Expanding the Maxpar Direct Immune Profiling Assay panel with additional cadmium-labeled antibodies and functional markers

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Introduction
CyTOF® technology, based on cytometry by time-of-flight, utilizes metal-labeled antibodies for single-cell detection by mass cytometry. A major advantage of mass cytometry is the ability to conduct comprehensive deep immune profiling studies using highly multiplexed panels comprising upwards of 50 markers without the signal spillover and compensation limitations of flow cytometry. The Maxpar® Direct® Immune Profiling Assay® (Cat. No. 201212) utilizes a ready-to-use dry-format 30-antibody staining panel for human whole blood and PBMC immunophenotyping by mass cytometry (Figure 1). Paired with Maxpar® Pathsetter software, users can automatically resolve this core 30 marker panel into 37 immune cell populations with reproducible results. This assay is ideal for use in longitudinal studies of immune response in the context of immune-mediated diseases and is already in use in COVID-19 research.1,2

Materials and Methods
Expansion of the Maxpar Direct Immune Profiling Assay to a 44-marker panel
Additional antibodies were selected in open channels to expand the 30-marker Maxpar Direct Immune Profiling Assay to a 44-marker panel. Markers were selected to further resolve monocyte subsets and to classify monophyly-derived suppressor cells (Mo-MDSCs). In addition, high-value functional markers were added to the panel to investigate their expression on cells in a disease model. Antibodies used to expand the panel consisted of custom conjugates using the MCPG C1 labeling kits and Fluidigm catalog reagents. Purified antibodies used for MCPG C1 labeling were purchased from Biolegend®. Antibodies were titrated on PBMC to determine optimal concentration in the panel.

Sample Preparation, Staining, and Analysis
The expanded 44-marker Maxpar Direct Immune Profiling Assay panel was tested on frozen human PBMC (STEMCELL® Technologies) from healthy donors and donors diagnosed with rheumatoid arthritis. This assay is highly customizable with the availability of more than 14 additional channels to provide panel flexibility (Figure 3). As shown, better background signal in these 14 channels is minimal, resulting in high resolution data for analysis.

Results
Validation of marker expression using Maxpar Pathsetter Cen-se′ plots
Reduced PD-L1 and OX40 up-regulation upon PHA stimulation in RA PBMC

Conclusions
The Maxpar Direct Immune Profiling Assay panel was expanded by adding 14 markers to create a 44-marker panel. The additional markers were incorporated into the Immune Profiling Assay model and report template in Maxpar Pathsetter to include 27 additional frequency measurements on various cell subsets. Relative expression levels of these 14 new markers can be visualized in Cen-se′ plots and cell progression plots generated in Maxpar Pathsetter. Frequencies of populations can be readily quantified using Maxpar Pathsetter. Increased frequencies of OX40 expressing CD4 T cells in PBMC from RA patients were observed, as previously reported by Jiang et al.1 Reduced CD40 and PD-L1 up-regulation was observed on CD4 T cell subsets upon PHA stimulation in PBMC from rheumatoid arthritis donors.

We demonstrated the flexibility of the Maxpar Direct Immune Profiling Assay and Maxpar Pathsetter software to accommodate expanded panels in order to further facilitate deep immune profiling studies.

References

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#P201

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