



## Simplifying High-Parameter Phenotypic and Functional Characterization of Cancer Immune Cells

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A simple and powerful workflow combines a backbone 30-marker immune profiling assay with panels designed to **deeply characterize NK and T cells** together with an intracellular panel enabling **comprehensive phenotypic and functional analysis of multiple myeloma cells**. This workflow can be applied to other disease states to focus deeper on specific biological questions.

### KEY TAKEAWAYS

- The addition of Expansion Panels to a backbone immune profiling assay enables deeper profiling of NK and T cells as well as analysis of cytokine responsiveness and cytotoxic potential.
- Important targets of immunotherapy such as PD-1, TIGIT, Tim-3, ICOS and 4-1BB could be quantified.
- The use of optimized and reproducible assays is critical in clinical and translational studies to ensure data variability is minimized and results are reliable.

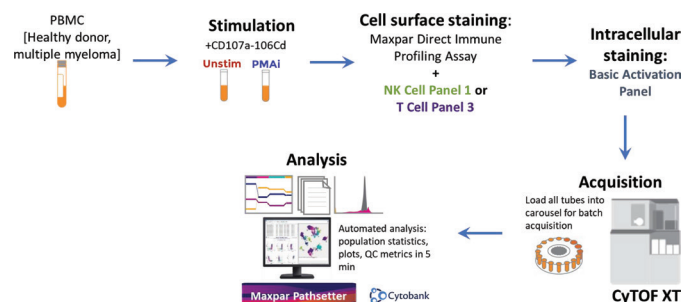
### Background

Interrogating immune cell composition and function in patients with cancer is crucial for making disease prognoses, monitoring efficacy of immunotherapies and identifying novel therapeutic targets.

Cellular and antibody-mediated immunotherapeutic approaches, including CAR T cells and monoclonal antibodies, have been developed to treat multiple myeloma. Since NK and T cells can also indirectly impact CAR T cell or antibody-based immunotherapies, characterizing these cells using optimized and reproducible assays is critical.

### Study Design

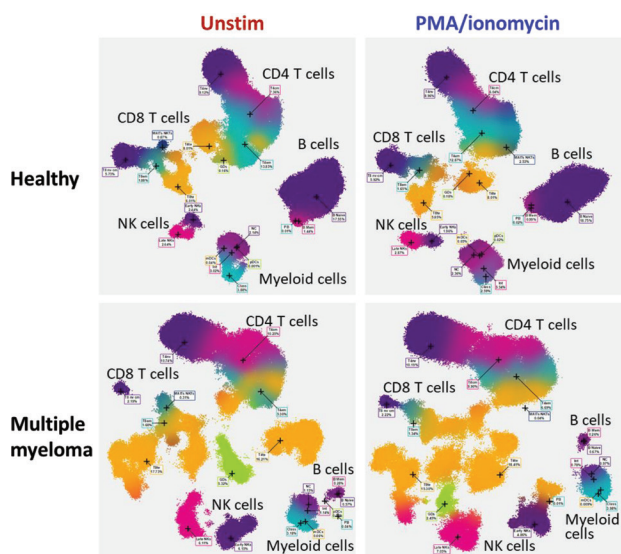
A validated 30-marker Maxpar® Direct™ Immune Profiling Assay™ for resolving 37 immune populations was expanded to two 43-marker panels by combining either the cell-surface T Cell Panel 3 or NK Cell Panel 1 with the intracellular Basic Activation Panel.



Peripheral blood mononuclear cells (PBMC) from healthy donor and multiple myeloma were stimulated, stained and acquired on a CyTOF® XT™ instrument. Normalized FCS files were analyzed with Maxpar Pathsetter™ software and Cytobank.

### Results

- NK and T cell differentiation correlated with markers of degranulation, activation and exhaustion.
- Selective response of effector and memory CD4 and CD8 T cells was shown in response to stimuli in healthy donor and multiple myeloma PBMC.
- Detection of malignant plasma cells demonstrates that disease-specific processes can be identified and further characterized.



Pathsetter automatically identifies and enumerates 37 populations of immune cells in peripheral blood leukocytes stained with the Maxpar Direct Immune Profiling Assay. A Cen-se™ (variant of t-SNE) plot from automated Pathsetter analysis of healthy donor and multiple myeloma patient PBMC showing distribution and frequencies of major immune cell populations.

## PROTOCOL

# Cancer Immune Cell Profiling

### Panel Information

Maxpar Direct Immune Profiling Assay						
30-marker single-tube backbone panel	CD45	CD8	CD161	CD183/CXCR3	TCR $\gamma$ 6	CD20
	CD196/CCR6	CD11c	CD194/CCR4	CD185/CSCR5	CD294	CD66b
	CD123/IR-3R	CD16	CD25	CD28	CD197/CCR7	HLA-DR
	CD19	CD45RO	CD27	CD38	CD14	IgD
	CD4	CD45RA	CD57	CD56	CD3	CD127

NK Cell Expansion Panel 1						
7-marker Expansion Panel	CD181	NKp30	NKp46	NKG2A	ICOS	TIGIT
	PD-1					

T Cell Expansion Panel 3						
7-marker Expansion Panel	OX40	TIGIT	CD69	PD-1	Tim-3	ICOS
	4-1BB					

Basic Activation Expansion Panel						
6-marker Expansion Panel	IL-2	TNF $\alpha$	IFN $\gamma$	CD107a	Perforin	Granzyme B

### Ordering Information

Product Name	Product Number	Product Name	Product Number
Maxpar® Direct™ Immune Profiling Assay™	201325	Maxpar Direct NK Cell Expansion Panel 1	201404
Maxpar Pathsetter™ v3.0	401019	Maxpar Direct T Cell Expansion Panel 3	201407
		Maxpar Direct Basic Activation Panel	201408



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