TRENDING NOW

CYTOF® FLOW CYTOMETRY PUBLICATIONS

March 2022

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

This month's edition features work using the Maxpar Direct Immune Profiling Assay and demonstrates its contribution to accelerating translational and clinical research around the world.

WHAT'S NEW IN BEST-IN-CLASS CYTOMETRIC IMMUNE PROFILING

Immunophenotyping made simple in recent research using the Maxpar Direct Immune Profiling Assay

Immune profiling is an extremely powerful tool for understanding an individual's unique immunologic response to disease or therapeutic interventions. When performed longitudinally on the same patient, or cohort of patients, a timeline of immune response is generated. This timeline provides researchers and clinicians with rich immunologic information that can be used to better understand disease pathogenesis, progression and resolution, or even to predict response to therapeutics.

In translational and clinical research, immune profiling studies often require collaborations involving multiple sites, possibly around the globe. This presents challenges in study design to ensure harmonized sample processing and data analysis, as well as sample storage and shipping, if needed. Much time and energy is spent prior to the start of every study in the design and testing of an immune profiling approach, as well as in how to preserve valuable samples and generate reproducible high-quality data.

The Maxpar® Direct™ Immune Profiling System provides a standardized, high-parameter cytometric assay with proven run-to-run and site-to-site performance ideal for multi-site and longitudinal studies. The 30-marker backbone panel comes in a dry, single-tube format and can be used to analyze whole blood, PBMC or even cultured cells. At least 37 immune cell populations can be quantified in less than 5 minutes with Maxpar Pathsetter™ software. An additional bonus is that stained samples can be stored for extended periods of time and even shipped for analysis at distant sites if needed.

This CyTOF® based system is the first complete sample-to-answer solution for high-dimensional immune profiling and has now been successfully used in a variety of settings. The publications and National Clinical Trials listed here exemplify the range of research areas and applications to which the assay is being applied.

Figure 1. Maxpar Direct Immune Profiling System

Simple standardization for clinical trials

Since the Maxpar® Direct™ Immune Profiling Assay™ includes a dried 30-marker panel designed to identify all major circulating immune cell subsets, it is an easy go-to assay for clinical trials that require a high level of standardization, run-to-run and site-to-site. In addition, the specificity of metal-tagged antibodies facilitates panel modifications and the ability to customize the assay for expanded mass cytometry panels, as recently described in the latest edition of *Methods in Molecular Biology* (**Petes et al.**).

IMPACC (Immunophenotyping Assessment in a COVID-19 Cohort) Clinical Study

The Immunophenotyping Assessment in a COVID-19 Cohort (**IMPACC**) longitudinal study is underway. This study is designed to identify immunologic, virologic, proteomic, metabolomic and genomic features of COVID-19-related susceptibility, severity and disease progression in 1,000 hospitalized patients with COVID-19 (**NCT04378777**). The goal is to detail in depth the relationship of pathogen dynamics and immune response with disease severity and pathogenesis, as well as identify biomarkers and initiate effective therapeutic development.

CyTOF technology and bulk RNA transcriptomics are being used to identify distinct immune cell populations and quantify changes in gene expression and activation markers. The CyTOF workflow employs the Maxpar Direct Immune Profiling Assay, which allows samples to be fixed, cryopreserved and shipped to the IMPACC core labs for barcoded batched processing. This includes labeling with a supplemental panel of 14 additional antibodies targeting fixation-resistant epitopes to resolve additional dynamic changes in cell phenotype. The Maxpar Direct assay was chosen specifically for this study to reduce sample usage, streamline sample processing and minimize experimental variability.

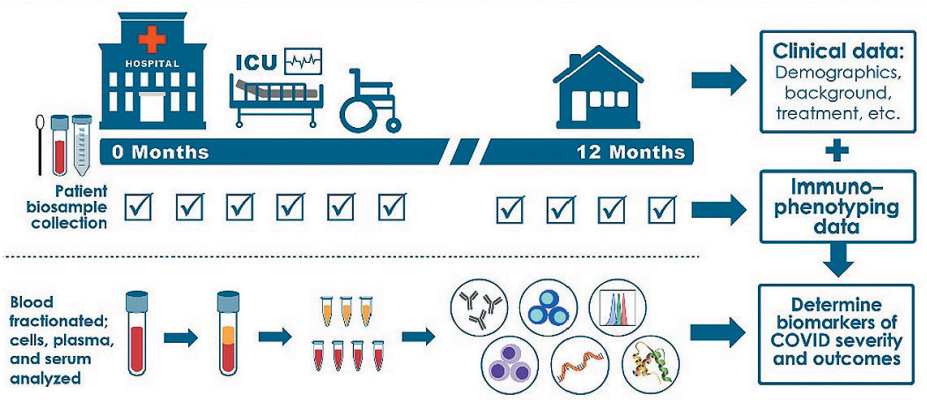

Figure 2. IMPACC study overview (*Science Immunology* 2021)

COntAGIouS (COvid-19 Advanced Genetic and Immunologic Sampling) Trial

The COntAGIouS trial is a prospective study aimed at characterizing clinical and immunological features of severe COVID-19 infection compared to mild cases (**NCT04327570**). The study could contribute to our understanding of the underlying pathophysiology of the disease and help to identify at-risk patients, guiding better treatment strategies for this group.

The transdisciplinary approach identifies host factors resulting in hypersusceptibility to SARS-CoV-2 infection that can more directly support targeted medical interventions. The team performs multiparametric analysis using mass cytometry with the Maxpar Direct Immune Profiling Assay for longitudinal immune profiling.

Watch a webinar in which Frederik De Smet reports on use of the Maxpar Direct Immune Profiling System in the COntAGIouS COVID-19 trial.

A Complete Immune Monitoring Solution with CyTOF | Frederik De Smet, MSc, PhD

Antidepressant Trial with P2X7 Antagonist JNJ-54175446 (ATP)

An ongoing clinical trial investigating the relationship between inflammation and depression is set to be completed later this year (**NCT04116606**). Since depression is a significant cause of disability and treatments are only moderately effective, The Cambridge Clinical Trials Unit (CCTU)-Core in collaboration with Janssen Pharmaceuticals is testing whether the anti-inflammatory drug JNJ-54175446, which blocks the activity of the P2X7 receptor, could have antidepressant effects on patients with moderate to severe depressive symptoms.

The group conducts immune profiling by mass cytometry, using the Maxpar Direct Immune Profiling Assay, to monitor the immune system before, during and after treatment. With any observed response, further study could identify associated immune biomarkers. Patients will also complete additional blood tests, questionnaires and magnetic resonance imaging brain scans throughout the trial to more comprehensively understand anti-inflammatory effects on the immune system and the brain.

Read more about this trial from first author Lori Turner in this **customer spotlight**.

COVID-19: Pediatric Research Immune Network on SARS-CoV-2 and MIS-C (PRISM)

A prospective, multicenter, observational cohort study, Pediatric Research Immune Network on SARS-CoV-2 and MIS-C (PRISM), is evaluating the short- and long-term health outcomes of SARS-CoV-2 infection and multisystem inflammatory syndrome in children (MIS-C) by characterizing associated immunologic pathways (**NCT04588363**).

The study goals are to determine the proportion of children with severe or fatal infections and identify immune signatures associated with the disease spectrum over the course of a one-year follow-up. Using mass cytometry with the Maxpar Direct Immune Profiling Assay supports the cross-site collection and processing of samples, maintaining a standardized protocol that allows for direct comparison of data.

Profiling infectious disease

The onset of an infection is typically a rapid process, meaning that any research aimed at studying disease progression must keep pace. Ready-to-use assays like the Maxpar Direct Immune Profiling System offer a quick approach that can be adopted immediately to collect, stain and store samples, enabling rapid response without compromising quality results.

Molecular and cellular immune features of aged patients with severe COVID-19 pneumonia

Immunity is known to wane in the elderly; thus, aging is a major risk factor in the development of severe COVID-19. Immune system remodeling via immunosenescence and inflammaging can cause predispositions to infection, including by SARS-CoV-2. Work led by the University of Modena and Reggio Emilia (**Lo Tartaro et al.**) profiled the main immune characteristics in older patients with severe SARS-CoV-2 infection, compared to subjects younger than 60 years of age.

The team used a 38-parameter mass cytometry panel, including the Maxpar Direct Immune Profiling Assay plus 6 drop-in Standard BioTools™ catalog antibodies and 2 custom-conjugated mAbs, focusing on the T lymphocyte, B cell and monocyte compartments. Results showed that immune response in older patients was characterized by higher plasma levels of pro-inflammatory cytokines and a profound dysregulation in several immune compartments.

Underlying co morbidity reveals unique immune signatures in type II diabetes patients infected with SARS-CoV-2

Given that SARS-CoV-2 infection in patients with comorbidities can be a major challenge, scientists at the Institute of Life Science in India compared immune response, viral loads and clinical parameters among COVID-19 patients with and without type II diabetes as well as in healthy controls to better understand their clinical pathology (**Sengupta et al.**). Whole blood immunophenotyping was performed using the Maxpar Direct Immune Profiling Assay in addition to plasma cytokine, chemokine, antibody isotyping and viral load determination.

The study established that type II diabetes patients exhibited higher inflammatory markers and a dysregulated immune response versus patients without diabetes. Analysis of these results suggests that chronic low-grade inflammation and an associated dysregulated immune response might be responsible for the higher level of inflammatory response in COVID-19 patients that leads to severe infection.

A monocyte/dendritic cell molecular signature of SARS-CoV-2-related multisystem inflammatory syndrome in children with severe myocarditis

Multisystem inflammatory syndrome in children (MIS-C) develops as an acute pathology of SARS-CoV-2 infection, though a better understanding of disease pathophysiology is needed. Researchers at the Imagine Institute in Paris combined cytokine measurements, deep immune cell phenotyping and transcriptomics analyses at the single-cell level to investigate SARS-CoV-2-related conditions in children (**de Cevins et al.**).

The team compared acute infection to post-acute hyperinflammation in order to analyze pathways and molecular signatures characteristic of the most severe form of MIS-C with myocarditis. Single-cell analysis by the Maxpar Direct Immune Profiling System and RNA sequencing correlated low monocyte and dendritic cell frequencies with post-acute hyperinflammatory illness, creating an inflammatory profile for severe cases in children.

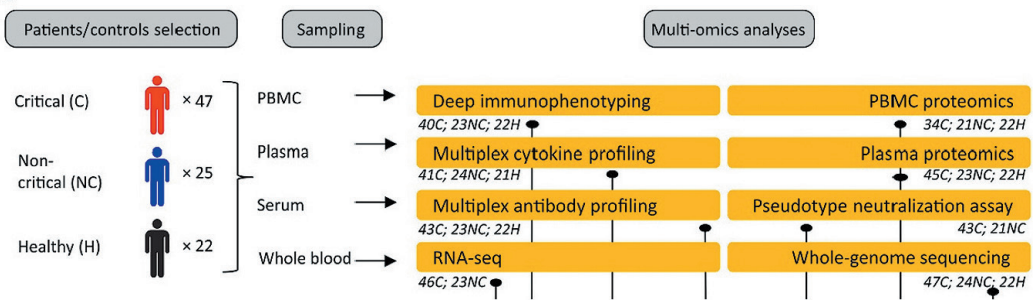

Figure 3. A multi-omics analytical strategy (Carapito et al.)

Identification of driver genes for critical forms of COVID-19 in a deeply phenotyped young patient cohort

Are drivers of severe COVID-19 different for the younger population? A collaboration from Université de Strasbourg and Harvard Medical School used multi-omics analysis and artificial intelligence to find a unique gene signature and characteristic virus-induced molecular changes that differentiate critical from noncritical patients (**Carapito et al.**).

The team used whole genome sequencing, whole blood RNA sequencing, plasma and blood mononuclear cell proteomics, cytokine profiling and high-throughput immunophenotyping with machine learning. The Maxpar Direct Immune Profiling Assay was used, where cells were stained and then stored at –80 °C until acquisition. The results detail the systemic immune response, define a transcriptomic signature that differentiates critical patients and offer potential diagnostic, prognostic and therapeutic targets to combat COVID-19.

Changes in immune response to cancer progression and immunotherapy

The longitudinal assessment of biospecimens can be crucial to identifying key players in the progression of cancer or immune response to treatment. With sample collection, staining, storage and shipping easily enabled with the Maxpar Direct Immune Profiling Assay, immune monitoring studies retain data integrity over the course of the disease or treatment period.

Immune profiling mass cytometry assay harmonization: multicenter experience from CIMAC-CIDC

The goal of the Cancer Immune Monitoring and Analysis Centers – Cancer Immunologic Data Commons (CIMAC-CIDC) Network is to identify relevant biomarkers in response to cancer immunotherapies for use in clinical trials. Mass cytometry is a primary platform used in these studies and is performed at all CIMAC laboratories.

Multistep cross-site harmonization for this research was an important quality control step to ensure CyTOF data could be directly compared across sites (**Sahaf et al.**). The Maxpar Direct Immune Profiling Assay supported this effort with a standardized assay and protocol that could easily be adapted to the needs of the study—using the base 30-antibody panel that can be augmented with multiple additional antibodies of interest to particular studies within the program.

Watch this presentation in which Holden Maecker describes the results and lessons learned from the study's harmonization efforts, as well as plans for using the Maxpar Direct Immune Profiling Assay in future CIMAC studies.

CyTOF in the Cancer Immune Monitoring and Analysis Centers (CIMAC) | Holden Maecker, PhD

30-marker* panel with clones and metals

Antibody	Clone	Metal
CD45	HI30	^{89}Y
Live/dead indicator	N/A	^{103}Rh
CD196 (CCR6)	G034E3	^{141}Pr
CD123	6H6	^{143}Nd
CD19	HIB19	^{144}Nd
CD4	RPA-T4	^{145}Nd
CD8a	RPA-T8	^{146}Nd
CD11c	Bu15	^{147}Sm
CD16	3G8	^{148}Nd
CD45RO	UCHL1	^{149}Sm
CD45RA	HI100	^{150}Nd
CD161	HP-3G10	^{151}Eu
CD194 (CCR4)	L291H4	^{152}Sm
CD25	BC96	^{153}Eu
CD27	O323	^{154}Sm
CD57	HCD57	^{155}Gd

Antibody	Clone	Metal
CD183 (CXCR3)	G025H7	^{156}Gd
CD185 (CXCR5)	J252D4	^{158}Gd
CD28	CD28.2	^{160}Gd
CD38	HB-7	^{161}Dy
CD56 (NCAM)	NCAM16.2	^{163}Dy
TCRγδ	B1	^{164}Dy
CD294	BM16	^{166}Er
CD197 (CCR7)	G043H7	^{167}Er
CD14	63D3	^{168}Er
CD3	UCHT1	^{170}Er
CD20	2H7	^{171}Yb
CD66b	G10F5	^{172}Yb
HLA-DR	LN3	^{173}Yb
IgD	IA6-2	^{174}Yb
CD127	A019D5	^{176}Yb

* 31 markers including the ^{103}Rh live/dead indicator

18 open channels for panel customization

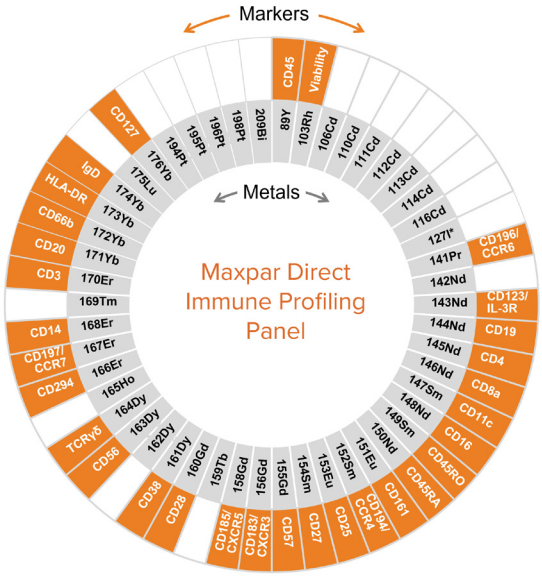

Figure 4. Maxpar Direct Immune Profiling Assay setup (from Holden Maecker)

Humoral and cellular correlates of a novel immune-related adverse event and its treatment

A group at the University of Texas Southwestern Medical Center examined whether humoral and cellular immune biomarkers may inform toxicity monitoring and management in patients receiving immune checkpoint inhibitor therapies (**Gonugunta et al.**). Using high-dimensional mass cytometry with the Maxpar Direct Immune Profiling Assay, the team performed longitudinal immune cell profiling to compare patients with immune-related adverse events after treatment to those with no toxicity and healthy controls. Their CyTOF panel contained the 30 standard markers plus 6 additional markers customized to relevant cell types.

They determined that preceding any toxic response, patients can develop broad increases in cytokines, autoantibodies, CD8 T cells and plasmablasts. Additionally, the study captured the biologic effects of corticosteroids on these parameters, showing profound decreases and indicating effective administration.

Mass cytometry: a robust platform for the comprehensive immunomonitoring of CAR-T-cell therapies

To address the challenge of defining the immune mechanisms that underlie the success of CAR T cell therapy, scientists (**Corneau et al.**) at the Hôpital Pitié-Salpêtrière presented the first application of mass cytometry to the comprehensive immune monitoring of CAR T cell therapies.

The team customized the standardized Maxpar Direct Immune Profiling Assay panel by developing metal-tagged antibodies that identify CAR T cells. The panel was then validated to support comprehensive immune monitoring after CAR T cell administration. The combination of antibodies in the customized panel allowed characterization of CAR and non-CAR immune cells and their functional states, in a single pass and over time.

Watch a recent webinar on this publication:

Mass cytometry: A robust platform for the comprehensive immunomonitoring of CAR T cell therapies | Jonathan Caron, PhD

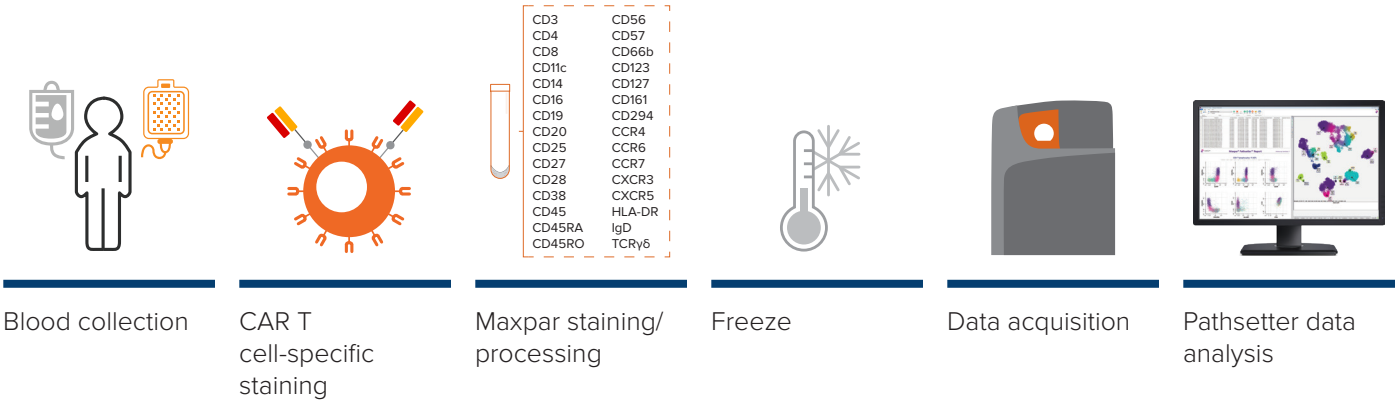

Figure 5. Immune profiling by cytometry workflow

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De Cevins, C. et al. “A monocyte/dendritic cell molecular signature of SARS-CoV-2-related multisystem inflammatory syndrome in children with severe myocarditis.” *Med* 2 (2021): 1,072–1,092.e7.

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Maxpar Direct Immune Profiling System: Publications, Preprints, and National Clinical Trials, March 2022.

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Sahaf, B. et al. “Immune profiling mass cytometry assay harmonization: multicenter experience from CIMAC-CIDC.” *Clinical Cancer Research* 27 (2021): 5,062–5,071.

Sengupta, S. et al. “Underlying co morbidity reveals unique immune signatures in Type II diabetes patients infected with SARS-CoV2.” *medRxiv* (2021): doi:10.1101/2021.12.03.21267282.

National Clinical Trials

Antidepressant Trial with P2X7 Antagonist JNJ-54175446 (ATP) (NCT04116606) Sponsor: CCTU-Core

COntAGIouS (COvid-19 Advanced Genetic and Immunologic Sampling) Trial (NCT04327570) Sponsor: Universitaire Ziekenhuizen Leuven

COVID-19: Pediatric Research Immune Network on SARS-CoV-2 and MIS-C (PRISM) (NCT04588363) Sponsor: National Institute of Allergy and Infectious Diseases (NIAID)

IMPACC (Immunophenotyping Assessment in a COVID-19 Cohort) Clinical Study (NCT04378777) Sponsor: National Institute of Allergy and Infectious Disease (NIAID)

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CORPORATE HEADQUARTERS
2 Tower Place, Suite 2000 | South San Francisco, CA 94080 USA
Toll-free: 866 359 4354 in the US and Canada | Fax: 650 871 7152

SALES
North America | +1 650 266 6170 | info-us@standardbio.com
Europe/Middle East/Africa/Russia | +33 1 60 92 42 40 | info-europe@standardbio.com
Latin America | +1 650 266 6170 | info-latinamerica@standardbio.com
Japan | +81 3 3662 2150 | info-japan@standardbio.com
China (excluding Hong Kong/Macau) | +86 21 3255 8368 | info-china@standardbio.com
All other Asia-Pacific countries | +1 650 266 6170 | info-asia@standardbio.com

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