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

This month’s issue is the second in a two-part series focusing on the application of mass cytometry to vaccine development. **Part one** highlighted the area of infectious diseases. In this issue we focus on vaccine and immunotherapy development for cancer.
The development and testing of new treatment vaccines for cancer begins with a comprehensive understanding of tumor cell and immune cell behavior, made possible with mass cytometry.
What makes a cure and treatments for cancer so elusive? While our understanding of the unique and variable processes of cancer progression continues to develop, we have yet to create a clear picture of how cancer works and what we can do to stop it. Every causative mutation, cell surface change and immune evasion process can differ with and within a cancer type down to the individual level, making prevention, treatment and even basic understanding a challenge.

Vaccines provide new possibilities for cancer prevention and treatment by strengthening the immune system to recognize and react to antigens and destroy cancer cells. Immunotherapies based on immune checkpoint blockades (ICB) remain the most promising treatment vaccine approach to date. However, issues with specificity and tumor resistance plague ICB immunotherapies, resulting in uneven responses to treatment and unpredictable efficacy.

To improve current immunotherapies or develop new ones, more work must be done to better understand how immune checkpoint blockades affect and activate immune cells. Deep immune monitoring using CyTOF® technology allows a comprehensive view of all cells involved in immune response and within a tumor microenvironment, exposing cell-cell interactions and identifying new tumor-specific neoantigens. An in-depth look at these tumor-specific T cells and neoantigens can reveal why these cells are inconsistent in their ability to eradicate a tumor, and what can be done to develop more reliably effective treatments.

Here we review several publications and insightful abstracts from 2020 that outline new work using mass cytometry for immune monitoring to identify predictive biomarkers, develop novel therapy approaches and evaluate vaccine efficacy for a variety of cancers. They are among a multitude of publications, in addition to more than 100 clinical trials, that attest to the success of mass cytometry in vaccine research and development.

Figure 1. The immune checkpoint blockade effect on T cell activation, typically acting through PD-1/PD-L1
How CyTOF specificity helps identify predictive biomarkers

Immunotherapy has received considerable attention as a treatment for cancers harboring tumor-specific neoantigens, a class of immunogens that confer tumor specificity and immune activation.

Currently, technologies that can detect tumor specificity of antigens stand out as a much-needed solution to distinguishing immune cells specifically activated by the vaccine from other activated cells. The significance of this distinction lies in the ability to improve efficacy and specificity of a vaccine. Mass cytometry holds the unique ability to identify neoantigen-specific T cells in the context of high-parameter phenotyping, as well as with high-parameter tetramer use.

A University of California, San Francisco clinical research study focused efforts on vaccine development for diffuse midline gliomas (DMGs), a fatal pediatric brain cancer (Mueller et al. NCT02960230). Even with treatment, survival outcomes for this type of cancer are dismal, with less than 10% of patients surviving beyond two years. Recently, known mutations in genes encoding histone 3 variants have become a tumor-specific target for immunotherapy.

In a multi-site study conducted through the Pacific Pediatric Neuro-Oncology Consortium (PNOC), researchers evaluated the safety, immunoreactivity and efficacy of a vaccine based on a specific H3 mutation in patients with DMGs containing the variant. The vaccine was administered to newly diagnosed patients on an ongoing schedule. Immune monitoring and imaging were performed every three months, with immune responses assessed in PBMC using mass cytometry.

This research validates the power of CyTOF technology for high-dimensional immune monitoring of immunotherapies based on CD8+ T cells.

The group combined the use of HLA dextramers with CyTOF technology to analyze multiple immune subsets to better explore associations between targeted antigen-reactive CD8+ T cells triggered by the vaccine and prolonged overall survival. Dextramers exhibit high affinity and specificity for epitope-specific CD8+ T cell populations, allowing identification and evaluation of epitope-specific CD8+ T cell responses in cancer patients treated with peptide-based vaccines.

Results indicated that the vaccine is safe for use, while the study itself demonstrated the significance of immune monitoring for identification of predictive biomarkers, detectable across any location (cell surface, cytoplasm, nucleus). This research validates the power of CyTOF technology for high-dimensional immune monitoring of immunotherapies based on CD8+ T cells.

Figure 2. Metal-tagged tetramers enable identification of antigen-specific T cells in the context of deep immune profiling with 40 or more markers using mass cytometry.
Using mass cytometry to support immunotherapy development

Mass cytometry’s ability to deeply characterize tumor and immune cells and their interactions empowers a new level of insight into how tumor-specific immune cells can relate to clinical response to therapies, identifying infiltrating behavior and specific immune signatures.

A joint effort by ImmunoScape, Fred Hutchinson Cancer Research Center and Genentech examined the nature and function of tumor-specific T cells, known to play a role in tumor rejection, aiming to support the development of immunotherapy strategies. Their new study (Abel et al.) leverages the high-dimensional immune profiling capabilities of CyTOF technology to identify and characterize circulating neoantigen-specific T cells in lung carcinoma patients undergoing atezolizumab treatment.

Combinatorial peptide-MHC tetramer staining and high-performance dimensional analysis tools, applied together with mass cytometry, revealed 13 unique neoantigens eliciting T cell reactivity among responding patients. Additionally, seven neoantigens were recognized in patients who did not respond to treatment, and differentiated T cell phenotypes were observed between the two patient groups. This broad and sensitive map of T cell reactivity against MHC-class I epitopes and simultaneous in-depth characterization of these rare antigen-specific T cells suggest that tumor-reactive T cell signatures could be associated with clinical response to anti-PD-L1 treatment.

“By providing insight into the nature and function of tumor-specific T cells, ImmunoScape’s unique high-dimensional immune profiling platform is a valuable tool to guide the development of novel immunotherapy strategies through assessment of drug biological activity, definition of mechanism of action, and identification of biomarkers of patient response,” the paper states.

A new approach to identifying tumor-specific neoantigens and investigating the role of neoantigen-specific T cells could be a significant step for designing promising cancer immunotherapies. Since immune checkpoint receptors such as PD-1 and CTLA-4 and the associated T cell checkpoint blockade have no inherent tumor specificity, response to these treatments is variable at best.

Researchers at Fred Hutchinson Cancer Research Center applied combinatorial tetramer staining by CyTOF technology to study an immunotherapy-resistant mouse model (Li et al.) in order to learn more about how immune checkpoint blockade affects tumor-specific T cells and why these cells are unsuccessful in attacking tumor cells after treatment.

The team identified a new tumor-specific neoantigen in Lewis lung carcinoma, activated tumor-specific CD8+ tumor-infiltrating lymphocytes (TILs). However, even with cell expansion after treatment, the cells did not appear to affect tumor regression. The approach enabled the group to discover novel cell types and behaviors associated with both immune checkpoint blockade and neoantigen peptide vaccination, providing a model for studying neoantigens in tumors. Further investigation into the role of neoantigen-specific T cells in ICB could have a significant impact on the design of future immunotherapeutic strategies.
Measuring vaccine efficacy by immune monitoring

Immune cell infiltration into tumors provides evidence of the level of immune response activated by a vaccine. Mass cytometry offers unparalleled analysis of the tumor immune cell infiltrate, generating detailed information on cell type, behavior and interactions from limited samples.

Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer. Though curative resection can be successful in tumor removal, recurrence of HCC remains high. Having demonstrated safety and efficacy of a novel therapeutic that induces antigen-specific cytotoxic T lymphocytes, researchers at Yamaguchi University in Japan conducted further study of the same vaccine as a perioperative immunotherapy option in patients with resectable HCC (Nakajima et al.).

Patients received 10 injections of adjuvant immunotherapy over four months before liver resection surgery. Researchers used mass cytometry to analyze liver samples and PBMC, choosing 66 antibodies across several panels to monitor T cell exhaustion, T cell activation and effector T regulatory cell induction. Three response types were identified based on the level of tumor immune cell infiltration observed: “hot cellular immunity,” “intermediate humoral immunity” and “cold response.” CyTOF analysis also revealed a difference in certain T cell subtypes in the tumor specimens, not seen in the PBMC, suggesting tumor-infiltrating lymphocyte analysis is critical to understanding immune response.

This novel immunotherapy induced sustained immune cell infiltration into tumor microenvironments, confirming the ability to convert progressive tumors into tumors containing PD-1+ lymphocytes and indicating the potential to combine the therapy with PD-1 antibodies.

In an effort to find new combination therapies to prolong overall survival for pancreatic ductal adenocarcinoma (PDA), an aggressive and lethal malignancy with poor prognosis, a team from Johns Hopkins University attempted a Phase 2 study to evaluate potential vaccine efficacy (Wu et al.). The combination therapy given after frontline chemotherapy consisted of granulocyte-macrophage colony-stimulating factor-allogeneic pancreatic tumor cells (GVAX) and ipilimumab.

While the study was discontinued after interim analysis, important clinical responses and immune cell effects were observed. The study used a 32-marker CyTOF panel including both surface and intracellular markers to monitor immune response after treatment and vaccination. The combination vaccine resulted in T cell differentiation into effector memory phenotypes in all samples and increased M1 macrophages in the tumor.

Even though the therapy did not improve overall survival in patients, the study demonstrates the use of mass cytometry for unraveling immune response to treatment and the feasibility of testing novel combinations in the maintenance treatment of metastatic PDA.

![Figure 4. Immune infiltration into tumors](image)
Mass cytometry’s impact on targeted vaccine research

Mass cytometry’s unique ability to detect a large array (50 or more) of diverse targets in a single tube of either tumor digests or peripheral blood samples makes it highly efficient for obtaining a comprehensive immune picture quickly and reliably. It enables simultaneous screening of numerous T cell antigen-specific epitopes as well as biomarkers, regardless of their cellular location, that indicate progression or improvement of disease. As the scientific community works tirelessly toward effective development of treatment vaccines for certain types of cancers, mass cytometry is helping bring research closer to success.

References


Please see these additional references using mass cytometry in therapeutic research

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.