Accelerating Immuno-Oncology Research with Flexible Workflows Using Modular Flex-Fit CyTOF Panels

Michael Cohen, Ling Wang, Shakir Hasan, Stephen Li, Lauren Tracey, Christina Loh

CYTO 2025

Introduction

Deep functional profiling of immuno-oncology markers and cytokines in whole blood (WB) reveals immune signatures that inform disease prognoses, immunotherapy selection and outcomes in cancer patients. WB is best analyzed within 24 hours of collection to capture clinically relevant immune signatures; however, WB collection and cytometric analysis are often performed at different sites, which can lead to significant delays. WB stabilizers can overcome this challenge, yet not all antibody panels are compatible with these reagents. To enable flexible experimental workflows for deep functional profiling in WB, we developed modular Flex-Fit CyTOF™ panels compatible with stabilizers.

High-parameter flow cytometry mediated by CyTOF technology enables single-tube staining to identify 50-plus markers at the single-cell level. CyTOF technology provides exceptional resolution without spectral unmixing or compensation, while Flex-Fit™ CyTOF panels allow simple and quick panel design by combining a customizable selection of pre-validated subpanels. Importantly, CyTOF technology demonstrates superior detection and signal resolution of intracellular markers compared with full spectrum flow cytometry (see Oral Presentation titled "Novel functional diversity of human T cells is revealed through the unprecedented resolution of intracellular cytokines, transcription factors and phosphoproteins using mass cytometry (CyTOF)") and thus is ideally suited for measuring immune functionality.

In this study, we analyze two high-parameter panels across two workflows, fresh WB staining and PROT1-stabilized WB staining, using frozen antibody cocktails, and demonstrate that deep functional profiling can be achieved with either workflow.

Frozen antibody cocktails | **Modular** panels | **Flexible** staining workflows | **Deep** functional profiling

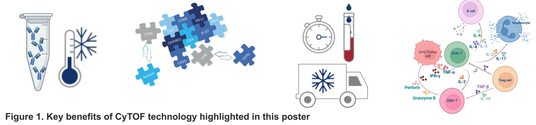

Figure 1. Key benefits of CyTOF technology highlighted in this poster

Methods and materials

Figure 2. Methods overview. Antibody cocktails were premixed and frozen until the time of staining to reduce experimental variability. To assess expression of inducible markers, fresh whole blood was stimulated with either (1) phytohaemagglutinin (PHA) and stained with the Immuno-Oncology Panel (Table 1), or (2) phorbol 12-myristate 13-acetate (PMA) and ionomycin (PMA/I) and stained with the Cytokine Panel (Table 2). Two donors were used for each condition. Refer to the QR codes (bottom of poster) for more information on antibody cocktail freezing and Flex-Fit modular panels.

	Flex-Fit Panel	Markers	Cell Types and Functional Insights	Part Number
Immuno-Oncology Panel 21 markers	TBMNK+G	CD45, CD56, CD3, CD19, CD14, CD16, CD66b, CD4, CD8	Immune cell profiling: granulocytes, B cells, natural killer cells (mature, immature), monocytes (classical and transitional), CD4+ T cells, CD8+ T cells, double negative T cells	201338
	Immune Checkpoint Core	PD-1, PD-L1, CTLA-4, CD40L, CD95, TIM-3*, TIGIT, OX40, 4-1BB	Immune checkpoint markers that play essential roles in the regulation of T cell receptors	201341
	Immune Checkpoint Expansion 2	DNAM-1, GITR, ICOS	Immune checkpoint molecules that exert stimulatory effects	201343
	Drop-in antibodies	CD244	Immune checkpoint molecules that exert inhibitory effects	3165046
	* TIM-3 was not included in this experiment because it is not compatible with PROT1.			

Table 1. The 21-marker Immuno-Oncology Panel, built from ready-to-use, pre-validated Flex-Fit modules plus one drop-in antibody

	Flex-Fit Panel	Markers	Cell Types and Functional Insights	Part Number
Cytokine Panel 30 markers	Human Broad Immune Profiling	CD45, CD56, CD3, CD19, CD14, CD16, CD66b, CD4, CD8, CD45RA, CD45RO, CD27, CD28, CD38, CD161, CD20, CD123, CD11c, HLA-DR, CD49d	Immune cell profiling: granulocytes, B cells (naive, memory, plasmablasts), natural killer cells (mature, immature), monocytes (classical, transitional), CD4+/CD8+ T cell subsets, double negative T cells, dendritic cells (plasmacytoid, conventional)	201339
	Cytokine Core	IL-2, IL-4, TNFα, IFNγ, IL-17A	Th1, Th2, Th17 cells; T helper cell cytokines with important implications in immunopathologies (autoimmune diseases, cancer)	201344
	Cytokine Expansion	IL-5*, IL-10, IL-21	Th2, Treg, T follicular helper cells (Tfh); immunomodulatory cytokines	201346
	Cytotoxic Mediators	IL-6, perforin, granzyme B	Cytotoxic lymphocytes; perforin and granzyme B are components of cytotoxic granules; IL-6 is a pleiotropic cytokine	201345
	* IL-5 was not included in this experiment because it was not compatible with PROT1.			

Table 2. The 30-marker Cytokine Panel, built from ready-to-use, pre-validated Flex-Fit modules, identifies >30 cell populations.

Key takeaways

- CyTOF systems **enable unique workflows** to maximize **flexibility in panel design** (Flex-Fit panels) and experimental procedures (frozen cocktails and fixative-stabilized WB) allowing **faster time to insights**
- High-parameter antibody panels using flexible workflows **enable deep functional profiling** of immune cells by identifying immuno-oncology markers and cytokines
- CyTOF offers **superior detection and signal resolution** of intracellular markers; thus, this technology is ideal for **clinical studies** to identify **functional immune signatures** that inform disease prognoses, immunotherapy selection and outcomes

Results

Fresh WB staining workflow

Immuno-oncology markers are easily identified in PHA-stimulated fresh WB

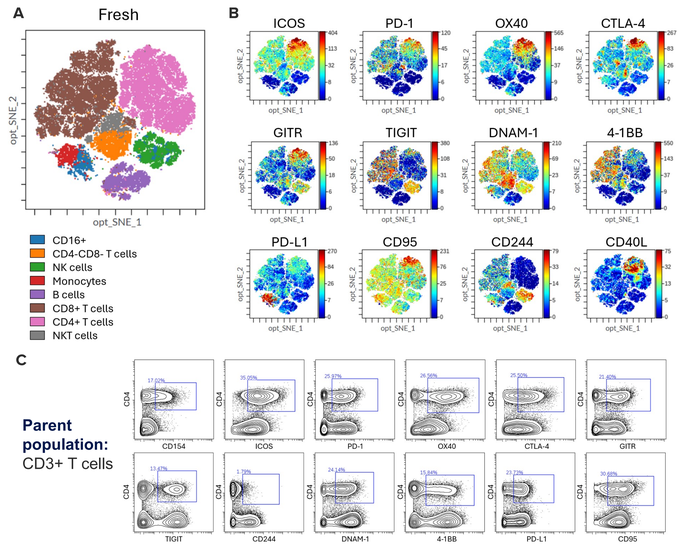

Figure 3. CyTOF analysis of the Immuno-Oncology Panel using the fresh WB staining workflow identifies cell type-specific expression patterns of IO markers.

A) opt-SNE, a dimensionality reduction tool, was applied to CD45+ cells and plotted. Overlaid colors correspond to gated cell populations. 60,000 CD45+ events were proportionally sampled across two donors, and one representative donor is shown in all plots. Clear discrimination of lymphocyte subsets is observed.

B) opt-SNE plots overlaid with signal intensity levels of the marker indicated above each plot. The population legend in Figure 3A can be used to track expression of individual markers across cells. For example, ICOS is most highly expressed in CD4+ T cells (pink population from Figure 3A), and its expression overlaps with PD-1, PD-L1 and CTLA-4, among others, demonstrating an exhaustive state.

C) Biaxial plots showing CD3+ T cells. The Y-axis shows CD4 expression, and the X-axes show expression of 10 functional markers included in the Immuno-Oncology Panel. CD4+ and CD4– subsets are clearly delineated for each IO marker shown.

Cytokines are readily detected in PMA/I-stimulated fresh WB

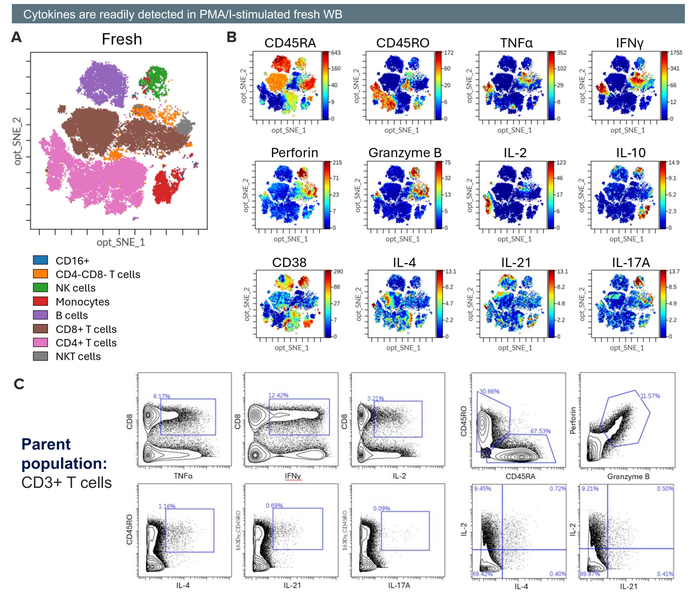

Figure 4. CyTOF analysis of the Cytokine Panel using the fresh WB staining workflow shows expression of nine cytokines across major immune populations.

A) opt-SNE was applied to CD45+ cells and plotted. Overlaid colors correspond to gated cell populations. 50,000 CD45+ events were proportionally sampled across two donors, and one representative donor is shown in all plots. Clear discrimination of lymphocyte subsets is observed.

B) opt-SNE plots overlaid with signal intensity levels of the marker indicated above each plot. The population legend in Figure 4A can be used to track expression of individual markers across cells, as well as co-expressed cytokines. For example, perforin, granzyme B, CD38, IL-17A and IL-10 are co-expressed in NK cells (green population from Figure 4A), informing functional dynamics of NK cells.

C) Biaxial plots showing CD3+ T cells. Cytokine expression in various T cell subsets are highlighted, including rare IL-17A+ Th17 cells, IL-2+IL-4+ cells and IL-2+IL-21+ cells. CD45RO and CD45RA expression is mutually exclusive in T cells and can be used to identify effector T cell subsets. Co-expression of perforin and granzyme B identifies effector T cells with cytotoxic granules.

PROT1-stabilized WB staining workflow

Expression of immuno-oncology markers is robust in PROT1-stabilized, PHA-stimulated WB

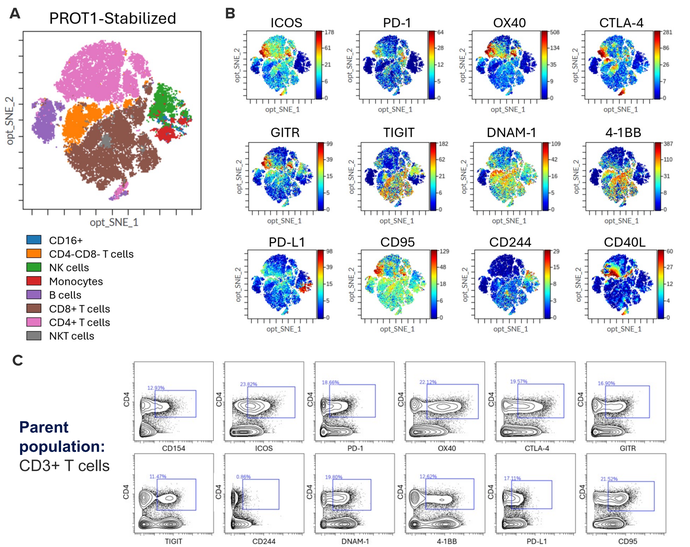

Figure 5. CyTOF analysis of the Immuno-Oncology Panel using the PROT1-stabilized WB staining workflow demonstrates robust expression of IO markers.

A) opt-SNE was applied to CD45+ cells and plotted. Overlaid colors correspond to gated cell populations. 50,000 CD45+ events were proportionally sampled across two donors, and one representative donor is shown in all plots. Clear discrimination of lymphocyte subsets is observed in stabilized WB samples.

B) opt-SNE plots overlaid with signal intensity levels of the marker indicated above each plot. The population legend in Figure 5A can be used to track expression of individual markers across cells. For example, TIGIT is most highly expressed in CD8+ T cells (brown population from Figure 5A), and its expression overlaps with DNAM-1, 4-1BB and CD95, highlighting the dynamic regulation of these IO markers.

C) Biaxial plots showing CD3+ T cells. The Y-axis shows CD4 expression, and the X-axes show expression of 10 functional markers included in the Immuno-Oncology Panel. CD4+ and CD4– subsets are clearly delineated for each IO marker shown in stabilized WB.

Cytokine expression signatures are captured in PROT1-stabilized, PMA/I-stimulated WB

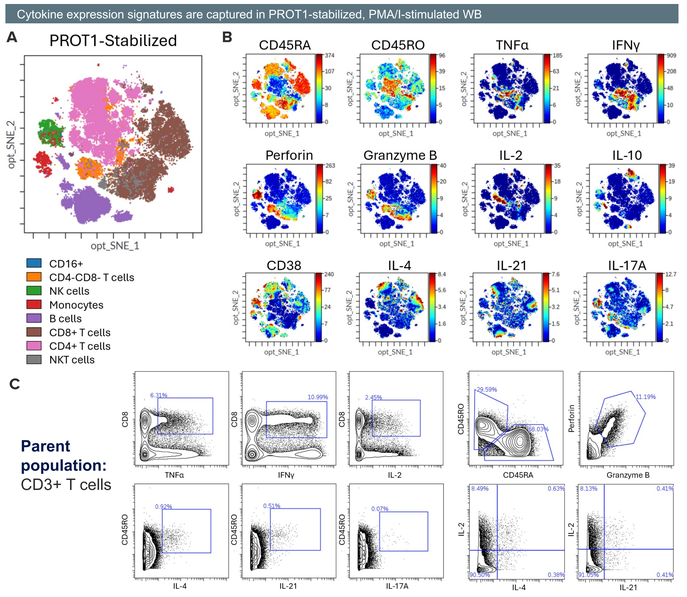

Figure 6. CyTOF analysis of the Cytokine Panel using the PROT1-stabilized WB staining workflow shows expression of nine cytokines across major immune populations.

A) opt-SNE was applied to CD45+ cells and plotted. Overlaid colors correspond to gated cell populations. 50,000 CD45+ events were proportionally sampled across two donors, and one representative donor is shown in all plots. Clear discrimination of lymphocyte subsets is observed.

B) opt-SNE plots overlaid with signal intensity levels of the marker indicated above each plot. The population legend in Figure 6A can be used to track expression of individual markers across cells, as well as co-expressed cytokines. For example, IL-2, CD45RO and TNFα are co-expressed in CD4+ T cells (pink population from Figure 6A), revealing an activated memory T cell population.

C) Biaxial plots showing CD3+ T cells. Cytokine expression in various T cell subsets are highlighted, including rare IL-17A+ Th17 cells, IL-2+IL-4+ cells and IL-2+IL-21+ cells. CD45RO and CD45RA expression is mutually exclusive in T cells and can be used to identify effector T cell subsets. Co-expression of perforin and granzyme B identifies effector T cells with cytotoxic granules. All functional markers are reliably detected in stabilized WB.

Standard BioTools Inc.
2 Tower Place, Suite 2000, South San Francisco, CA 94080 USA
+1 650 266 6000 • Toll-free in the US and Canada: 866 359 4354
standardbio.com

Flex-Fit CyTOF panels flyer

Antibody cocktail freezing poster

Download this poster