APPLICATION NOTE

Bruker Proteomic Product Suite for Neuroinflammation

Bruker's functional proteomics reveal unique secretomic signatures and insights into neuroinflammation

In this Application Note we outline:

- Overcoming challenges in neuroinflammation
- Understanding the mechanism of disease progression in neuroInflammation
- The neural impact of T cells and inflammatory cytokines in Alzheimer's disease
- The role of innate immunity in Multiple Sclerosis (MS) pathogenesis
- Revealing biomarkers of neurotoxicity and Immune Related Adverse Events (IRAEs)
- Insights from functional phenotyping of microglia in neuroinflammation
- Neurological manifestations of COVID-19 in patients
- Informing targeted combination therapy to overcome resistance in Glioblastoma (GBM) with single-cell intracellular proteomics

BRUKER

Prep, Run, Analyze

High Level Challenges and Applications

Application 1: Understanding Mechanism of Disease Progression in NeuroInflammation

Application 2: Neural Impact of T Cells and Inflammatory Cytokines in Alzheimer's Disease

Application 3: The Role of Innate Immunity in MS Pathogenesis

Application 4: Biomarkers of Neurotoxicity and IRAEs

Application 5: Functional Phenotyping of Microglia in Neuroinflammation

Application 6: Neurological Manifestations of COVID-19 in Patients

Application 7: Informing Targeted Combination Therapy to Overcome Resistance in GBM

Bruker Product Types That Address These Challenges:

Single-Cell Secretome

CodePlex Secretome

Single-Cell Intracellular Proteome

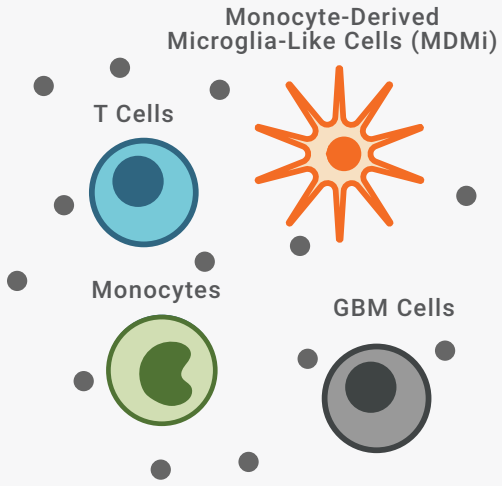
- **Challenge 1-5: Requires Single-Cell Secretome Solution**
- **Challenge 6: Requires Highly Multiplexed CodePlex Secretome Solution**
- **Challenge 7: Requires Single-Cell Intracellular Proteome Solution**

Overcoming Challenges in Neuroinflammation

Neuroinflammation is a complex biological response to nervous tissue injury involving many inflammatory mediators, such as cytokines, chemokines, and many different cell types. It is well accepted that neuroinflammation plays a key pathogenetic role in a number of neurological disorders and several neurodegenerative diseases.

Heterogeneity in cytokine production and aberrant cytokine signatures can create challenges in assessing neuroinflammation. Bruker's platform is helping leaders accelerate their workflows by functionally defining each cell type to inform more effective therapies.

Cell Types and Cytokines Implicated



Functionally defining each cell type involved in the immune response for neuroinflammation

Why Cell Subsets for Multiplexing Cytokines Matter in Neuroinflammation

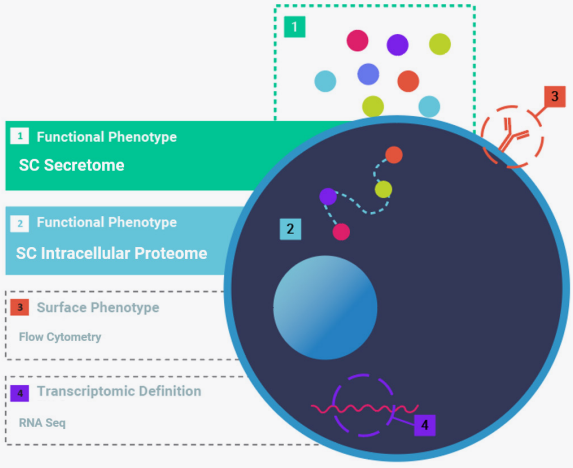
Traditional technologies average serum protein information from all cells. Bruker's cellullar functional phenotyping uncovers cellular differences to identify functional mechanisms in neuroinflamation and neurotoxicity..

Understanding Cellular Immune Function is Critical for Understanding Pathways of Neurotoxity and Neuroinflammation

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 which specific cytokines are produced by each heterogenous immune cell matters, and Bruker's cellullar 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 neuroinflammatory 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 and intracellular phenotypic differences that reveal the biological drivers of patient response may be missed.

Prep, Run, Analyze

Detecting Multiplexed Serum Protein from Ultra Low Sample Volume is Critical in Predicting Response in Neuroinflammation

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 20-40 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 and novel therapies in neuroinflammation.

CodePlex Secretome Panels

Panel Menu

Granzyme B, IFN-y, MIP-1a, Perforin, TNF-a, TNF-B, GM-CSF, IL-2, IL-5, IL-7, IL-8, IL-9, IL-12, IL-15, IL-21, CCL11, IP-10, MIP-1B, RANTES, IL-4, IL-10, IL-13, IL-22, sCD137, sCD40L, IL-1B, IL-6, IL-17a, IL17F, MCP1, MCP-4, IL-18, TGF-a, BCA-1, IL-12-p40, MIF, EGF, PDGF-BB

Human Adaptive Immune

IL-17A, MIP-1a, IL-9, MIP-1b, IL-6, IL-7, IL-8, IFN-y, IP-10, GM-CSF, IL-4, IL-5, IL-10, TNF-a, MCP-1, IL-13, IL-2, Perforin, sCD137, TNF-b, Granzyme B, IL-15

Human Innate Immune

IL-17A, MIP-1a, MIP-1b, IL-6, IL-7, IL-8, IFN-y, IP-10, GM-CSF, IL-4, IL-5, IL-10, TNF-a, MCP-1, IL-2, Perforin, sCD40L, sCD137, TNF-b, Granzyme B, IL-15, PDGF-BB

Human Cytokine Storm Panel

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

Stem Cell Signaling

IL-17A, MIP-1a, MIP-1b, IL-6, IL-8, IFN-y, GM-CSF, IL-4, IL-10, TNF-a, MCP-1, IL-2, IL-15, Rantes (MPN), IL1a, IL1b, IL12, CCL2, CXCL5 * (MPN)

Cancer Signaling

IL-6, IL-7, IFN-y, IL-4, IL-5, IL-10, TNF-a, MCP-1, IL-13, IL-2, EGF, PDGF-BB, Rantes (MPN), MIF, FGF, HGF, IL1a, IL1b, IL12

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
X	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

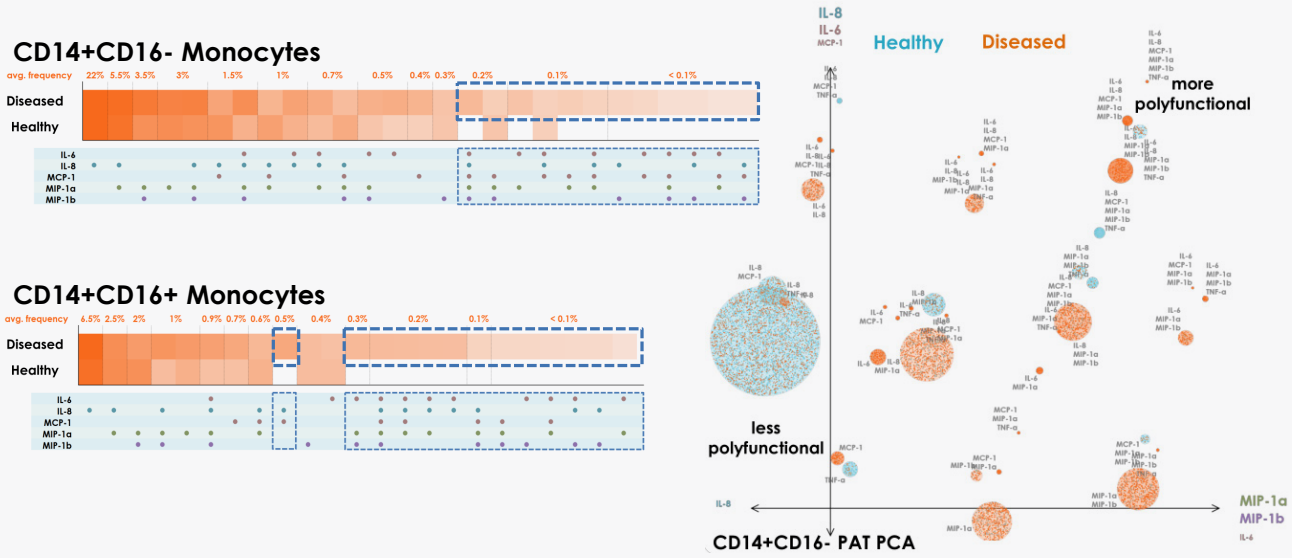
Application 1 – Understanding Mechanism of Disease Progression in NeuroInflammation

Functional Phenotyping Reveals Differences between Circulating Monocytes in Patients with Frontotemporal Degeneration (FTLD)

Products Used

Single-Cell Secretome

Polyfunctional Responses of CD14+ Monocytes are Strongly Associated with FTLD



Increased inflammatory polyfunctional monocyte subsets with unique cytokine signatures provide a potential basis for biomarkers for peripheral immune pathology unique to FTLD.

Applying Single-Cell Secretome to FTLD

- Single-cell polyfunctional heatmap reveals a more diverse secretion profile of both CD14+CD16- and CD14+CD16+ monocytes within diseased patients.
- Single-Cell Secretome analysis revealed increased polyfunctional profiles of monocyte subsets in FTLD patients compared to healthy control.
- Increased inflammatory polyfunctional monocyte subsets with unique cytokine signatures provide a potential basis for biomarkers for peripheral immune pathology unique to FTLD.

Liu et al. Single-cell Multiplex Proteomics Identifies Functional Differences between Circulating CD14+ CD16- and CD14+CD16+ Monocytes in Patients with Frontotemporal degeneration, Providing Basis for Understanding Disease Progression. FOCiS, 2019.

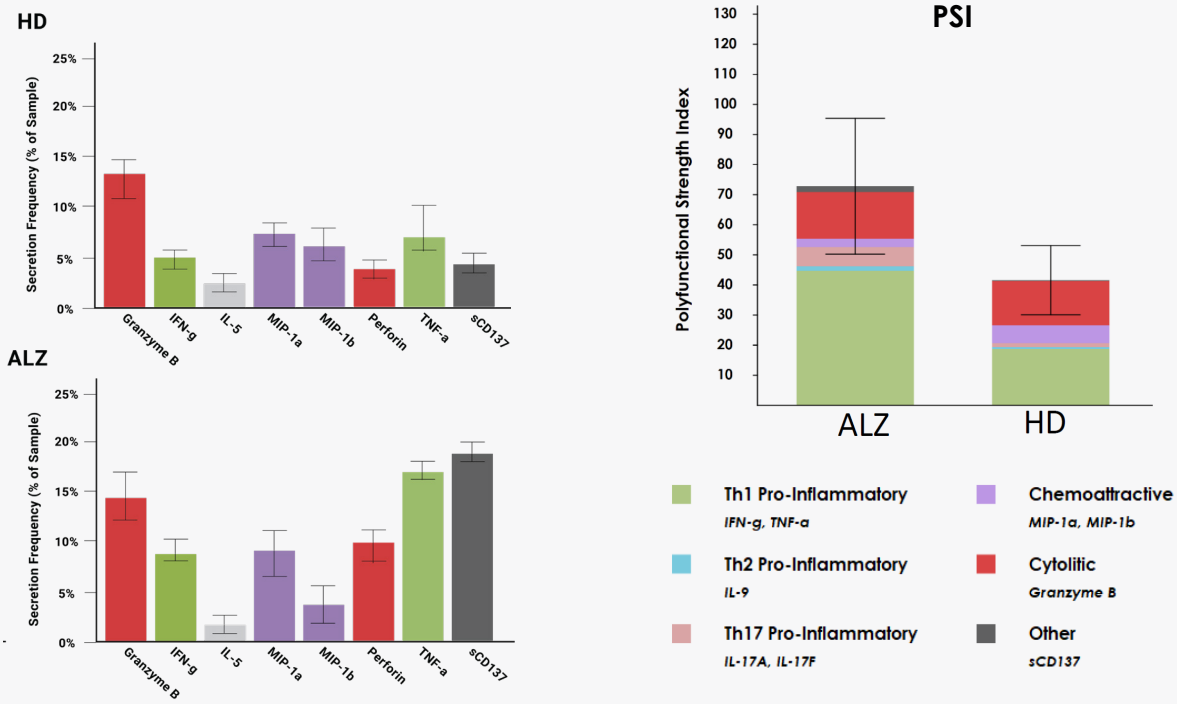
Application 2 – Neural Impact of T Cells and Inflammatory Cytokines in Alzheimer's Disease

Functional Phenotyping Reveals Biomarkers for the Immune Monitoring of Alzheimer's

Products Used

Single-Cell Secretome

Biomarker Identification in Alzheimer's Disease



Left: Cytokine secretion frequency of CD8 T cells in healthy donors (top) versus Alzheimer's patients (bottom). Right: Polyfunctional Inflammation Index of CD8 T cells in Alzheimer's patients versus healthy donors.*

Highlights of Bruker's Functional Proteomics and Biomarker Discovery in Alzheimer's Disease

- Single-Cell Secretome revealed increased polyfunctionality in Alzheimer's disease versus age matched healthy donors.
- Upregulation of Th1 and Th17 pro-inflammatory cytokines and downregulation of chemoattractive cytokines drove polyfunctional heterogeneity.

***Internal Bruker data.**

Application 3 – The Role of Innate Immunity in Multiple Sclerosis (MS) Pathogenesis

Assessing Dysfunctional Innate Immune Cell Subsets in MS with Functional Phenotyping

Products Used

Single-Cell Secretome

Highlights of Identifying Inflammation of Subsets of Monocytes in MS

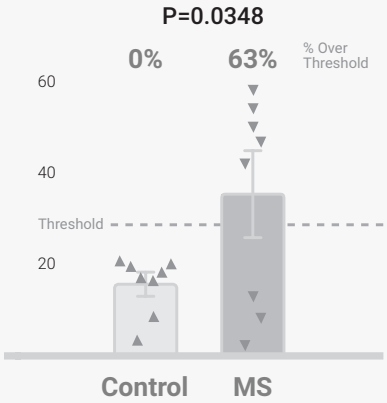
- Single-Cell Secretome identified a pathologically and therapeutically relevant Toll-like receptor (TLR) innate immune abnormality previously uncharacterized in MS.
- Data suggests that the highest frequency of enhanced TLR2 responders within the MS cohort may be found among the patients with progressive forms of the disease.
- The ability to stratify MS patients based on monocyte functionality may be especially powerful given the observed differences in therapeutic response for those with the different forms of the disease.

Fujiwara M, et al. Enhanced TLR2 Responses in Multiple Sclerosis. Clinical and Experimental Immunology, 2018

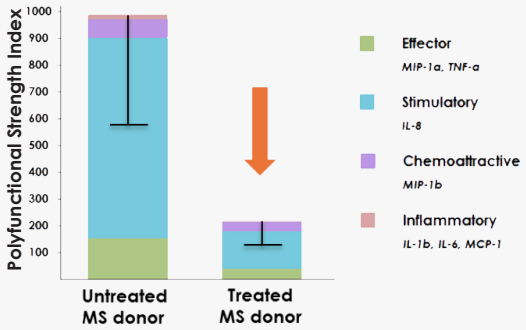
Zhou, et al. ImmunoTx., 2018

Inflammation of Subsets of Monocytes Exist in an Upregulated Manner in MS Patients

Monocytes in MS



Treatment Response in MS



On-treatment MS patients showed deep downregulation of the monocyte polyfunctionality. PSI correlated as an indicator of on treatment/off treatment response in MS.

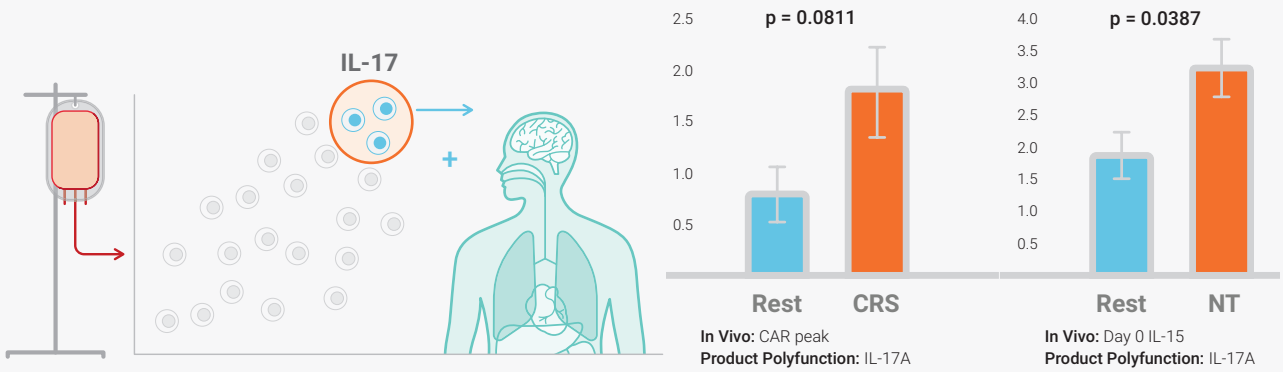
Application 4 – Biomarkers of Neurotoxicity and Immune Related Adverse Events (IRAEs)

Preinfusion CAR-T Cell Product Metrics Uniquely Correlate with Grade 3+ Cytokine Release Syndrome (CRS)

Products Used

Single-Cell Secretome

Elevated Inflammatory Functional Phenotype Shows Significant Correlation to Grade 3 CRS in CD19 CAR-T Therapy From Preinfusion Product



Association between inflammatory functional phenotype in conjunction with either CAR peak or pretreatment *in vivo* IL-15 levels in blood, and grade 3+ NT.

Highlights of Bruker's Functional Proteomics and Correlation to CRS

- Single-Cell Secretome revealed association between inflammatory functional phenotype in conjunction with either CAR peak or pretreatment *in vivo* IL-15 levels in blood, and grade 3+ NT.
- IL-17A functional phenotype combined with IL-15 levels at day 0 had a statistically significant association with grade ≥3 NT, suggesting a critical role for IL-17A producing polyfunctional cell subsets in neurologic toxicities.
- Secretion profiles from single peripheral immune cells fundamentally impact the understanding of the underlying mechanism of adverse events correlated with immunotherapies.

Rossi J, Paczkowski P, Shen Y, Morse K, Flynn B, Kaiser A, Ng C, Gallatin K, Cain T, Fan R, Mackay S, Heath JR, Rosenberg SA, Kochenderfer JN, Zhou J, and Bot A., Preinfusion Polyfunctional Anti-CD19 Chimeric Antigen Receptor T Cells Associate with Clinical Outcomes in NHL. Blood, 2018

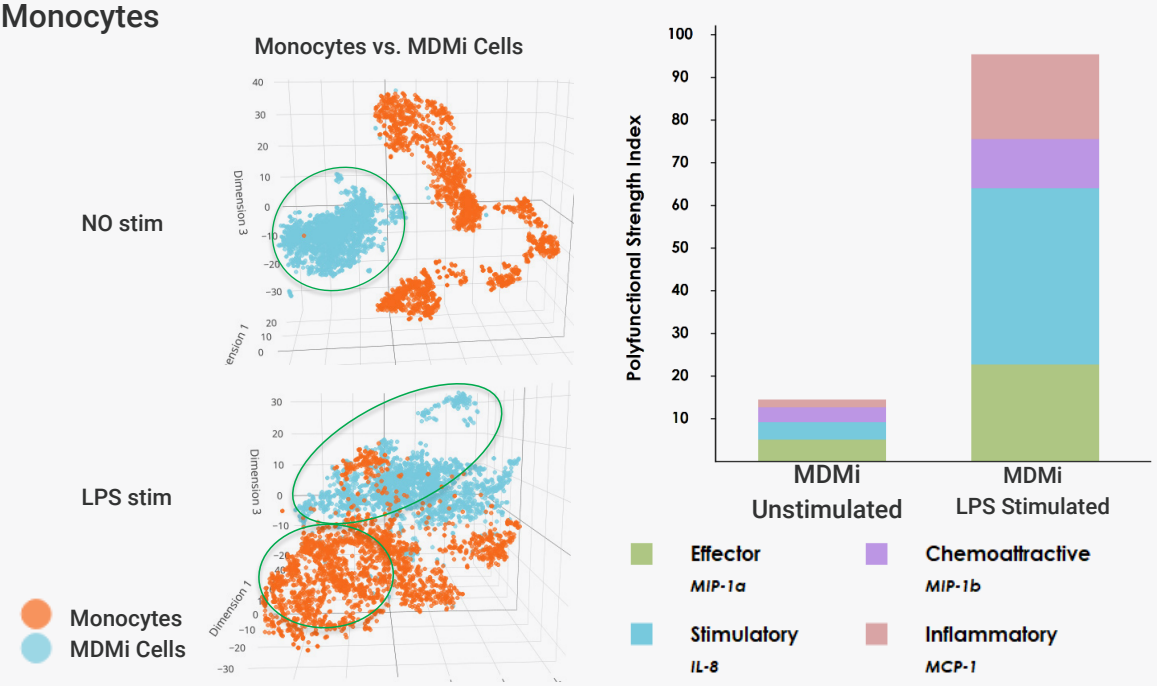
Application 5 – Functional Phenotyping of Microglia in Neuroinflammation

Bruker's Functional Proteomics Reveals Distinct Polyfunctional Profiles from MDMi Cells

Products Used

Single-Cell Secretome

MDMi Cells Exhibited a Greater Polyfunctional Profile with More Diverse Secretions than Monocytes



Left: High-dimensional t-SNE analysis of unstimulated MDMi cells versus stimulated MDMi cells. Right: Single-cell polyfunctional cytokine analysis of. unstimulated MDMi cells versus stimulated MDMi cells showing upregulation in dominant functional groups.*

Highlights of Bruker's Functional Phenotyping in Microglia

- Single-cell functional phenotyping data showed that MDMi cells exhibited a greater polyfunctional profile with more diverse secretions than monocytes.
- Enhanced polyfunctional cell subsets with distinct combinatorial cytokine secretions were observed most prominently in the MDMi LPS stimulated and Monocyte LPS stimulated samples.
- Single-Cell Secretome revealed an upregulation of PSI across MDMi cells, with a greater increase and more diverse secretions in MDMi cells in response to LPS stimulation compared to the unstimulated cells.

*Internal Bruker data.

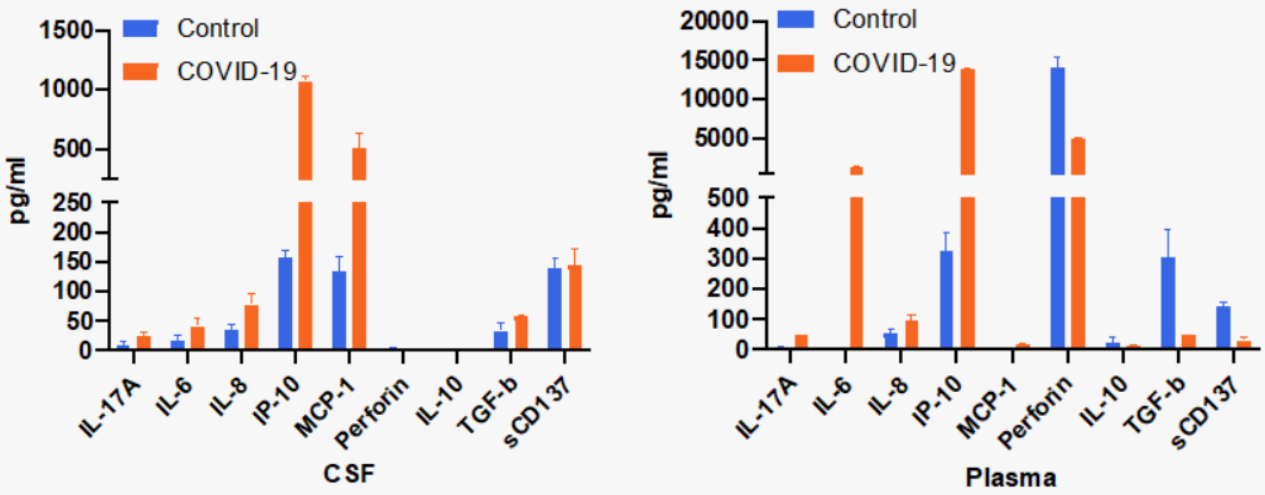
Application 6 – Neurological Manifestations of COVID-19 in Patients

Bruker's Functional Proteomics Reveals Secretomic Signatures in CSF and Plasma

Products Used

CodePlex Secretome

Acute Encephalopathy with Elevated CSF Inflammatory Markers as the Initial Presentation of COVID-19



Single-Cell Secretome secretion profiles of CSF and plasma in COVID-19 samples versus control.

Highlights of Findings of Unique Secretomic Signatures Revealed by CodePlex Secretome

- CodePlex Secretome reveals unique cytokine signatures in plasma and CSF from patients presenting with neurological disorders in COVID-19.
- Findings suggest that neurologic symptoms such as encephalopathy and seizures may be the initial presentation of COVID-19.
- Central nervous system inflammation may associate with neurologic manifestations of disease.

Farhadian, S et al. Acute encephalopathy with elevated CSF inflammatory markers as the initial presentation of COVID-19. BMC Neurology 2020.

Application 7 – Informing Targeted Combination Therapy to Overcome Resistance in Glioblastoma (GBM) with Single-Cell Intracellular Proteomics

Single-Cell Phosphoproteomics Identifies Adaptive Mechanism of Resistance

Products Used

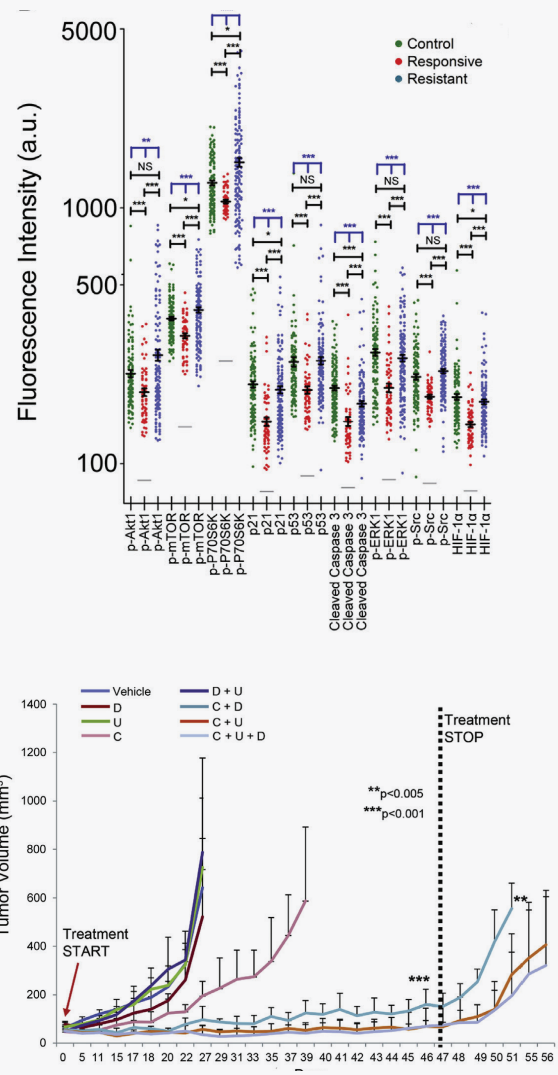
Single-Cell Intracellullar Proteome

Highlights of Informing Targeted Combination Therapies with Single-Cell Intracellular Proteomics

- Single-cell intracellular proteomics uncovers rewiring of signaling pathways, revealing dominant mechanism of resistance.
- Single-cell intracellular proteomics identifies changes in signaling nodes missed by genomic analysis.
- Targeting these signaling nodes before treatment blocks resistance, demonstrating the importance of single-cell intracellular proteomics and network re-wiring for predicting cancer treatment responses.

Wei W, et al. Single-Cell Phosphoproteomics Resolves Adaptive Signaling Dynamics and Informs Targeted Combination Therapy in Glioblastoma. Cancer Cell, 2016

Informing Better Combination Therapies to Overcome Resistance



Top: Single-Cell Intracellular Proteome Analysis of Glioblastoma. Bottom: Results for the seven monotherapy or combination therapies based upon the predictions from the Single Cell Intracellular Proteomics. All seven predictions proved correct

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Challenges & Applications	Solutions
Application 1: Understanding Mechanism of Disease Progression in NeuroInflammation	• Single-Cell Secretome reveals differences between circulating monocytes in patients with FTLD.
Application 2: Neural Impact of T Cells and Inflammatory Cytokines in Alzheimer's Disease	• Single-Cell Secretome reveals biomarkers for the immune monitoring of Alzheimer's .
Application 3: The Role of Innate Immunity in MS Pathogenesis	• Single-Cell Secretome enables assessment of dysfunctional innate immune cell subsets in MS
Application 4: Biomarkers of Neurotoxicity and IRAEs	• Single-Cell Secretome reveals association between inflammatory functional phenotype and CRS
Application 5: Functional Phenotyping of Microglia in Neuroinflammation	• Single-Cell Secretome reveals distinct polyfunctional profiles from MDMi cells
Application 6: Neurological Manifestations of COVID-19 in Patients	• CodePlex Secretome reveals secretomic signatures in CSF and plasma
Application 7: Informing Targeted Combination Therapy to Overcome Resistance in GBM with Single-Cell Intracellular Proteomics	• Single-Cell Intracellular Proteome identifies adaptive mechanism of resistance