Welcome to the March issue of Trending Now, a quarterly anthology of recent impactful publications by researchers using CyTOF technology.

This month’s issue focuses on research efforts to determine how immunotherapy success and efficacy are impacted by the immune response for a variety of cancers.
WHAT’S NEW IN MASS CYTOMETRY

An understanding of cell behavior in the immune response to cancer and to immunotherapy, made possible with mass cytometry, is a significant stride in broadening access to effective treatments for more patients.

The immuno-oncology community has achieved remarkable progress in the exploration of immune responses to cancer and in the development of treatments leveraging the immune system to fight cancer. There is a burgeoning confidence that we can defeat such an infiltrative disease by relying on the creativity in approaches from the knowledge we have gained from such technologies as mass cytometry.
The tremendous promise of immuno-oncology

Cancer cells have the capacity to evade or suppress surrounding immune cells, allowing their proliferation. By stimulating different facets of the immune system with adoptive cell therapy, vaccines, immunomodulation or targeted antibodies, reinvigorated immune cells become our most effective cancer fighters. These therapies offer the potential for durable clinical responses and long-term survival in patients who react positively.

Despite these successes, most patients do not respond to immunotherapy, a result of the complex and variable nature of the immune landscape between individuals. The overarching question then becomes how to broaden the correct functioning of immunotherapies in more patients.

Robust biomarker development and in-depth characterization of cell-cell interactions within the tumor microenvironment are bringing us closer to the answers we seek. Incorporating relevant technologies for biomarker discovery and validation or facilitated immune monitoring of patient response can accelerate patient stratification and combination therapy design. The Society for Immunotherapy of Cancer resource document\(^1\) has recently been updated to support and guide research aspiring to these goals.

High-dimensional analysis of all cells within a tissue microenvironment, using such technologies as mass cytometry and single-cell RNA sequencing, has contributed to understanding cellular composition and signaling, bringing us ever closer to greater success in this field. For example, one of the latest editions\(^2\) of Methods in Molecular Biology highlights experimental approaches to the understanding and characterization of myeloid-derived suppressor cells. The book outlines techniques to distinguish these heterogeneous cells within a tumor microenvironment, employing mass cytometry for better definition of their role in the prognosis and physiopathology of various tumors.

The unique ability of CyTOF\(^\circledast\) technology to identify and compare the diversity of cells in one experiment enables easier standardization of protocols for more trusted clinical research outcomes and better-informed treatment development and decisions. Here, we review several new insights revealed using mass cytometry to aid in the advancement of immuno-oncology.

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**Figure 1.** T cell differentiation states and subset specifications (Ferrarini et al.). CD4\(^+\) T cells differentiate from naive to effectors (EFF), effector memory (EM) and central memory (CM) cells. CD8\(^+\) T cells progress from naive to CM, EM and terminal effector memory re-expressing CD45RA (TEMRA)\(^3\).
Prediction of immunotherapy response based on cell signature

T and natural killer (NK) cells are known to constitute much of the immune response in the tumor microenvironment, but their roles can be hampered by coordinated immune evasion. Proliferating cancer cells are able to influence response and tolerance in these cells based on mutations that affect the balance of inhibitory and activation mechanisms. In classic Hodgkin lymphoma, for example, several studies using mass cytometry and Imaging Mass Cytometry™ have indicated regulation or immunosuppression of these immune cell types by targeted cancer cell signaling activity.

A recent study from the National Cancer Institute compared response outcomes in melanoma patients treated with adoptive cell transfer (ACT), manipulating a patient’s own T cells to fight cancer. Using a 37-marker CyTOF panel to analyze tumor-infiltrating lymphocytes (TILs) in responders vs. nonresponders, they identified a memory-progenitor CD39-CD69- stem-like phenotype associated with complete cancer regression and TIL persistence. Conversely, a terminally differentiated CD39+CD69+ phenotype was associated with poor TIL persistence.

Knowledge of the mechanisms behind immune cell differentiation triggered by a therapeutic empowers new research directed to stratifying patients most likely to respond.

In an effort to clarify which critical factors impact clinical response to therapy, researchers at Washington University School of Medicine in St. Louis used mass cytometry to explore NK cell type and behavior in patients with acute myeloid leukemia (AML) in a Phase 1 clinical trial. NK cells are a strong contender for cell therapy, but little is known about the repercussions of donor NK cell and host factors on treatment response or resistance.

A comprehensive analysis highlighted differences between responders and nonresponders to cytokine-induced memory-like (ML) NK cellular therapy. Using three CyTOF panels for phenotype (37 markers), function (36 markers) and resistance (22 markers), the team identified a unique NKG2A+CD8+ NK cell signature associated with treatment failure after ML NK cell therapy. Defining the multidimensional dynamics of donor ML NK cells in vivo revealed new opportunities to enhance the efficacy of NK cell therapeutics and enabled discovery of mechanisms important for ML NK cell function.
Determining efficacy of an immunotherapy

Additional therapies such as vaccines, kinase inhibitors and checkpoint inhibitors can help extend long-term survival benefits beyond the use of chemotherapy and resection. Mass cytometry offers a unique advantage in determining treatment efficacy by more comprehensively characterizing induced T cell responses post-treatment.

A study out of Duke University looked at the efficacy and overall survival rates of vaccination with alphaviral replicon particles for tumor-associated antigens in patients with metastatic or resected cancer. Patients with stage 3 and stage 4 colorectal cancer who had completed their initial postoperative adjuvant chemotherapy were given the vaccine every three weeks for four immunizations. Immune monitoring by mass cytometry using a 28-marker CyTOF panel revealed that the vaccine stimulated antigen-specific effector T cells and decreased T regulatory cells, suggesting favorable immune modulation and possible improved overall survival.

Mass cytometry and Imaging Mass Cytometry readily allow for investigations with extraordinary depth and breadth into immune function and spatial dynamics. Johns Hopkins researchers applied both techniques to perform an unbiased integrated analysis of local and systemic immune responses in hepatocellular carcinoma (HCC) patients treated with a combination therapy before resection surgery. The study establishes the first case of a patient who had unresectable HCC downstaged with cabozantinib and nivolumab treatment, allowing for resection and leading to long-term disease-free survival.

Their 27-marker CyTOF analysis on peripheral blood samples and 26-marker IMC assessment on tissue samples revealed immune infiltration into the tumor microenvironment (TME) in nonrandom, organized cellular neighborhoods. Systemically, the combination therapy activated effector cytotoxic T cells and effector memory helper T cells as well as increasing NK cell abundance and CD8+ cell density. This latter change suggests that the treatment appears to direct the type of T cells activated within the TME.

Improvements in cell production for immunotherapy

It is challenging to manufacture multitudes of immune cells that can be administered without toxicity and can show in vivo persistence. A clinical study (NCT01040026) on the feasibility of using NK cell immunotherapy for plasma cell myeloma (PCM), a malignant disease characterized by the neoplastic proliferation of plasma cells, describes a protocol for the in vitro expansion of high numbers of activated haploidentical NK cells.

This trial analyzed phenotypic and functional characteristics of NK cell expansion cultures using mass cytometry, with barcoding to allow for bulk analysis. Though high doses of NK cells could be produced and safely administered, no clinical benefit was observed. The team continues to optimize the expansion protocol to resolve phenotypic changes in the expanded NK cells, such as increased expression of certain inhibitory and activating receptors, substrate receptors and effector molecules, including NKG2A.
University of Sydney researchers developed a novel manufacturing method for preferentially expressed antigen in melanoma (PRAME)-specific T cells from healthy donors for adoptive immunotherapy. Detailed phenotypic and functional analysis and cytokine response following antigen re-exposure were conducted on selected cultures. A 38-marker CyTOF panel was used along with flow cytometry, enzyme-linked immunospot and supernatant cytokine detection, showing favorable in vivo function and persistence. The method is part of an ongoing Phase I clinical trial (NCT02895412) on immunotherapy following allogeneic stem cell transplant for patients with AML.

Back to basics: functional biology of the immune response

As an important immune checkpoint therapeutic target, PD-1 therapy can reactivate cytotoxic T cells. But there is much to learn about T cell subset phenotype and function, particularly regarding T cell exhaustion. A study out of Shanghai demonstrates the effectiveness of mass cytometry in analyzing cells by phenotype, function and regional distribution and in identifying new potential prognostic markers and optimal combinations of current therapies based on immune response.

Mass cytometry analysis on immune cells in matched tumor- and nontumor-adjacent tissues and blood samples from patients with HCC provided regional distribution and functional differences of CD8+PD-1+CD161+/- T cells. Given this information, the group determined better prognosis with CD8+PD-1+CD161+ T cell enrichment and association of CD161- with exhaustion, providing new insights into the heterogeneous immune environment.

While T cell exhaustion is a common predictor of immune evasion by cancer cells, the signaling events that lead to exhaustion are still in question. A group from Michigan State University investigated the drivers behind T cell exhaustion and the known role that TCR signaling plays, with a focus on a malignancy marker, SLAMF7. Reanalysis of a CyTOF dataset comprising TME profiles for 73 clear cell renal cell carcinoma patients showed SLAMF7 expression patterns correlating exhausted T cells with SLAMF7+ tumor-associated macrophages (TAMs) and a unique subset of SLAMF7/CD38 TAMs. SLAMF7 appears to play an active role in T cell functional modulation, reprogramming T cells into exhaustion and acting as a prognostic indicator in cancer progression.

Figure 4. Single-cell mass cytometry workflow and a potential regulation mechanism

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A strong foundation for progress

High-parameter phenotyping extends the possibilities for immune monitoring. With a need for more sensitive and selective tools to identify rare cells in a mixed population and to investigate functional changes induced by immunotherapies, mass cytometry brings a level of speed, precision and reproducibility to cell analysis not enabled by other single-cell platforms. Recognition of the power mass cytometry can bring to the development and assessment of immunotherapies is reflected in its recent adoption as the high-parameter cytometry approach of choice by the Cancer Immune Monitoring and Analysis Centers and the Cancer Immunologic Data Commons (CIMAC-CIDC). This consortium is an established network of four academic centers and a data coordinating center focused on strategies to develop assay harmonization and validation for cross-trial and cross-site immune monitoring studies. Through biomarker discovery and correlation with clinical outcome, data from the network could significantly accelerate advances in cancer therapy.

References


Explore mass cytometry at go.fluidigm.com/cytof

Listen to recorded seminars on the tumor microenvironment given by Joshua Brody, Jonathan Irish and Evan Lind.

Learn more about decoding immune cell heterogeneity with mass cytometry in an interview with Evan Newell.