

Flexible and Comprehensive Phenotyping of Tumor and Peripheral Blood Mononuclear Cells in **Endometrial Carcinoma**

27 samples

10 individuals

0.3M–2M cells/sample

Connie Inlay, Deeqa Mahamed, Geneve Awong, Jennifer Frahm Standard BioTools Inc., Markham, ON, Canada

Introduction

Cancer biomarkers have revolutionized management and treatment of the disease, leading to remarkable advancements in personalizing medicine and determining optimal therapeutic combinations. However, researchers face significant challenges in identifying biomarkers in the context of extreme biological heterogeneity, a common feature of many cancers. While immune profiling of peripheral blood is a common approach, many critical disease processes are only evident within the tumor environment. These processes can provide valuable prognostic and diagnostic insights or reveal therapeutic targets that are not detectable in the peripheral immune system.

Endometrial carcinoma (EC) is the most prevalent form of uterine cancer, with its incidence rising in developed countries due to factors such as population aging and increasing obesity rates. Despite a relatively favorable prognosis, with an 80% survival rate at 5 years post-diagnosis, the primary curative treatment remains the total removal of the uterus, ovaries and fallopian tubes. This underscores the need for identifying biomarkers that can lead to more personalized medicine approaches and the development of immunotherapies, particularly for aggressive EC subtypes.

Mass cytometry is a high-plex proteomic technology that simultaneously resolves phenotypic and functional markers, enabling researchers to implement large-scale immunophenotyping strategies that span biological heterogeneity and sample types. A combination of immune and functional profiling is key to elucidating disease mechanisms and revealing predictive biomarkers. Mass cytometry conducted on the CyTOF[™] XT PRO system uniquely enables higher-parameter, precise immunophenotyping at greater throughput without the data artifacts introduced by compensation and spectral deconvolution. The ability to easily and rapidly design and modify 50-plus-marker panels, along with flexible sample staining and acquisition workflows and the use of sample multiplexing, makes mass cytometry the premier choice for large and complex clinical studies and drug discovery programs.

Objective

To provide a means to achieve deep phenotyping and functional characterization of multiple clinical samples in a single tube by leveraging modular, ready-to-use Flex-Fit[™] panels and the enhanced throughput of the CyTOF XT PRO system. By showcasing characterization of both immune and non-immune cells in tumor tissue and PBMC, this method highlights a rapid, high-plex workflow that provides valuable insights for cancer research and potential therapeutic targets from minimal sample amounts.

- Key advantages of mass cytometry on the CyTOF XT PRO system for clinical research
- Capturing phenotypic and functional variation in a single CyTOF panel generates unique biomarkers that can reveal mechanisms of disease activity, drug response and prognostic potential
- Pre-optimized modular panels combined with the enhanced throughput of the CyTOF XT PRO system enables fast implementation of large-scale immune profiling studies
- Sample multiplexing using a variety of barcoding reagents (Pd, CD45, TeMal) is a powerful method to harmonize sample sets, reduce batch effects, and improve standardization in multi-site and longitudinal studies
- The CyTOF XT PRO system addresses regulatory requirements with **21 CFR Part 11 compliance-enabling software** ensuring user management, user audit trails and integrity of output files

Materials and methods

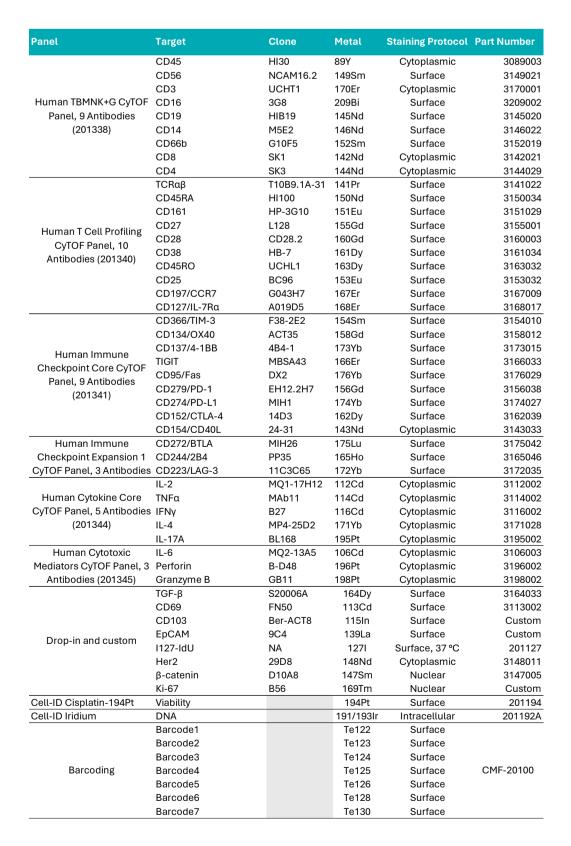


 Table 1. CyTOF panel of 57 parameters
composed of 47 antibodies, viability, DNA and barcoding reagents

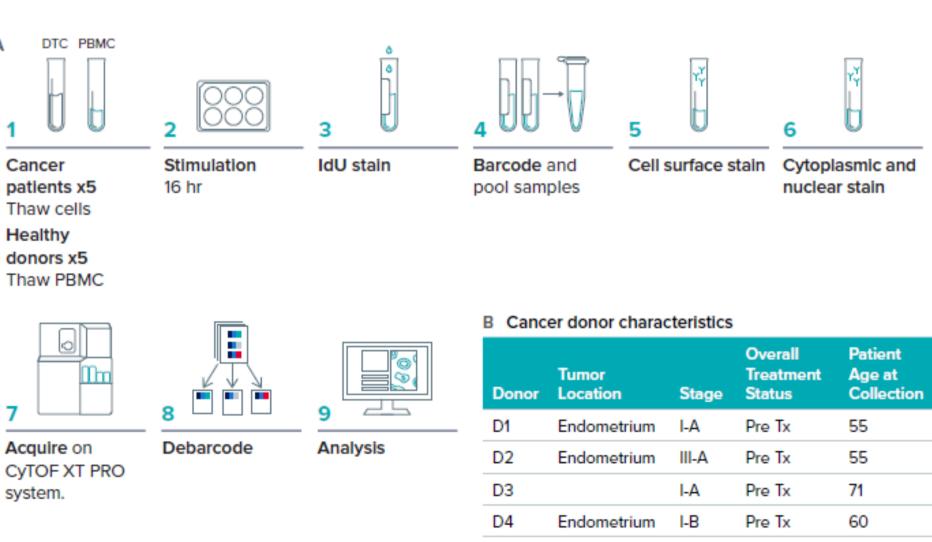


Figure 1. A) Sample stimulation, barcoding and staining workflow. B) Summary of the patient characteristics (age, tumor stage, tumor location if known) of the five sample donors Sample stimulation and staining. Matched PBMC and dissociated tumor cells (DTCs) from EC patients and healthy donor PBMC (n=5 each) were purchased from Discovery Life Sciences. Cells were stimulated with 25 ng/mL PMA and 500 ng/mL ionomycin in complete RPMI in the presence of brefeldin A and monensin (BioLegend) for 16 hours at 37 °C in a humidified incubator with 5% CO2. After labeling with 25 µM Cell-ID[™] 127 IdU for 15 minutes at 37 °C, samples were harvested before live-cell universal barcoding using a tellurium-based 7-choose-3 strategy. The pooled and barcoded samples were then stained for viability, surface, cytoplasmic and nuclear targets before labeling with 15.6 nM Cell-ID Intercalator (iridium), followed by washing in Maxpar[™] Cell Staining Buffer and Maxpar Cell Acquisition Solution (CAS) Plus according to the Maxpar Cell Staining with Fresh Fix User Guide (FLDM-01319).

Data acquisition and analysis. Samples were acquired on a CyTOF XT PRO instrument with a redesigned injector capable of increased throughput at >500 events/second, allowing for rapid acquisition of the barcoded sample after resuspension in Maxpar CAS Plus and addition of EQ[™] Six Element Calibration Beads. Data normalization and debarcoding were performed to obtain sample-specific cell frequencies and marker expression profiles using Cytobank (Beckman Coulter) or CellEngine (CellCarta). Dimensionality reduction using an opt-SNE algorithm in Cytobank was performed for samples with at least 10,000 live singlets. Heat maps and correlation matrices were generated with Morpheus (Broad Institute).

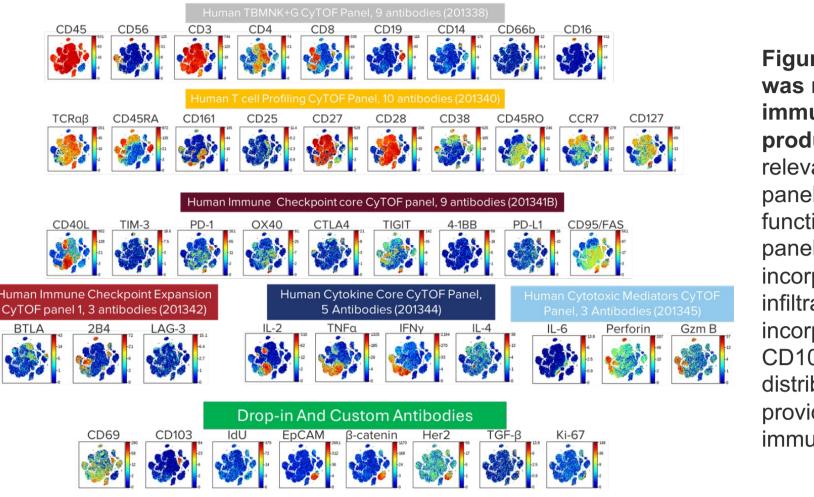
Standard BioTools Inc.

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

	Stage	Overall Treatment Status	Patient Age at Collection
m	I-A	Pre Tx	55
m	III-A	Pre Tx	55
	I-A	Pre Tx	71
m	I-B	Pre Tx	60
	1	Pre Tx	85



A 47-marker CyTOF panel for broad immune cell phenotyping, deep T cell profiling, cytokine production and cell proliferation



Tellurium-based live-cell barcoding for sample multiplexing of immune and nonimmune cells enables fast sample processing and reduces batch effects

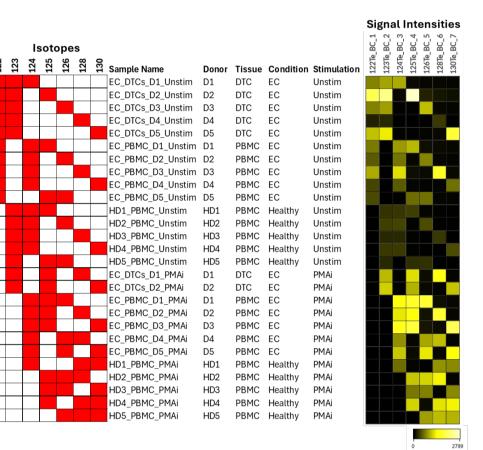


Figure 3. Cell type-agnostic barcoding to reduce technical variation and batch effects across distinct sample types. Tellurium isotopologues (TeMal) were used to multiplex 27 samples using a 7-choose-3 barcode scheme, reducing on time and ensuring consistent and comparable data across sample types. The samples were then pooled and stained in a single tube for surface, intracellular and nuclear targets before acquisition on a CyTOF XT PRO instrument. After acquisition, the multiplexed sample was debarcoded using CyTOF Software v9.2 with a barcode separation threshold of 0.3 and a Mahalanobis distance of 10 (representing no Mahalanobis filtering). About 15% of events were unassigned, including EQ6 Beads.

Cell composition and functional heterogeneity in PBMC and tumor-derived cells from endometrial cancer patients

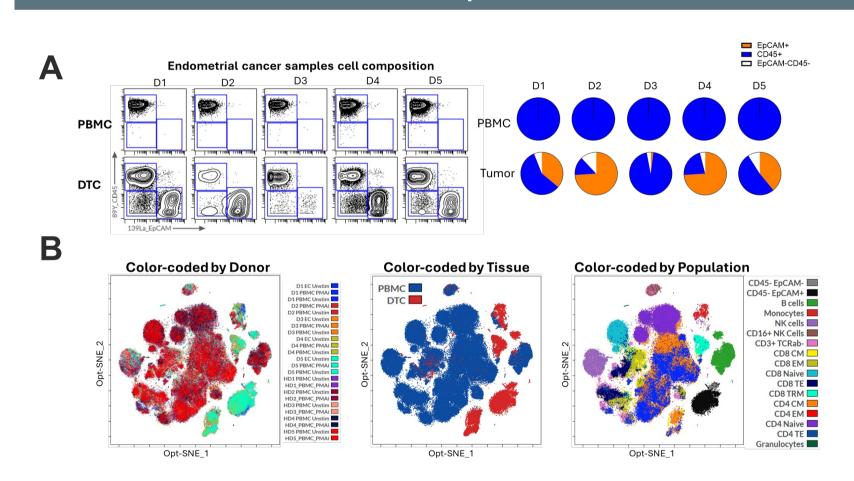
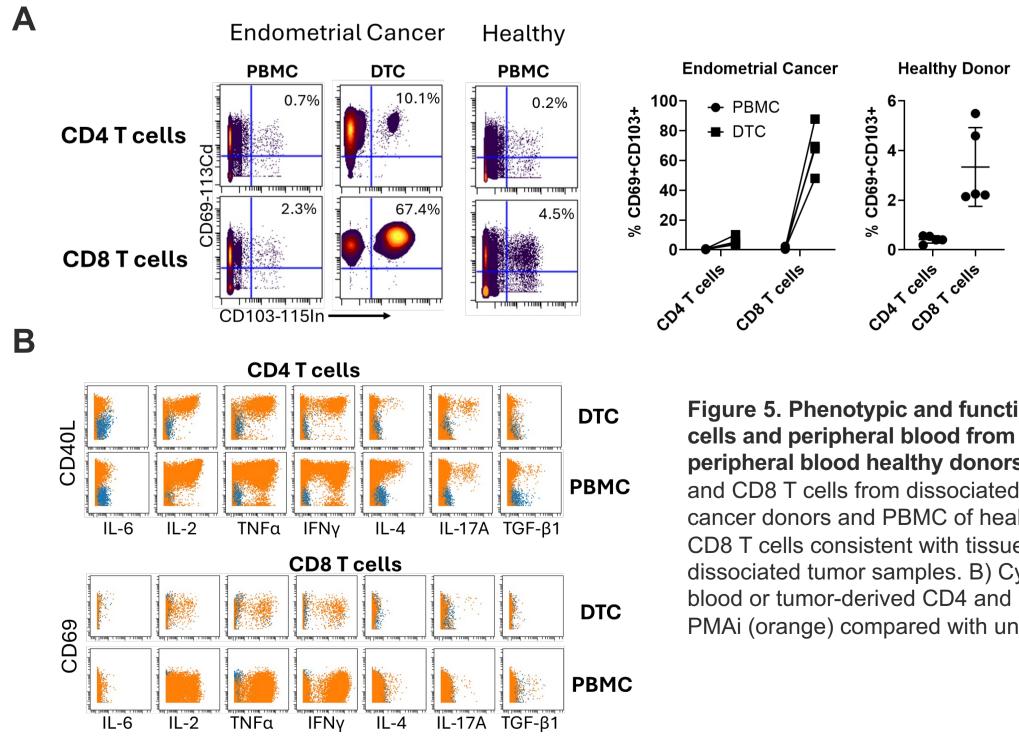


Figure 4. Cell composition and functional heterogeneity in PBMC and tumor-derived cells from endometrial cancer patients. A) Cell composition in PBMC and dissociated tumor cell (DTC) samples from five endometrial cancer patients (D1–D5). B) Cells projected onto opt-SNE plots and colored based on donor identity, tissue type or gated population identity for samples with at least 10,000 live singlets. t-SNE plots were generated from 10,000 events per sample using all the Flex-Fit panels and drop-in antibodies (47 markers). C) Expression of EpCAM, βcatenin, HER2, PD-L1, CD95/Fas, IdU and Ki-67 in unstimulated dissociated tumor cells in the five EC patients.



For Research Use Only. Not for use in diagnostic procedures.

Patent and License Information: www.standardbio.com/legal/notices. Trademarks: www.standardbio.com/legal/trademarks. Any other trademarks are the sole property of their respective owners. ©2025 Standard BioTools Inc. All rights reserved.

Figure 2. A comprehensive CyTOF panel measuring 47 markers was rapidly designed by combining pre-optimized modules for immune cell phenotyping, T cell profiling and cytokine production and cytotoxicity detection. Eight additional antibodies relevant to endometrial cancer were integrated, creating a tailored panel that reveals immune and non-immune cell phenotypes functions and interactions with minimal optimization effort. This panel expansion included assessment of cell proliferation using IdU incorporation and Ki-67 staining. Additionally, tumor cells and tumorinfiltrating leukocytes (TILs) were further characterized by incorporating the key markers EpCAM, HER2, β -catenin, CD69, CD103, PD-L1, and TGF-β1. In combination with the 39 markers distributed across six predefined panels, these add-on markers provide a highly customized panel for probing immune cell and nonimmune cell phenotypes, functions and potential interactions.

- Two stimulation conditions
- Two tissue types

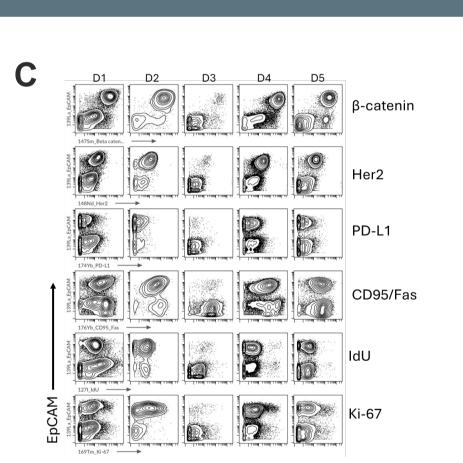
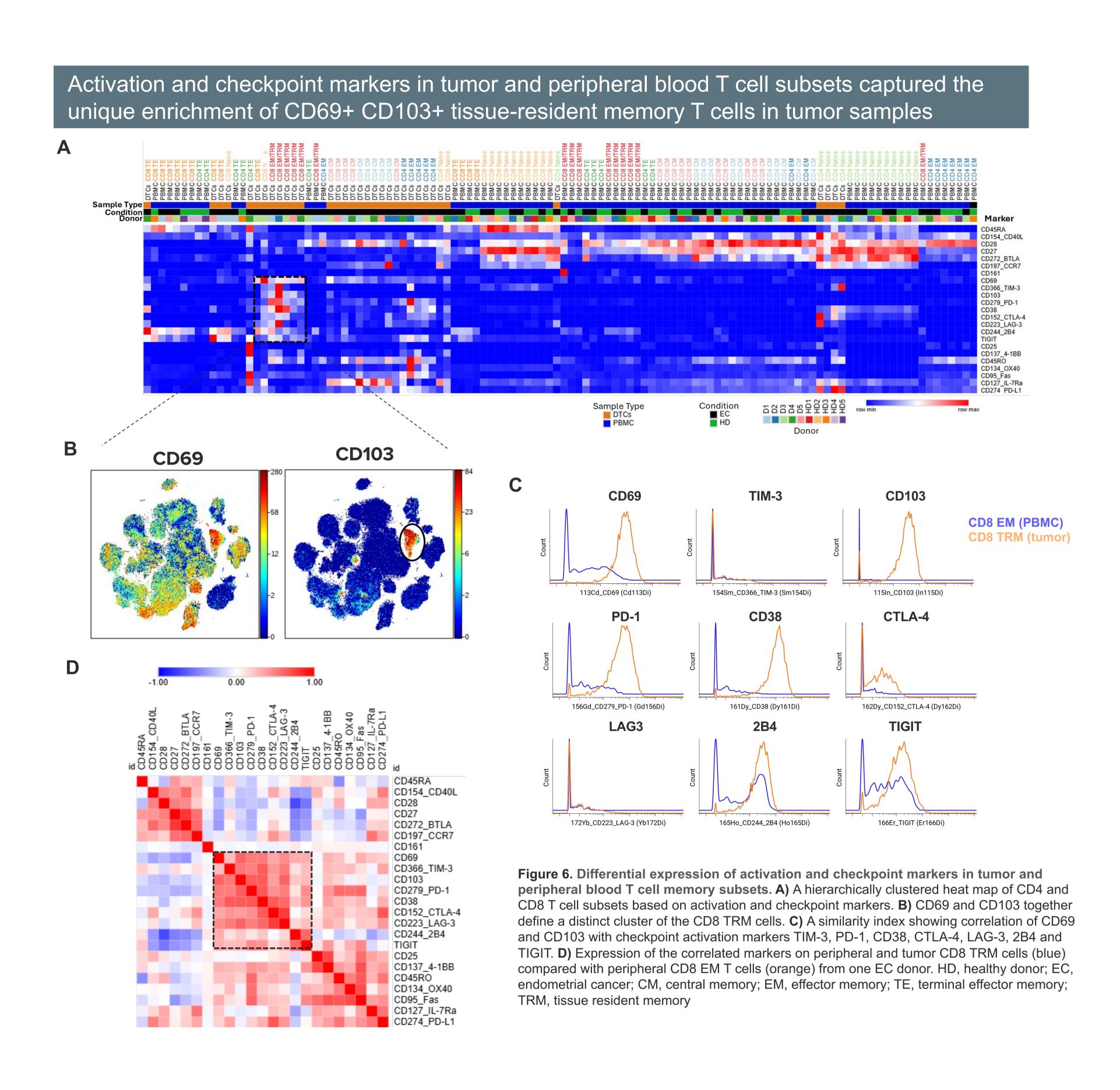


Figure 5. Phenotypic and functional profiling of T cells in tumor-derived cells and peripheral blood from endometrial cancer donors and in peripheral blood healthy donors. A) CD69 and CD103 expression on CD4 and CD8 T cells from dissociated tumor cells (DTC) and PBMC of endometrial cancer donors and PBMC of healthy donors. An increase in CD69+CD103+ CD8 T cells consistent with tissue residency phenotype is observed only in the dissociated tumor samples. B) Cytokine response in EC donor peripheral blood or tumor-derived CD4 and CD8 T cells after *in vitro* stimulation with PMAi (orange) compared with unstimulated samples (blue).



Conclusions

CYTO 2025

• This panel enables cell composition analysis of immune and non-immune cells including frequencies of T cells, B cells, NK cells, granulocytes, monocytes and epithelial cells

• By using modular and flexible panel design, deeper characterization of T cells and NK cells (memory, exhaustion, response to stimuli, proliferation) revealed disease- or tissue-specific processes

• This approach can be used for tracking the homing, activation and functional responsiveness of engineered cell therapy products such as TILs and CAR T cells at the tumor site

• The response to other immune-directed therapies such as checkpoint inhibitors, antibody-drug conjugates, cancer vaccines or bispecific T cell engagers can be monitored

• Tellurium-based barcoded enabled sample-sparing multiplexed staining and acquisition to reduce batch effects and streamline the sample preparation, staining and acquisition workflow

1. Lheureux, S. et al. "Translational randomized phase II trial of cabozantinib in combination with nivolumab in advanced, recurrent, or metastatic endometrial cancer." The Journal for ImmunoTherapy of Cancer

2. Anderson, A.C. et al. "Lag-3, Tim-3, and TIGIT: Co-inhibitory receptors with specialized functions in immune regulation." *Immunity* 44 (2016): 989–1,004.

3. Tymon-Rosario, J.R. et al. "Targeted therapies in the treatment of uterine serous carcinoma." Current Treatment Options in Oncology 23 (2022): 1,804–1,817. 4. Liu, Y. et al. "Understanding the versatile roles and applications of EpCAM in cancers: From bench to bedside." *Experimental Hematology & Oncology* 11 (2022): 97.

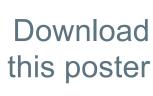
5. Luke, J.J. et al. "WNT/β-catenin pathway activation correlates with immune exclusion across human cancers." *Clinical Cancer Research* 25 (2019): 3,074–3,083.

6. Kumar, B.V. et al. "Human tissue-resident memory T cells are defined by core transcriptional and functional signatures in lymphoid and mucosal sites." Cell Reports 20 (2017): 2,921–2,934.

8. Duhen, T. et al. "Co-expression of CD39 and CD103 identifies tumor-reactive CD8 T cells in human solid tumors." *Nature Communications* 9 (2018): 2724.

9. Accelerating Immuno-Oncology Research by Profiling Cell Function with Flex-Fit CyTOF Panels Application Note (FLDM-01375)







References

^{10 (2022):} e004233.

^{6.} Willis, L.M. et al. "Tellurium-based mass cytometry barcode for live and fixed cells." *Cytometry Part* A 93 (2018): 685–694.

^{10.} Maxpar Cell Staining with Fresh Fix User Guide (FLDM-01319)