APPLICATION NOTE

Scale Immunotherapy Clinical Research with Rapid, High-Plex Mass Cytometry: Endometrial Carcinoma Tumor and PBMC Profiling Using the CyTOF XT PRO System

Introduction

Cancer biomarkers have revolutionized management and treatment of the disease, leading to remarkable advancements in personalized medicine and determining optimal therapeutic combinations. However, researchers face significant challenges in identifying biomarkers in the midst 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.

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 immunophenotyping at greater speeds 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.

Key advantages of mass cytometry on the CyTOF XT PRO system for clinical research

- Pre-optimized, modular panels combined with the CyTOF XT PRO system's enhanced throughput mode enables fast implementation of large-scale immune profiling studies
- Capturing phenotypic and functional biological variation in a single CyTOF panel generates unique biomarkers to reveal mechanisms of disease activity, drug response and outcome prediction
- Advancements in downstream analysis of multiplexing using a variety of barcoding reagents (Pd, CD45, TeMal), which is a powerful method that harmonizes sample sets, reduces batch effects and improves 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

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[1].

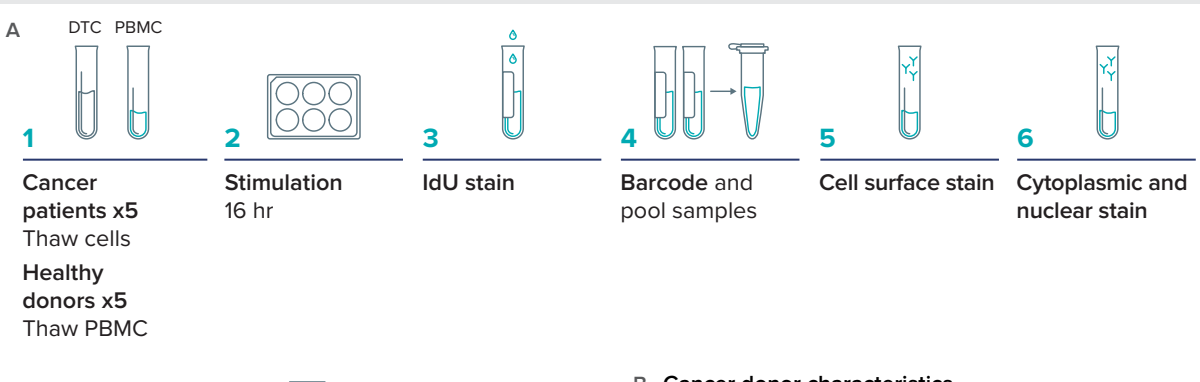
Objective

By leveraging modular, ready-to-use Flex-Fit™ panels and the enhanced throughput of the CyTOF XT PRO system, we showcase a means to achieve deep phenotyping and functional characterization in a single tube. Using both immune and non-immune cells in tumor tissue and peripheral blood mononuclear cells (PBMC), this application highlights a rapid, high-plex workflow that provides valuable insights for cancer research and potential therapeutic targets from minimal sample amounts.

Study design

A comprehensive CyTOF panel measuring 47 markers was rapidly designed by combining pre-optimized modules for immune cell phenotyping, T cell profiling and cytokine/cytotoxicity detection (Appendix A). Eight additional antibodies relevant to endometrial cancer were seamlessly integrated, creating a customized panel that thoroughly examines 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 tumor-infiltrating leukocytes (TILs) were further characterized by incorporating markers such as EpCAM, Her2, β-catenin, CD69, CD103, PD-L1, FoxP3 and TGF-β1. These markers play significant roles in the progression, diagnosis, immune response and/or treatment of EC and many other cancers (Box 1). In combination with the 39 markers distributed across 6 predefined panels, these add-on markers provide a highly customized panel for probing immune cell and non-immune cell phenotypes, functions and potential interactions.

A



B **Cancer donor characteristics**

Donor	Tumor Location	Stage	Overall Treatment Status	Patient Age at Collection
D1	Endometrium	I-A	Pre Tx	55
D2	Endometrium	III-A	Pre Tx	55
D3		I-A	Pre Tx	71
D4	Endometrium	I-B	Pre Tx	60
D5		I	Pre Tx	85

Figure 1. Sample stimulation, barcoding and staining workflow. A) PBMC and DTCs collected prior to treatment from 5 EC patients were purchased from Discovery Life Sciences. Samples were stimulated with PMAi for 16 hr, then IdU-labeled, barcoded, pooled and stained for surface, cytoplasmic and nuclear markers. The pooled sample was acquired on a CyTOF XT PRO instrument at 500 events/sec, debarcoded using CyTOF Software and analyzed for sample-specific cell frequencies and marker expression using CellEngine and Cytobank. B) Summary of the patient characteristics (age, tumor stage, tumor location if known) of the 5 sample donors.

For these experiments, the use of tellurium maleimide (TeMal), a species and cell type agnostic barcoding reagent, was used to reduce technical variation and batch effects across 3 distinct sample types: healthy donor PBMC, EC PBMC and EC dissociated tumor cells. To test the panel, healthy donor and EC PBMC and EC dissociated tumor cells were stained *ex vivo* or following stimulation with phorbol-12-myristate 13-acetate (PMA) and ionomycin (PMAi). TeMal was used to multiplex live samples using a 7-choose-3 barcode scheme. Samples were then stained for surface, intracellular and nuclear targets before streamlined acquisition on a CyTOF XT PRO instrument, ensuring consistent and comparable data across sample types (Figure 1).

Highlight of results

Tellurium-based live-cell barcoding for sample multiplexing of immune and non-immune cells

Sample multiplexing provides a major advantage in mass cytometry, as it enables staining and/or acquisition of multiple samples in a single tube, reducing potential variability that can be introduced during sample processing. The choice of which barcode reagent and scheme to use depends on the sample type, species, number of samples and compatibility of the antibody panel with fixation. Human and mouse anti-CD45 antibodies can be used when immune cells are the cells of interest, while the Cell-ID™ 20-Plex Pd Barcoding Kit is ideal for fixation-compatible panels or following surface staining if the panel is fixation-sensitive. For heterogenous tissue samples in which a fixation-sensitive panel may be required, the tellurium-based TeMal 7 isotopes bundle is ideal, as it does not require cell fixation and labels all cell types[6]. TeMal was thus used here to enable barcoding and pooling of both tumor- and blood-derived cells (Appendix B). As post-thaw cell recovery of dissociated tumor cells (DTCs) was lower than expected, different starting numbers of cells (0.5M–2M) were barcoded, leading to variable signal intensities across the samples as projected. Nevertheless, barcoding was highly efficient, with over 85% of events assigned to samples out of the total 3.2 million events acquired. The unassigned events were attributed to cell-cell doublets and cell-bead doublets.

Distinct determination of immune and non-immune tumor sample cell composition

Leveraging the enhanced throughput of the CyTOF XT PRO system, several cell types, including endometrial dissociated tumor cells, PBMC from the same patient sample and healthy donor PBMC, were assessed and comprehensively analyzed. Dissociated tumor samples from EC patients had variable immune cell composition, while PBMC samples were >98% CD45+ as expected (Figure 2A). The majority of CD45– cells were EpCAM+, although 1–15% of cells were CD45–EpCAM–, likely composed of fibroblasts, endothelial cells, stem cells and cancer stem cells. Use of 47 antibodies from 6 pre-designed CyTOF panels along with several drop-in antibodies revealed distinct clustering by projecting events onto t-SNE dimensionality reduction plots. EC donors are largely clustered together, while major islands of cells are segregated based on tissue source and cell type (Figure 2B). This separation may reflect specific disease processes in the tumor microenvironment (TME) that can enrich for specific cell types based on expression of the functional and phenotypic markers in this panel.

Within the non-immune cell compartment, β-catenin, Her2 and CD95/Fas were expressed on most EpCAM+ cells, while PD-L1 showed variable expression (Figure 3). Proliferating cells were detectable at low frequencies based on IdU incorporation or Ki-67 expression, with higher Ki-67 expression observed. This is expected, as Ki-67 labels all cycling cells (G1, S and G2 but not G0 phases) in comparison to IdU, which is only incorporated into cells actively synthesizing DNA (S phase).

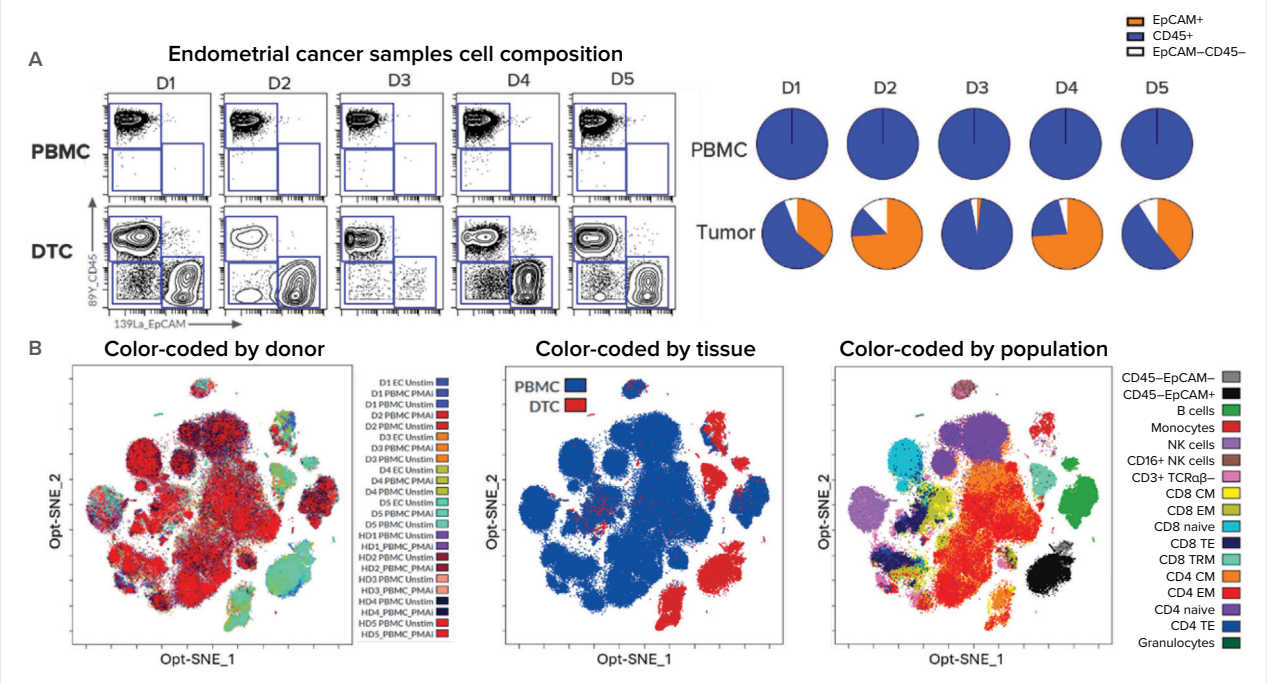


Figure 2. Cell composition and functional heterogeneity in PBMC and tumor-derived cells from EC patients. A) Cell composition in PBMC and dissociated tumor samples from 5 EC patients (D1–D5). B) Cells projected onto opt-SNE plots and colored based on donor identity, tissue type or gated population identity (Appendix C) 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).

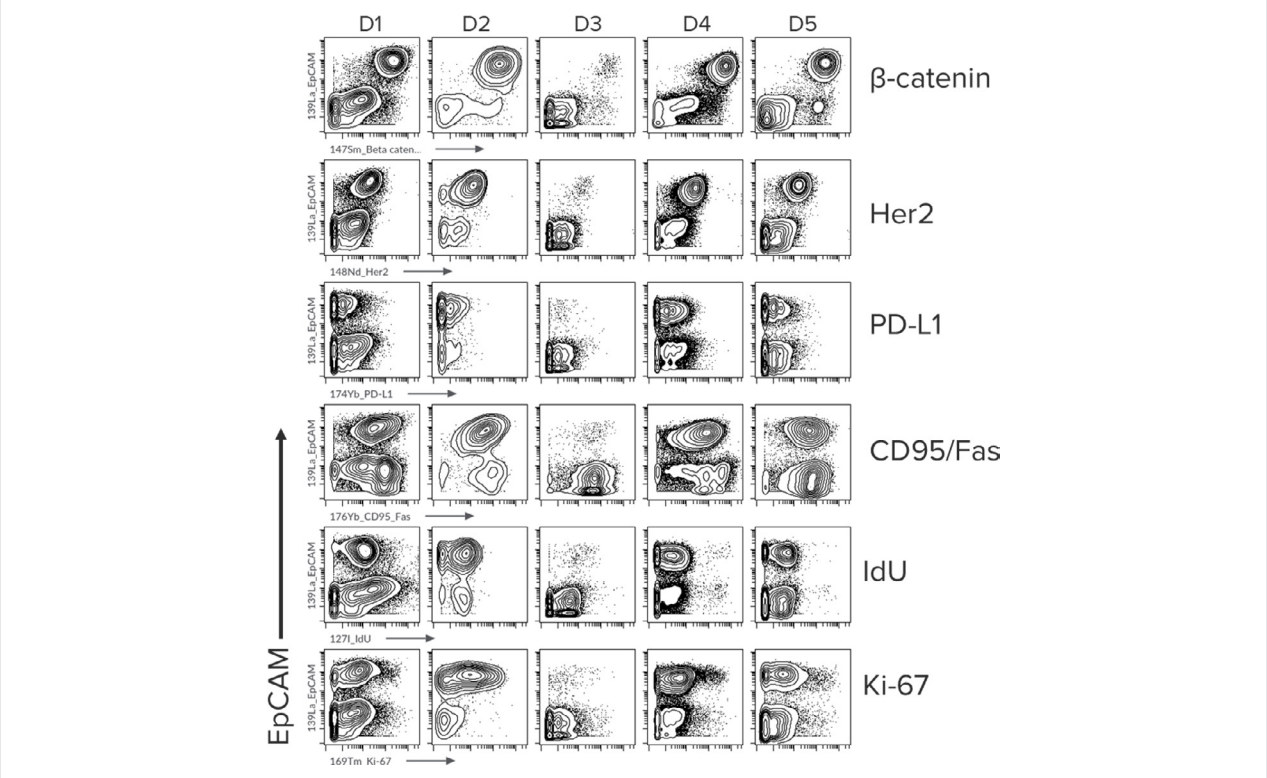


Figure 3. Expression of EpCAM, β-catenin, Her2, PD-L1, CD95/Fas, IdU and Ki-67 in unstimulated dissociated tumor sample cells in the 5 EC patients

The tumor-derived T cells from EC patients exhibited a tissue residency phenotype

Tissue-resident memory T cells (TRM cells) are a specialized subset of memory T cells that reside permanently in tissues rather than circulating through the bloodstream, distinguishing them from other T cell memory subsets such as naive, central memory T cells (TCM) and effector memory T cells (TEM). They play a crucial role in the immune response to infection, particularly in barrier tissues such as the skin and mucosa, by providing rapid and localized responses within the tissues they inhabit[7]. High levels of TRM cells have been linked to improved survival rates in patients with various types of cancer, including ovarian, lung and breast cancers. The expression of CD69 and CD103 is a defining characteristic of CD8 TRM cells[8], while on CD4 TRM CD69 and CD103 expression is more variable. Mass cytometry, with its ability to detect a large number of parameters at the single-cell level without spectral overlap, offers significant advantages in assessing TRM cells compared with fluorescence flow cytometry. In this study, the tumor-derived CD8 T cells from EC patients exhibited a tissue residency phenotype with 50–80% of CD8 T cells doubly positive for CD69 and CD103 compared with <5% in the peripheral blood (Figure 4A).

Clear detection of high- and low-frequency cytokines produced by tumor-derived T cells from EC patients

The high-parameter panel used in this experiment contained both surface markers and numerous intracellular cytokines assessed from a single tube. The functional capacity of T cells to respond to stimuli can be assessed by measuring cytokine responses to activation. PMAi activation of PBMC and DTCs led to IL-2, IFNγ, TNFα, IL-4, IL-17A and TGF-β production in CD4 T cells, while CD8 T cells produced IL-2, TNFα and IFNγ (Figure 4B). The ability to detect and clearly resolve many cytokines from a single tube is significant, as this is challenging to achieve with fluorescence flow cytometry and is a unique capability of CyTOF technology. Not only was CyTOF technology able to detect high-frequency cytokines, but low-frequency cytokines in this study, like IL-17 and TGF-β, were also clearly detected alongside the others in a single tube. Given the importance of these cytokines in shaping the immune response in the TME, identifying which cytokines are induced in response to stimuli can inform design of immunotherapy drugs and allow patient stratification based on likelihood of response to existing and novel immunotherapy targets.

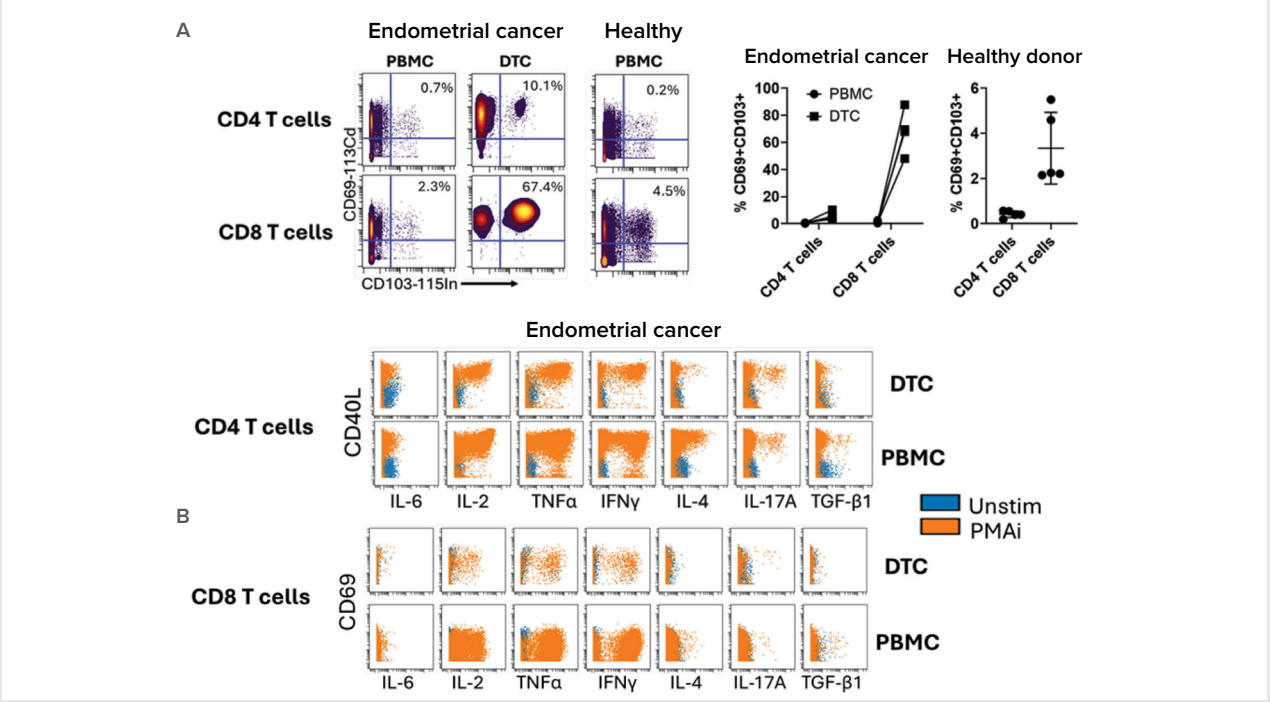


Figure 4. T cell phenotyping in tumor-derived cells and peripheral blood from EC donors and in peripheral blood healthy donors (HD). A) CD69 and CD103 expression on CD4 and CD8 T cells from DTC and PBMC of EC donors and PBMC of HDs. An increase in CD69+CD103+ CD8 T cells consistent with tissue residency 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).

High-parameter detection highlights differential expression of activation and checkpoint markers in tumor and peripheral blood T cell memory subsets

Given the differences in distribution, tumor antigen exposure, homing and retention signals, and interaction with other immune cells and the extracellular matrix, it is likely that expression of important biomarkers of activation, exhaustion and responsiveness to stimulation will differ between T cells in the peripheral blood compared with tissue resident T cells. Implementation of the high-parameter panel used in this study would allow such comprehensive functional profiling and allow for further understanding of T cell biology within the tumor vs. peripheral blood compartments. Hierarchical clustering of CD4 and CD8 T cell subsets showed highly correlated modules of markers that clustered all 5 EC donor CD8 TRM cell subsets based on immune activation and checkpoint markers such as TIM-3, PD-1, CD38, CTLA-4 and LAG-3 (Figure 5A). These important markers were highly enriched in expression in the CD8 TRM subsets compared with peripheral effector memory CD8 T cells (Figure 5B and 5D), and in turn they closely correlated with one another (Figure 5C). These observations highlight the importance of profiling the TME to identify specific cell subsets that can serve as targets of immunotherapy or biomarkers predicting response to therapy.

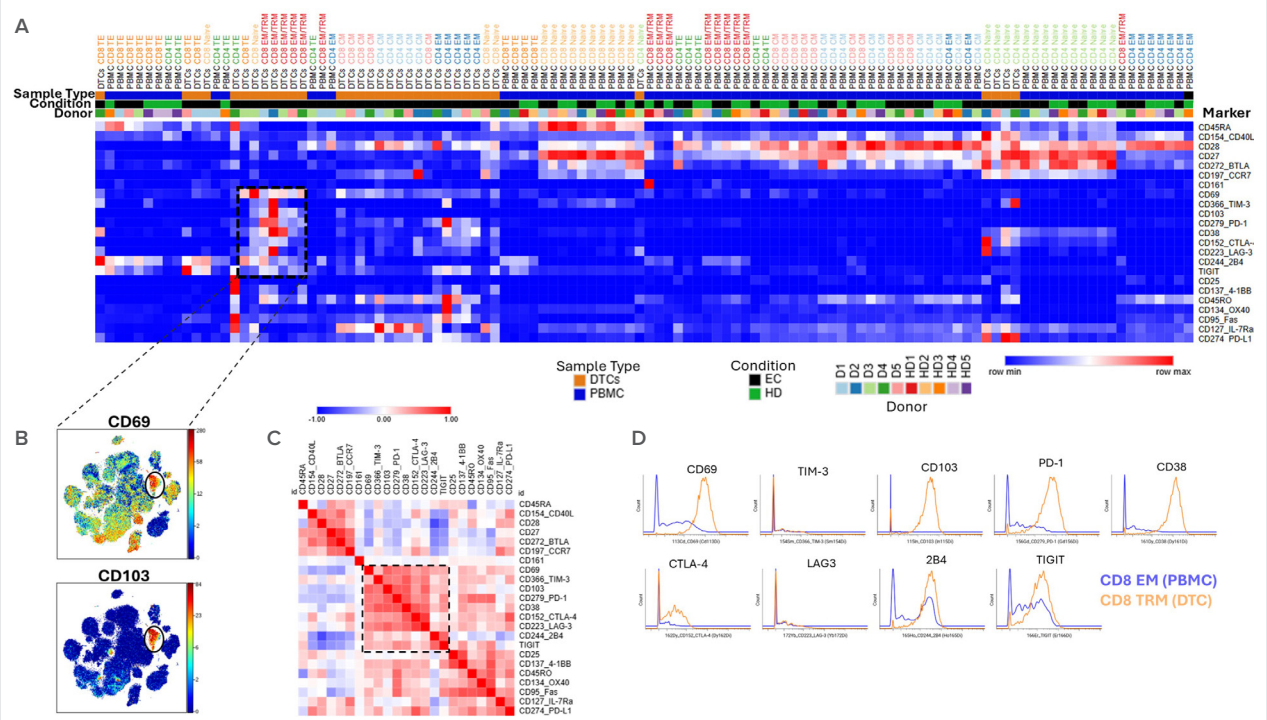


Figure 5. Differential expression of activation and checkpoint markers in tumor and peripheral blood T cell memory subsets. A) A hierarchically clustered heat map of CD4 and CD8 T cell subsets based on activation and checkpoint markers. B) CD69 and CD103 together define a distinct cluster of the CD8 TRM cells. C) A similarity index showing correlation of CD69 and CD103 with checkpoint activation markers TIM-3, PD-1, CD38, CTLA-4, LAG-3, 2B4 and TIGIT. D) Expression of the correlated markers on peripheral and tumor CD8 TRM cells compared with peripheral CD8 EM T cells from 1 EC donor. HD, healthy donor; EC, endometrial cancer; CM, central memory; EM, effector memory; TE, terminal effector memory; TRM, tissue resident memory.

Discussion and summary

The enhanced throughput of the CyTOF XT PRO system, coupled with the high-parameter panel used in this study, is significant for advancing cancer research through deep and rapid immune profiling. Both tools enabled an unprecedented phenotypic and functional analysis of circulating immune cells, tissue and tumor cells, and TILs from a single tube. The ability to use this panel with dissociated cells from tumor resections, biopsies and low-input samples like liquid or fine needle aspirate biopsies, when combined with live-cell universal barcoding, highlights its versatility.

By providing a flexible and modular assay capable of characterizing both immune and non-immune cells, this CyTOF panel holds significant potential for biomarker discovery and the acceleration of drug development in cancer therapeutics. The detailed profiling it offers can be used to research personalized treatment strategies for patients with EC. The panel can also be adapted to study immune and non-immune cells in other cancers, such as breast, lung and colorectal cancers. This can help identify unique biomarkers and potential therapeutic targets specific to each cancer type. By combining pre-optimized, modular panels, tissue- or disease-specific add-on markers (Figure 6), sample multiplexing and batch acquisition with enhanced throughput on the CyTOF XT PRO system, we demonstrate a powerful tool for comprehensive immune profiling in cancer research. The ability to provide high-dimensional data across various sample and cell types positions CyTOF technology as an invaluable asset for personalized medicine and cancer therapeutics.

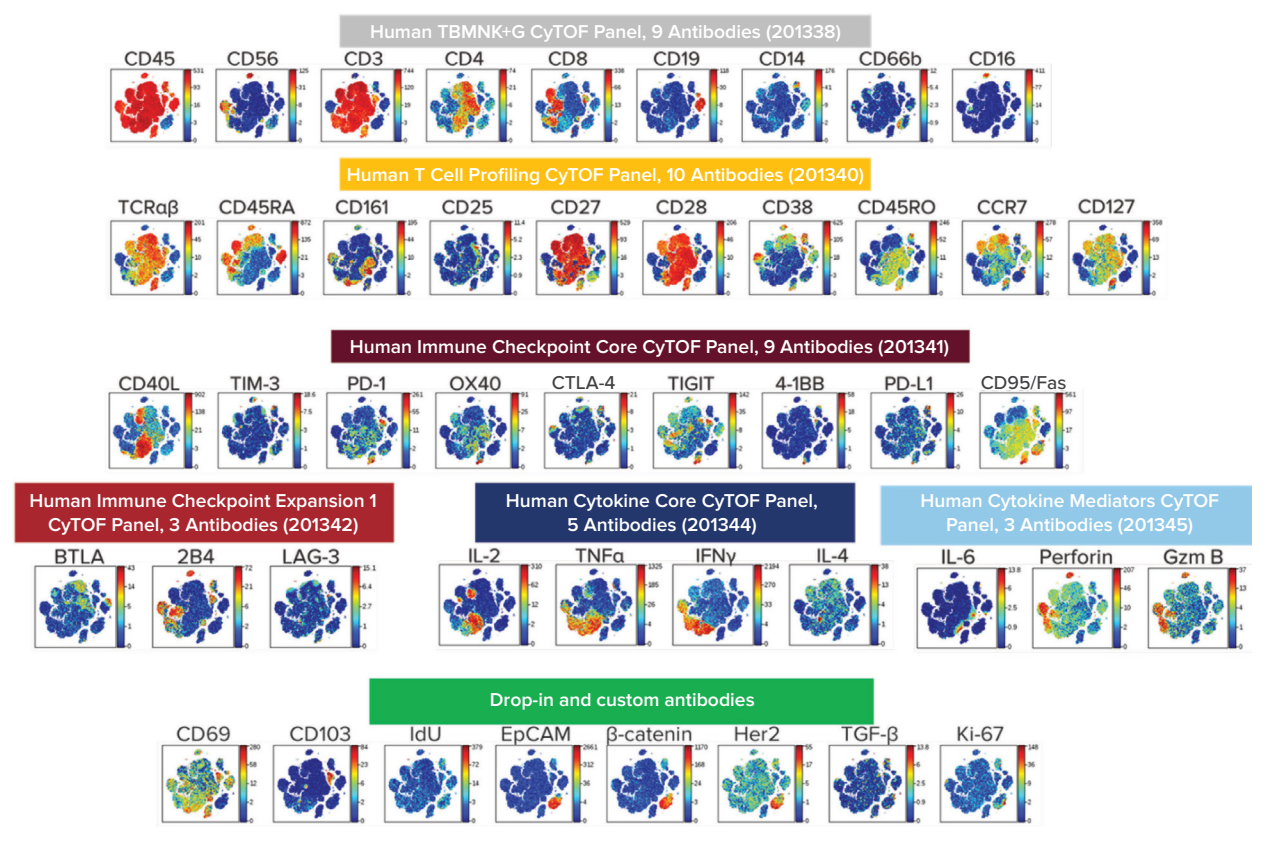


Figure 6. Expression of all the markers in the panels projected onto t-SNE plots generated from 10,000 live singlet events per sample. t-SNE projection was generated using all 48 antibodies, excluding viability and barcoding markers.

Potential applications of this panel:

- Cell composition analysis of immune and non-immune cells including frequencies of T cells, B cells, NK cells, granulocytes, monocytes and epithelial cells
- Specific and deeper characterization of T cells and NK cells (memory, exhaustion, response to stimuli, proliferation)
- Tracking homing, activation and functional responsiveness of engineered cell therapy products such as TILs and CAR T cells at the tumor site in comparison to peripheral response
- Monitoring the response to other immune-directed therapies such as checkpoint inhibitors, cancer vaccines or bispecific T cell engagers
- Sample-sparing multiplexed staining and acquisition to reduce batch effects and streamline the sample preparation workflow

Tips for success

- As with any high-parameter panel, titration of antibodies and viability and IdU reagents is critical for optimal staining for each application
- Viability staining is important to differentiate between live and dead cells when working with tissue samples and following stimulation. Monoisotopic Cell-ID Cisplatin-194Pt was used in this panel due to the presence of platinum-labeled antibodies in the panel.
- Stimulation with most reagents can downmodulate certain cell surface markers, such as CD4 and CD16. To achieve optimal resolution, consider increasing the antibody concentration or using intracellular staining. Simulation conditions should be considered during titration and optimization.
- TGF-β1 staining is performed during surface staining protocol due to tethering of TGF-β1 on the cell membrane
- Contact our Field Applications Scientists for information on how to optimize your experiment

Materials and methods

Sample stimulation and staining

Matched PBMC and DTCs from EC patients and healthy donor PBMC (n=5 each) were purchased from Discovery Life Sciences. The cells were thawed in complete RPMI (10% heat-inactivated fetal bovine serum, 1% penicillin/streptomycin/L-glutamine, Gibco) supplemented with CTL Anti-Aggregate Wash Solution (CTL). 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 hr at 37 °C in a humidified incubator with 5% CO_2. After labeling with 25 µM Cell-ID 127 IdU for 15 min at 37 °C, samples were harvested before live-cell universal barcoding using a tellurium-based 7-choose-3 strategy (Appendix B). 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 a correlation matrix were generated with Morpheus (**software.broadinstitute.org/morpheus**).

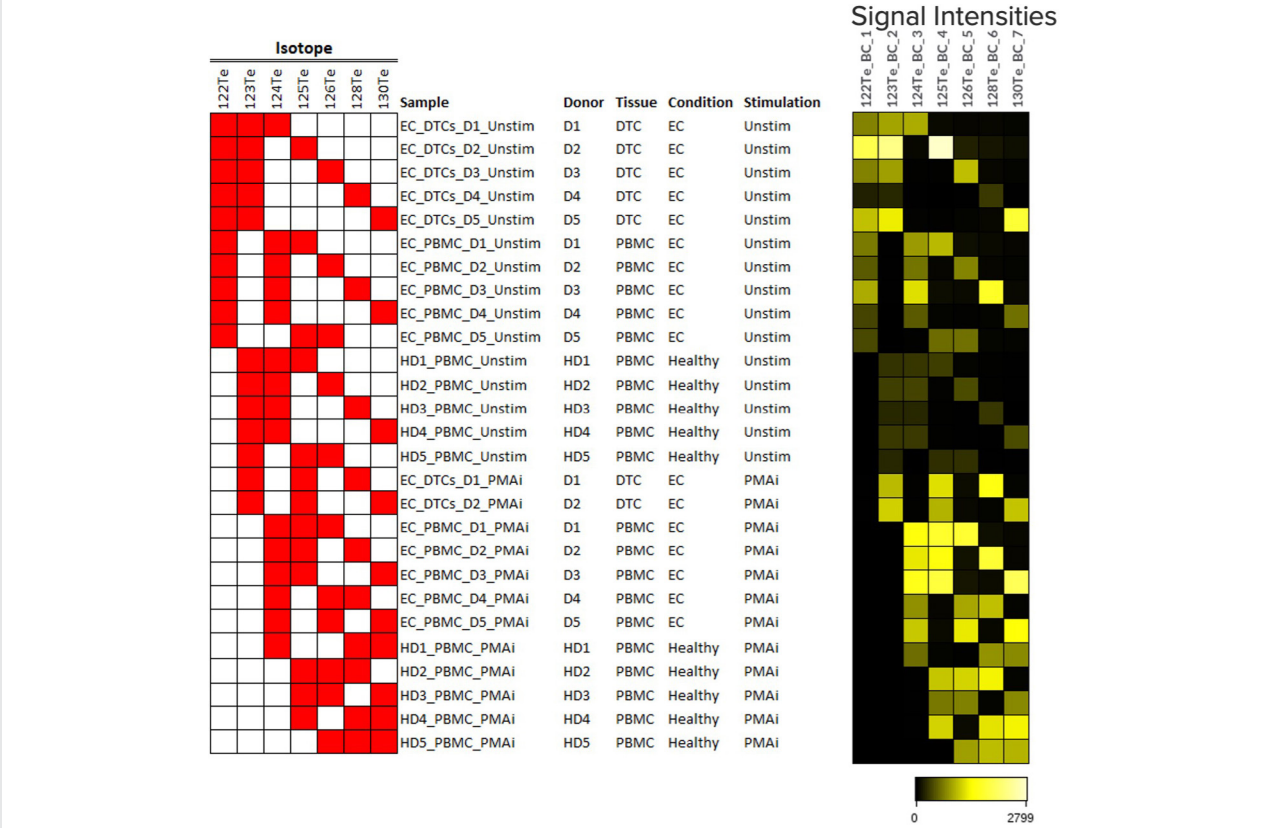
Appendix A: Antibody list

Panel	Antigen	Clone	Metal	Staining Protocol	Part Number
Human TBMNK+G CyTOF Panel, 9 Antibodies (201338)	CD45	HI30	89Y	Cytoplasmic	3089003
	CD56	NCAM16.2	149Sm	Surface	3149021
	CD3	UCHT1	170Er	Cytoplasmic	3170001
	CD16	3G8	209Bi	Surface	3209002
	CD19	HIB19	145Nd	Surface	3145020
	CD14	M5E2	146Nd	Surface	3146022
	CD66b	G10F5	152Sm	Surface	3152019
	CD8	SK1	142Nd	Cytoplasmic	3142021
	CD4	SK3	144Nd	Cytoplasmic	3144029
Human T Cell Profiling CyTOF Panel, 10 Antibodies (201340)	TCRαβ	T10B9.1A-31	141Pr	Surface	3141022
	CD45RA	HI100	150Nd	Surface	3150034
	CD161	HP-3G10	151Eu	Surface	3151029
	CD27	L128	155Gd	Surface	3155001
	CD28	CD28.2	160Gd	Surface	3160003
	CD38	HB-7	161Dy	Surface	3161034
	CD45RO	UCHL1	163Dy	Surface	3163032
	CD25	BC96	153Eu	Surface	3153032
	CD197/CCR7	G043H7	167Er	Surface	3167009
	CD127/IL-7Rα	A019D5	168Er	Surface	3168017
Human Immune Checkpoint Core CyTOF Panel, 9 Antibodies (201341)	CD366/TIM-3	F38-2E2	154Sm	Surface	3154010
	CD134/OX40	ACT35	158Gd	Surface	3158012
	CD137/4-1BB	4B4-1	173Yb	Surface	3173015
	TIGIT	MBSA43	166Er	Surface	3166033
	CD95/Fas	DX2	176Yb	Surface	3176029
	CD279/PD-1	EH12.2H7	156Gd	Surface	3156038
	CD274/PD-L1	MIH1	174Yb	Surface	3174027
	CD152/CTLA-4	14D3	162Dy	Surface	3162039
	CD154/CD40L	24-31	143Nd	Cytoplasmic	3143033
Human Immune Checkpoint Expansion 1 CyTOF Panel, 3 Antibodies (201342)	CD272/BTLA	MIH26	175Lu	Surface	3175042
	CD244/2B4	PP35	165Ho	Surface	3165046
	CD223/LAG-3	11C3C65	172Yb	Surface	3172035
Human Cytokine Core CyTOF Panel, 5 Antibodies (201344)	IL-2	MQ1-17H12	112Cd	Cytoplasmic	3112002
	TNFα	MAb11	114Cd	Cytoplasmic	3114002
	IFNγ	B27	116Cd	Cytoplasmic	3116002
	IL-4	MP4-25D2	171Yb	Cytoplasmic	3171028
	IL-17A	BL168	195Pt	Cytoplasmic	3195002
Human Cytotoxic Mediators CyTOF Panel, 3 Antibodies (201345)	IL-6	MQ2-13A5	106Cd	Cytoplasmic	3106003
	Perforin	B-D48	196Pt	Cytoplasmic	3196002
	Granzyme B	GB11	198Pt	Cytoplasmic	3198002

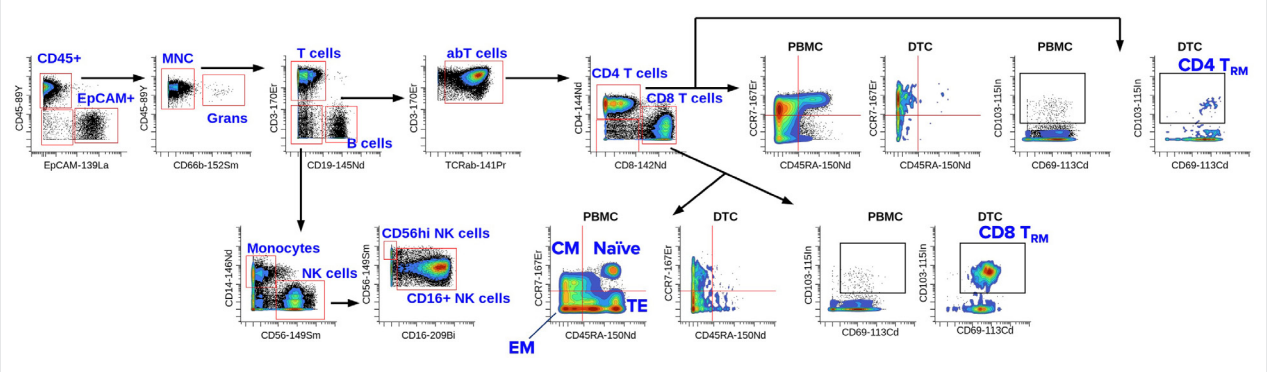
Panel	Antigen	Clone	Metal	Staining Protocol	Part Number
Drop-in and custom	TGF-β	S20006A	164Dy	Surface	3164033
	CD69	FN50	113Cd	Surface	3113002
	CD103	Ber-ACT8	115In	Surface	Custom
	EpCAM	9C4	139La	Surface	Custom
	I127-IdU	NA	127I	Surface, 37 °C	201127
	Her2	29D8	148Nd	Cytoplasmic	3148011
	β-catenin	D10A8	147Sm	Nuclear	3147005
	Ki-67	B56	169Tm	Nuclear	Custom
	FoxP3	PCH101	159Tb	Nuclear	3159039
Viability	Cell-ID Cisplatin-194Pt		194Pt	Surface	201194

Appendix B: TeMal barcoding scheme and results

Samples were barcoded following IdU staining as follows: Cells (0.5–2M/sample) were washed once with room temperature phosphate-buffered saline (PBS), then resuspended in 800 µL PBS. TeMal barcodes were thawed and 195 µL of PBS were added to each barcode. The barcodes were added to the cell suspension such that the final concentration of each barcode isotope was 2 µM in 0.05% v/v DMSO. Cells were incubated for 15 min, washed twice with Maxpar Cell Staining Buffer and then pooled. 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.



Appendix C: Gating scheme for major cell lineages on live single cells



Grans = granulocytes; MNC = mononuclear cells; CM = central memory; EM = effector memory; TE = terminal effector memory; TRM = T resident memory

Appendix D: Ordering information

Standard BioTools Reagents	Part Number
Human TBMNK+G CyTOF Panel, 9 Antibodies	201338
Human T Cell Profiling CyTOF Panel, 10 Antibodies	201340
Human Immune Checkpoint Core CyTOF Panel, 9 Antibodies	201341
Human Immune Checkpoint Expansion 1 CyTOF Panel, 3 Antibodies	201342
Human Cytokine Core CyTOF Panel, 5 Antibodies	201344
Human Cytotoxic Mediators CyTOF Panel, 3 Antibodies	201345
TeMal 7 Isotopes Bundle	CMF-20100
Cell-ID 127 IdU	201127
Maxpar Cell Surface Staining Kit, containing: • Cell-ID Intercalator-Ir, 12.5 µM – 50 µL • Maxpar Cell Acquisition Solution Plus – 200 mL • Maxpar Cell Staining Buffer – 500 mL • Maxpar PBS – 100 mL • Maxpar Fix and Perm Buffer – 25 mL	201601
Maxpar Nuclear Antigen Staining Buffer Set, containing • Maxpar Nuclear Antigen Staining Buffer Concentrate (4X) • Maxpar Nuclear Antigen Staining Buffer Diluent • Maxpar Nuclear Antigen Staining Perm 1X	201063
Maxpar PBS – 100 mL	201058
Maxpar Cell Staining Buffer – 500 mL	201068
Maxpar Fix I Buffer (5X) – 15 mL	201065
Maxpar Perm-S Buffer – 250 mL	201066

Box 1: Key functional and phenotypical markers

- The 11 markers of T cell and NK cell activation, exhaustion and co-inhibition – **PD-1, TIM-3, OX40, 4-1BB, TIGIT, CD95/Fas, CTLA4, CD40L, BTLA, 2B4** and **LAG-3** – are active immunotherapy and checkpoint inhibitor therapy targets[1,2,3]. The PD-1 inhibitor pembrolizumab is approved for treatment of recurrent microsatellite instability high cancers, including EC. For detailed characterization of these markers in their Flex-Fit panels, see the Accelerating Immuno-Oncology Research by Profiling Cell Function with Flex-Fit CyTOF Panels Application Note (FLDM-01375).
- **EpCAM** is a membrane protein frequently overexpressed in carcinomas, including EC. It is involved in cell adhesion, proliferation and differentiation. High EpCAM expression is often associated with poor prognosis and increased metastatic potential[4].
- **Her2**, a member of the epidermal growth factor receptor (EGFR) family, is overexpressed in many solid tumors, including a subset of EC (30% of uterine serous carcinomas), and is associated with aggressive disease and poor prognosis. The anti-Her2 monoclonal antibody trastuzumab has shown efficacy in treating advanced and/or recurrent ECs, while tyrosine kinase inhibitors such as lapatinib function by inhibiting Her1 and Her2 activity[3].
- Mutations in the *CTNNB1* gene, which encodes **β-catenin**, are common in endometrioid ECs. These mutations lead to the activation of the Wnt/β-catenin signaling pathway, promoting tumor growth and progression and inhibiting immune evasion in many cancers[5]. Thus β-catenin can serve as a prognostic marker and potential therapeutic target in cancers with Wnt pathway alterations.
- **CD69** is an early activation marker on T cells and other immune cells. Its expression is associated with tissue residency and immune response regulation. CD69 expression on TILs can indicate immune activation and potential exhaustion, impacting the effectiveness of immunotherapies.
- **CD103** is an integrin expressed on tissue-resident memory T cells (TRM) involved in the retention of these cells in epithelial tissues. High levels of CD103+ TILs are associated with better prognosis in various cancers, including EC. CD103+ TILs are considered a marker of effective antitumor immune response and are being studied for their role in enhancing immunotherapy outcomes.
- **PD-L1** is expressed on tumor cells and immune cells within the TME. It interacts with PD-1 on T cells to inhibit immune responses, allowing tumors to evade immune detection. PD-L1 expression is a key biomarker for selecting patients for immune checkpoint inhibitor therapies, such as durvalumab, which block PD-1/PD-L1 interaction to enhance antitumor immunity. The PD-1 inhibitor pembrolizumab is FDA approved for treating advanced EC in combination with lenvatinib, a multi-kinase inhibitor that targets several VEGFRs.
- High **Ki-67** expression is correlated with increased tumor aggressiveness and poor prognosis. It is used to assess the growth fraction of a tumor (Ki-67 index), helping predict the likely course of the disease and response to treatment. In EC, a high Ki-67 index can indicate a more aggressive tumor phenotype. In the immune system, Ki-67 staining can identify proliferating immune cells, such as T cells and B cells, which are actively responding to an infection or tumor. This can provide insights into the immune response within the TME, indicating the presence of active immune surveillance or immune evasion mechanisms.
- **IdU** (5-Iodo-2'-deoxyuridine) is a thymidine analog that gets incorporated into DNA during the S phase of the cell cycle, marking cells that are actively synthesizing DNA. IdU incorporation is used to measure cell proliferation rates in tumors. By staining for IdU, the fraction of cells undergoing DNA replication can be determined to estimate tumor growth and aggressiveness. This can be particularly useful in assessing the efficacy of anti-proliferative cancer therapies. In the context of the immune response, IdU staining can help identify proliferating immune cells, and thus reveal the dynamics of immune cell expansion in response to infections, vaccinations or cancer.
- **TGF-β1** is a potent immunosuppressive cytokine that can impair the recruitment and function of effector immune cells such as T cells and NK cells, while inducing differentiation of myeloid-derived suppressor cells and activation of cancer-associated fibroblasts. TGF-β can also induce epithelial-mesenchymal transition, a process in which epithelial cells acquire mesenchymal properties, leading to increased motility and invasiveness and thus promoting cancer metastasis.

References

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