APPLICATION NOTE

Bruker Proteomic Product Suite for Infectious Disease

Breakthroughs in Developing Vaccines, Protective Immune Monitoring, & Measuring Toxicity

In this Application Note we outline:

- The lethal impact of cytokine storm among infectious diseases
- Targeting cytokines for the prediction of cytokine storm
- Current challenges in vaccine development, immune monitoring of patients, and prediction of immune related adverse events.
- How single-cell proteomics provides predictive analysis of vaccines that create protective response
- Immune monitoring of vaccine response for early & predictive response
- Cellular & cytokine level monitoring for toxicities related to cytokine storm

BRUKER

Prep, Run, Analyze

High Level Challenges and Applications

Application 1: Vaccines developed to create protective T cell response

Application 2: Cellular immune monitoring for protective response early in patients

Application 3: Cellular prediction and cytokine level monitoring for toxicities related to cytokine storm

Bruker Product Types that Address These Challenges:

Single-Cell Adaptive Immune (Mouse and Human)

Single-Cell Innate Immune

CodePlex Secretome

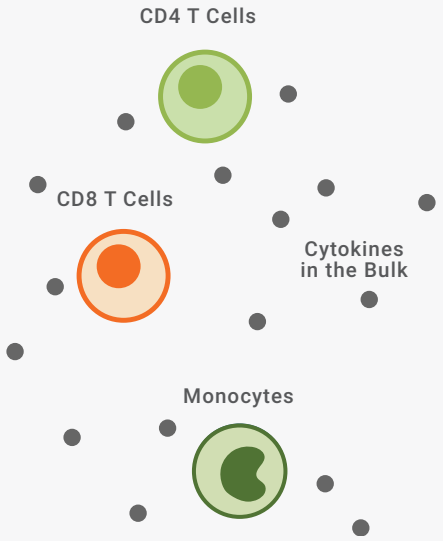
Overcoming Challenges in Infectious Disease

There are several challenges within infectious disease research and the development of vaccines, protective immune monitoring, and measuring toxicity. These challenges involve vaccines developed to create protective T cell response, cellular immune monitoring for protective response early in patients, and cellular prediction and cytokine level monitoring for toxicities related to cytokine storm. The IsoLight system provides a solution for the single-cell and accelerated population level functional proteomics required to overcome these challenges.

IsoLight is the only system that can enable researchers to get the highly multiplexed cytokine data with no advanced training, and no interaction with the samples. Furthermore, the IsoLight is also the only system to perform multiplexed proteomic detection of 30+ cytokine markers simultaneously, to provide early predictive metrics of these functional and inflammatory cytokines, in an automated, all-in-one system, for increased work-away time. This system can handle a smaller amount of sample volume if large blood draws are not possible, making it capable of handling a wider range of clinical sample sizes.

By functionally defining each cell type involved in the immune response, researchers can better understand the functional mechanisms for the development of patient biomarkers, vaccines, and novel therapies for infectious disease.

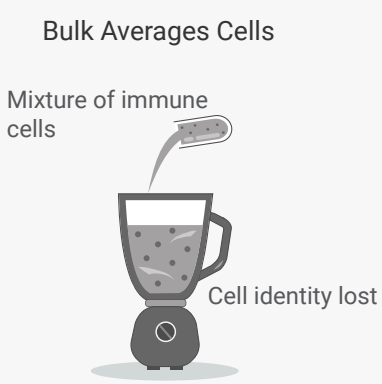
Cell Types and Cytokines Implicated

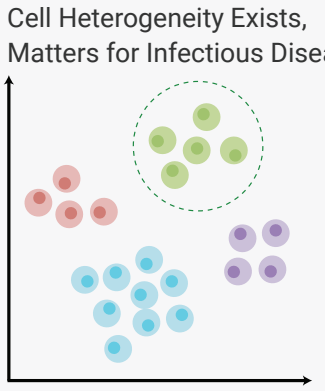


Functionally defining each cell type involved in the immune response for infectious disease.

- **Challenges 1 & 2: Requires Single-Cell Functional Proteomics**
- **Challenge 3: Requires Highly Multiplexed Codeplex Secretome Solution**

Why Cell Subsets for Multiplexing Cytokines Matters for Infectious Disease





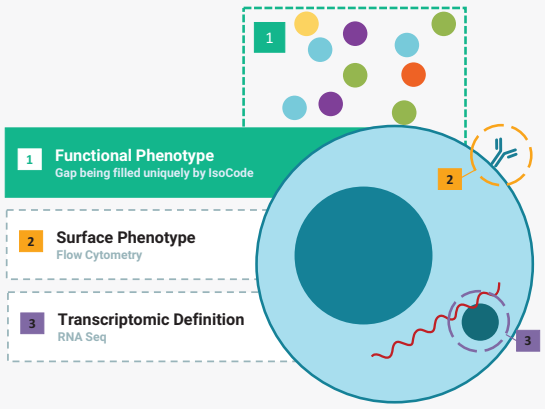
Traditional technologies average serum protein information from all cells. Bruker's cellular functional phenotyping uncovers cellular differences to identify functional mechanisms in the immune response for infectious disease.

Understanding Cellular Immune Function is Critical for Predicting Protective Immunity

Traditional technologies average serum protein information from all cells. In a variety of trials†, stratification of responders from non-responders is not possible with status quo technologies. Data shows that what specific cytokines are produced by each heterogenous immune cell matters, and Bruker's cellular functional phenotyping uncovers these cellular differences.

Through analysis of cellular RNA or surface phenotypes alone, you may be missing essential functional extracellular phenotypic differences that reveal the biological drivers of patient response. Bruker's single-cell functional proteomics fills the existing gap in complete cellular characterization.

Multiplexed Proteomic Characterization: Filling the Existing Gap in Full Cellular Characterization from Single-Cells



Through analysis of cellular RNA or surface phenotypes alone, functional extracellular phenotypic differences that reveal the biological drivers of patient response may be missed.

Prep, Run, Analyze

Detecting Multiplexed Serum Protein is Critical in Predicting Cytokine Storm & Toxicity

The IsoLight is the only system that enables researchers to obtain highly multiplexed cytokine data without advanced training and without interaction with the samples. Furthermore, the IsoLight is also the only system to:

(1) Perform multiplexed proteomic detection of the above 30+ cytokine markers simultaneously, to provide early predictive metrics of functional and inflammatory cytokines

(2) Provide an automated, all-in-one system, for increased work-away time

(3) Handle a smaller amount of sample volume if large blood draws are not possible, making it capable of handling a wider range of clinical sample sizes

By functionally defining each cell type involved in the immune response, researchers can better understand the functional mechanisms for the development of patient biomarkers, vaccines, and novel therapies for infectious disease.

CodePlex Secretome Cytokine Storm Panel

Panel Menu

IL-17A	IL-4
MIP-1α	IL-5
IL-9	IL-10
MIP-1β	TNF-α
IL-6	MCP-1
IL-7	IL-13
IL-8	IL-2
IFN-g	Perforin
IP-10	
GM-CSF	

Status Quo Multiplexed Bulk Analysis
X Up to 100-200 uL per sample (for replicates)
X 6-10 hours of hands-on sample prep time
X Workflow requires multiple steps and user interaction points
X Fill 96 samples before run
X Multiple systems required to generate and analyze data
√ Limit of Detection: 5-5000 pg/ml
X Data analysis and visualizations require much user input and are not automated

CodePlex Secretome
√ 11 uL per samples (for replicates)
√ 5 minutes of hands-on time
√ Completely automated workflow
√ Modular, load 8-64 samples per run
√ One system: The IsoLight
√ Limit of Detection: 5-5000 pg/ml
√ State-of-the-art data analysis software with advanced visualizations

The CodePlex Secretome Solution measures 30+ cytokines in bulk, automated on the IsoLight system, and can selectively run eight conditions a chip in “MacroChambers” across eight chips on a single run. Easily run replicates with a small sample volume: 5.5 uL per microwell (11 uL per sample replicate).

Prep, Run, Analyze

Application 1 – Accelerating Vaccine Development

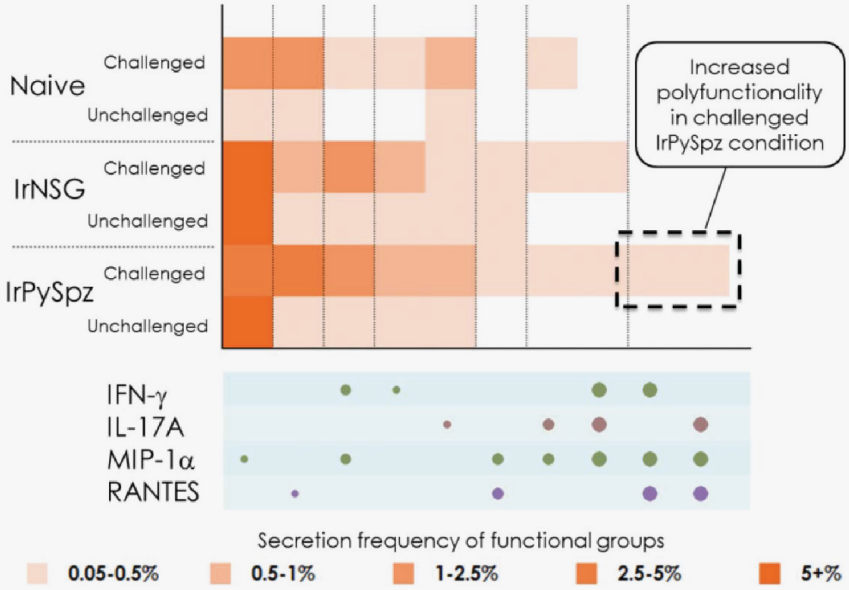
Single-Cell Proteomics Provides Predictive Analysis of Vaccines that Create Protective Response

Products Used

Single-Cell Adaptive Immune (Mouse)

Single-Cell Heatmap Predicts Malaria Vaccine Response with Highly Polyfunctional T Cell Subsets

Anti-Malaria Protection from Hepatic Polyfunctional CD8⁺ T Cells Induced by the Vaccine



Single-cell polyfunctional heatmap shows unique and highly polyfunctional hepatic CD8⁺ T cell subsets with the dominant profile of MIP-1α, RANTES, IFN-γ, and/or IL17A, which were induced in a group of mice that received IrPySpz immunization and live PySpz challenge [1].

Using More Predictive Single-Cell Proteomics in Mouse Models to Understand Correlative T Cell Potency in Infectious Disease Vaccines

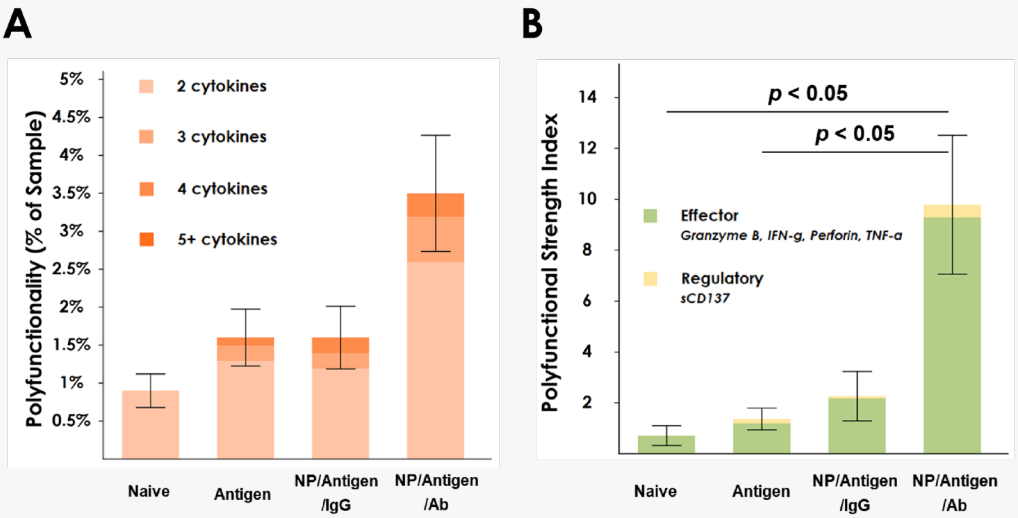
- Malaria vaccines are difficult to create & analyze in hepatic T cell response.
- Single-cell proteomics reveals predictive potency in polyfunctional subsets of cells.
- Heatmapping visualizations can pick which vaccines meet protective criteria.

Application 1 – Accelerating Vaccine Development (Cont.)

Products Used

Single-Cell Adaptive Immune (Human)

Single-Cell CD8⁺ Polyfunctionality is Upregulated in Nanoparticle Vaccinated Humanized Mouse Group, Correlating to *in vivo* Response



Single-cell CD8⁺ polyfunctionality and PSI are upregulated in NP-vaccinated mouse group relative to naïve controls. (A) A robust upregulation of polyfunctional (secreting multiple cytokines) human CD8⁺ T cells in HIS mice immunized with NP/Antigen/Ab was observed, compared to other groups of HIS mice. (B) Comparing the adaptive immune index (PSI) induced by NP/Antigen/Ab vaccine relative to other groups. We observed a statistically significant difference between the NP/Antigen/Ab group and both the naïve and antigen groups. The enhanced PSI was predominated by effector proteins [2].

Bruker System Used in Pre-Clinical Setting to Generate Predictive Data in Humanized Mice

- Nanoparticle-based melanoma vaccine demonstrated the capability of inducing a potent antigen specific human CD8⁺ T cell response in HIS mice.
- Single-cell functional proteomics sensitively reveals distinct polyfunctional cell subsets with human cytokine signatures that drove anti-tumor CD8⁺ T cell response by the NP vaccine in HIS mice.
- Polyfunctional human CD8⁺ T cell responses sensitively demonstrated successful induction of the tumor vaccine in HIS mice, correlating to *in vivo* response.

Application 2 – Immune Monitoring: Immune Monitoring of Vaccine Response for Early & Predictive Response in Publications

Products Used

- Single-Cell Innate Immune (Human)
- Single-Cell Adaptive Immune (Human)

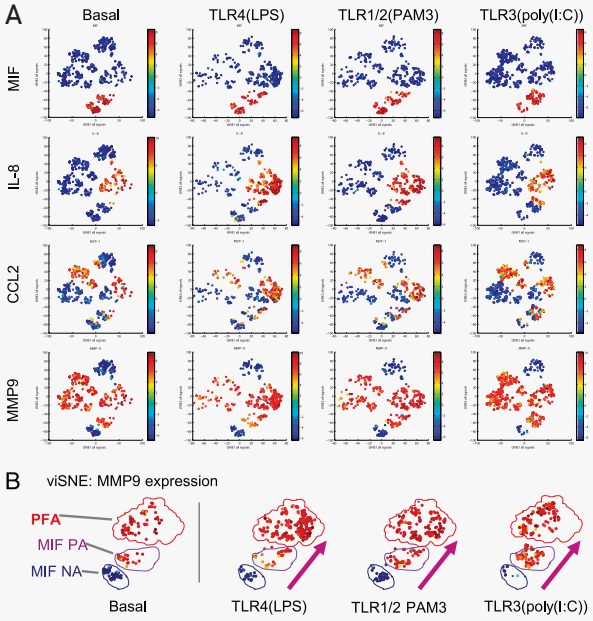
Highlights of Protective Response in Monitoring with Single T Cell Subsets

- Upregulation of polyfunctionality and PSI in CD4⁺ T cells after GVAX Vaccine treatment
- Patients showed varying levels of CD4⁺ T cell polyfunctional upregulation post vaccination
- Post- versus pre-vaccination fold-change of PSI significantly associated with patient overall survival (p = 0.001)

The therapeutic GVAX vaccine boosts the body's immune system T cells. However, It remains challenging to identify clinical correlates that can sensitively detect T cell functional kinetics in patients post GVAX vaccination.

Single-cell highly multiplexed proteomic profiling provides a comprehensive assessment of T cell function and identifies functional drivers between pre- and post-vaccination CD4⁺ T cells as a novel correlate to overall survival with GVAX treatment [3].

Macrophages, Early Responders to Infection, are Functionally Heterogeneous in Response to Pathogen



Profiling of the same single-cells before and after LPS stimulation identified a role for macrophage inhibitory factor (MIF) to potentiate the activation of LPS-induced cytokine production [4].

Highlights of Looking at Macrophage Functional Phenotyping, the Early Innate Responders, in Infectious Disease

- Advanced cellular visualizations and mapping revealed five distinct subpopulations of response to pathogenic ligands.
- Different pathogenic ligands induced varying levels of potency and response, and each resulted in upregulation of different cytokines.
- Phenotypically similar cell populations are potentially functionally and behaviorally heterogenous, further showing the need for single-cell functional phenotyping of immune activation in response to pathogens.

Application 3 – Predicting Cytokine Storm Infectious Disease with Cellular and Serum Cytokine Monitoring

Products Used

Single-Cell Innate Immune

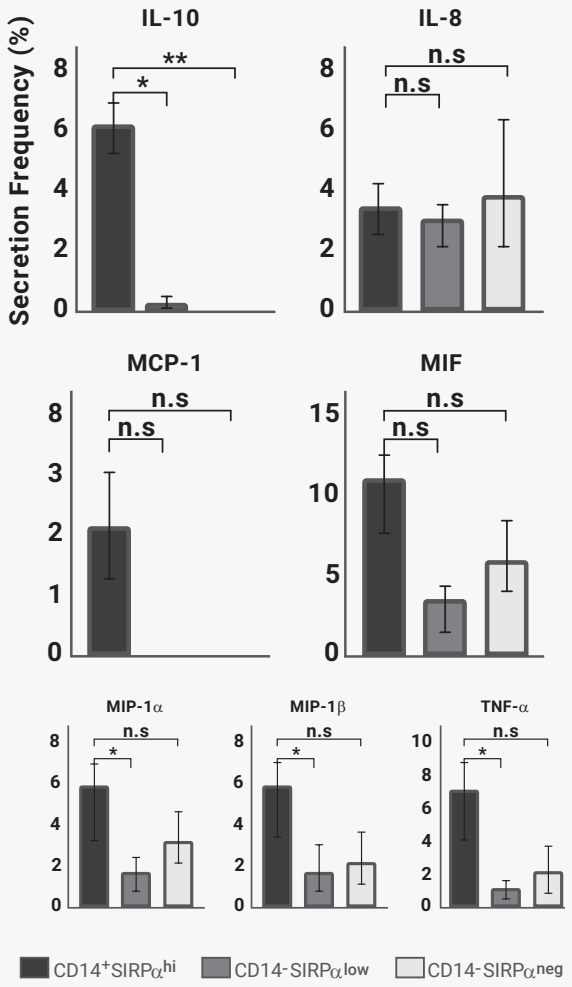
CodePlex Secretome

Bruker can uniquely measure the multiplexed cytokine based functionality of monocytes. Recent studies [7,8] further reveal the crucial roles of dysfunctional immune cells such as $CD4^+$ and $CD8^+$ T cells as well as monocytes/macrophages in the inflammation cascades and subsequent cytokine storm in the patients infected with COVID-19. For example, the data shows hyperactivated $CD4^+$ and $CD8^+$ T cells with marked increases of IL-17 secretion and cytotoxic granules, respectively, in blood. In addition, these aberrant $CD4^+$ T cells upon activation can become more pathogenic and release a large amount of GM-CSF, which therefore promotes activation of $CD14^+CD16^+$ monocytes. These inflammatory monocytes released high amounts of cytokines and chemokines such as IL-6 and GM-CSF, thus subsequently accelerating the inflammation leading to immune damages of organs [9].

Highlights of Prognostic Response with Single Monocyte Function

- Unique mechanisms displayed by the Bruker platform, and in particular the innate and myeloid panel, show monocyte functionality plays a key role in progression of disease
- Inhibitory mechanism of enhanced IL-10 in single-cell polyfunctional $CD14^+$ $SIRP\alpha^{hi}$ subsets for T cell proliferation revealed by Bruker's platform, provides the functional insights of Mo/MΦ subsets, which were implicated in inferior survival of patients. In this case, the cellular IL-10 level shows critical mechanistic importance of monocyte function in the interaction with T cell functional response, which correlated with patients who had inferior survival

Cytokine/Chemokine Production by $CD14+SIRP\alpha^{hi}$, $CD14-SIRP\alpha^{low}$, or $CD14-SIRP\alpha^{neg}$ cells



Cytokine/chemokine production by $CD14+SIRP\alpha^{hi}$, $CD14-SIRP\alpha^{low}$, or $CD14-SIRP\alpha^{neg}$ cells determined by the the Single-Cell Innate Immune solution. Results were expressed as the percentage of cells producing cytokines/chemokines. *p < 0.05; **p < 0.01; n.s. no significant difference. N = 3.[5].

Application 3 – Predicting Cytokine Storm (Cont.)

Fully Automated Functional Protemics for Predicting and Treating Cytokine Storm Impact of COVID-19 Disease

Lethal Impact of Cytokine Storm in COVID-19 Patients:
Recent published data [6-8] in The Lancet reports the clinical features of patients infected with COVID-19 and highlights the correlation of high levels of circulating inflammatory cytokines, or "cytokine storm," with severity of illness in the infected patients. The data demonstrates that the patients who were admitted to the intensive care unit (ICU), particularly those with severe disease, exhibited significantly higher levels of inflammatory cytokines compared to those that did not require ICU.

Stratifying predictive markers for ICU patients: IL-2, IL-7, IL-10, G-CSF, IP-10, MCP-1, MIP-1α, and TNF-α were higher in ICU patients than non-ICU patients, forming the basis for a manner to predict which patients would go to the ICU, with a highly multiplexed protein assay.

Stratifying predictive markers for challenged patients, vs. healthy adults: IL-1β, IL-1RA, IL-7, IL-8, IL-9, IL-10, basic FGF, G-CSF, GM-CSF, IFN-γ, IP-10, MCP-1, MIP-1α, MIP-1β, PDGF, TNF-α, and VEGF concentrations were higher in COVID patients than in healthy adults.

Defining Cytokine Storm and History in SARS & MERS:
"Cytokine storm" triggers a viral sepsis in coronavirus infection, where viral replication and excessive, uncontrolled systemic inflammation can lead to pneumonitis, acute respiratory distress syndrome, respiratory failure, shock, multiple organ failure, secondary bacterial pneumonia, and potentially death. This same correlation between cytokine storm and severity of illness was observed previously in both severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) patients with coronavirus infection.

Patients with Cytokine Storm, if Predicted, Can Be Assessed and Treated:
The prediction of cytokine storm can help in the assessment of potentially severely ill patients before they progress. Publications on cancer immunotherapy patients involving multiplexed protein analysis have shown the ability to predict cytokine storm [9]. Additionally, there are treatments available for cytokine storm, such as anti-IL6, which has been historically successful in cancer immunotherapy patients [10].

Challenges & Applications

Application 1: Vaccines developed to create protective T cell response

Application 2: Cellular immune monitoring for protective response early in patients

Application 3: Cellular prediction and cytokine level monitoring for toxicities related to cytokine storm

Solutions

- Single-cell proteomics provides predictive analysis of vaccines that create protective response
- Single-cell immune monitoring of vaccine response for early & predictive response using Bruker's functional proteomics
- Bruker's platform provides cellular & cytokine level monitoring for toxicities related to cytokine storm

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