets associated with clinical response.

Maxpar Direct Immune Profiling Assay data enabled analysis of total MDSC levels according to clinical response

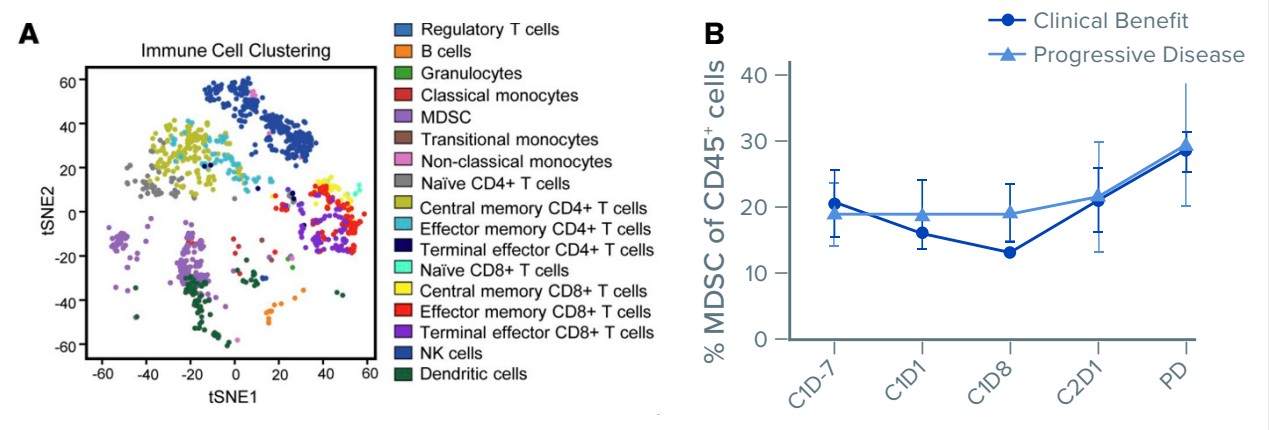


Figure 4: The Maxpar Direct Immune Profiling Assay detailed the peripheral immune landscape of patients receiving ibrutinib plus nivolumab. A) t-SNE plot of immune subset clustering. B) Frequency of MDSCs compared between patients with clinical benefit (n=8) vs. progressive disease (n=8). Unpaired Student's t-test

Study at a glance:

- Rapid immune profiling revealed an expansion of monocytic MDSCs, total NK cells and CD8 T cells in patients with clinical benefit receiving BTK inhibition

Case study

The Maxpar Direct Immune Profiling Assay reveals predictive markers of response to PD-1 blockade in neuroendocrine neoplasm (NEN) patients

The Maxpar Direct Immune Profiling Assay enabled rapid and accurate CyTOF immune profiling in a non-randomized Phase 2 clinical trial (NCT03728361) of patients with NEN receiving nivolumab and temozolomide[4].

Researchers employed high-dimensional CyTOF staining of PBMC with the Maxpar Direct Immune Profiling Assay to characterize the clinical response of 28 NEN patients. The median progression-free survival was 8.8 months, while the median overall survival was 32.3 months. Dimensionality reduction analysis with CyTOF technology revealed:

- A decrease in circulating CD4+ T cells
- An increase in CD8+ T cells 15 days post-treatment
- Reduced LAG-3+ T cell levels in patients with partial response compared with nonresponders
- A decrease in T cell PD-1 expression
- An increase in regulatory T cell frequencies

The Maxpar Direct Immune Profiling Assay facilitated rapid immunophenotyping with CyTOF technology, identifying key immune alterations in circulating T cells that correlated with clinical response in patients with NEN.

Key immune alterations identified by the Maxpar Direct Immune Profiling Assay that correlated with clinical response

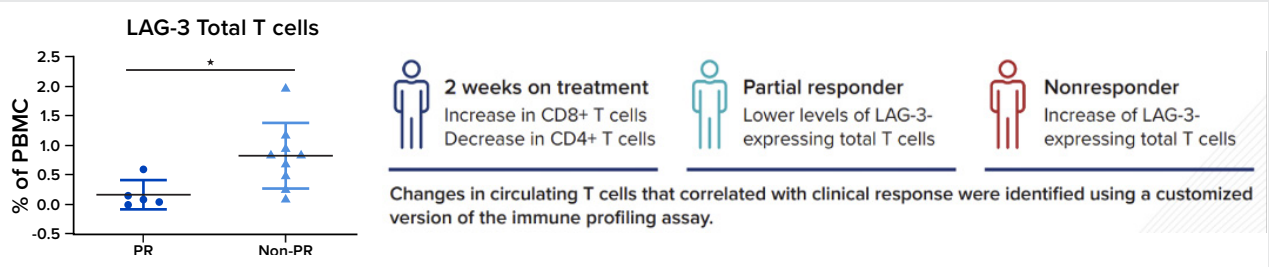


Figure 5. The Maxpar Direct Immune Profiling Assay reveals changes in LAG-3-expressing T cells between partial responders and nonresponders. (A) Schematic of clinical trial and patient response. (B) Changes in peripheral LAG-3-expressing total T cells in the entire study cohort shows significantly lower levels in patients with a partial response compared with patients that experienced a non-partial response.

Study at a glance:

- CyTOF immunophenotyping of PBMC identified a significant decrease in LAG-3+ T cells in patients with partial response receiving nivolumab and temozolomide combination therapy

Case study

Rapid high-dimensional immune profiling of patients reveals pathway-specific correlates of response in a Phase 1/2 multiple myeloma (MM) trial

Identifying immune biomarkers correlated with clinical response is essential for understanding the mechanisms of action and efficacy of novel immunotherapies, ultimately improving patient stratification and treatment outcomes.

In a recent Phase 1/2 MM trial (MyCheckpoint, NCT04150965)[5], 6 participants with relapsing/refractory MM were treated with either anti-LAG-3 or anti-TIGIT monoclonal antibodies. Both TIGIT and LAG-3 blockade are supported by preclinical evidence and are being tested in other trials. However, pathway-specific correlates to clinical response in MM remain unclear.

Researchers utilized the Maxpar Direct Immune Profiling Assay to perform unbiased high-dimensional profiling of peripheral blood and bone marrow mononuclear cells. This approach enabled the characterization of unique cellular pathways upregulated in patients receiving anti-TIGIT, which were correlated with the expression of DNAM-1, CD112 and NK cell activation. In contrast, the anti-LAG-3 cohort was most closely associated with the PD-1 axis, with notably higher PD-1 expression in T helper cells, cytotoxic T cells and regulatory T cells.

By leveraging the efficiency and comprehensive coverage of the Maxpar Direct Immune Profiling Assay, researchers were able to identify key pathway-specific correlates to clinical response in MM patients from limited samples, facilitating timely and informative analysis.

Biomarkers of response identified by the Maxpar Direct Immune Profiling Assay based on protein expression changes

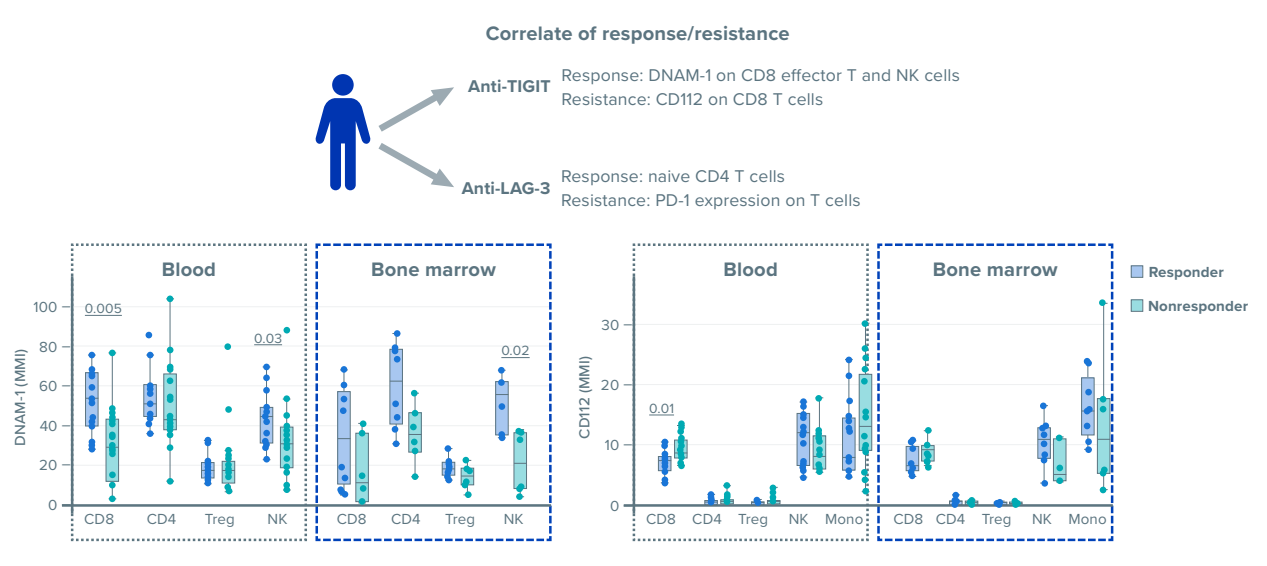


Figure 6. Maxpar Direct Immune Profiling Assay data correlating immune cell changes to clinical response. A summary of immune correlates of clinical response and data showing DNAM-1 and CD112 expression changes across cell types.

Study at a glance:

- Patients receiving anti-LAG-3 had increased peripheral PD1+ T cells, while the anti-TIGIT cohort showed elevated DNAM-1 and CD112 expressions with NK activation

Summary

- The Maxpar Direct Immune Profiling Assay significantly reduces the preparation and optimization steps in cytometry experiments by providing a pre-optimized, validated ready-to-stain panel of antibodies
- Key alterations in the peripheral immune system were observed with a customized Maxpar Direct Immune Profiling Assay panel in a Phase 2 clinical trial of solid tumor patients receiving BTK inhibition therapy
- The Maxpar Direct Immune Profiling Assay identified LAG-3 as the key biomarker of response in a Phase 2 clinical trial of patients with neuroendocrine neoplasms
- In a Phase 1/2 multiple myeloma trial, researchers demonstrated pathway-specific response correlates in patients receiving anti-TIGIT or anti-LAG-3 therapy

References

1. Deep Immune Profiling with the Maxpar Direct Immune Profiling System White Paper (FLDM-40047)
2. Awong, G. et al. "A Comparison of 37+ Immune Populations in Whole Blood by CyTOF Flow Cytometry Collected with 4 Different Preservation Reagents." SITC (2023).
3. Schwarz, E. et al. "Inhibition of Bruton's tyrosine kinase with PD-1 blockade modulates T cell activation in solid tumors." *JCI Insight* 9 (2024): e169927.
4. Owen, D.H. et al. "A Phase II clinical trial of nivolumab and temozolomide for neuroendocrine neoplasms." *Clinical Cancer Research* 29 (2023): 731–741.
5. Richard, S. et al. "Clinical response and pathway-specific correlates following TIGIT–LAG3 blockade in myeloma: the MyCheckpoint randomized clinical trial." *Nature Cancer* 5 (2024): 1,459–1,464.

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