APPLICATION NOTE

Bruker Proteomic Product Suite for Autoimmune Inflammation

Bruker's functional proteomics reveals unique secretomic signatures and insights into autoimmune inflammation

In this Application Note we outline:

- Overcoming challenges in autoimmune inflammation
- Cytokine storm and neurological manifestations of COVID-19 in patients
- The role of innate immunity in Multiple Sclerosis (MS) pathogenesis
- Altered cytokine production and functional mechanisms of IBD
- Biomarkers of neurotoxicity and Immune Related Adverse Events (IRAEs)
- Polyfunctional cytokine drivers of transplant rejection

BRUKER

APPNOTE-10 REV 1.0

Prep, Run, Analyze

High Level Challenges and Applications

Application 1: Cytokine Storm and Neurological Manifestations of COVID-19 in Patients

Application 2: The Role of Innate Immunity in Multiple Sclerosis (MS) Pathogenesis

Application 3: Altered Cytokine Production and Functional Mechanisms of IBD

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

Application 5: Polyfunctional Cytokine Drivers of Transplant Rejection

Bruker Product Types that Address These Challenges:

Single-Cell Secretome

CodePlex Secretome

Overcoming Challenges in Autoimmune Inflammation

The inflammatory response is driven by the inflammatory cytokine contribution from various immune cell types, though it remains challenging to find early biomarkers of inflammatory reaction & autoimmune progression.

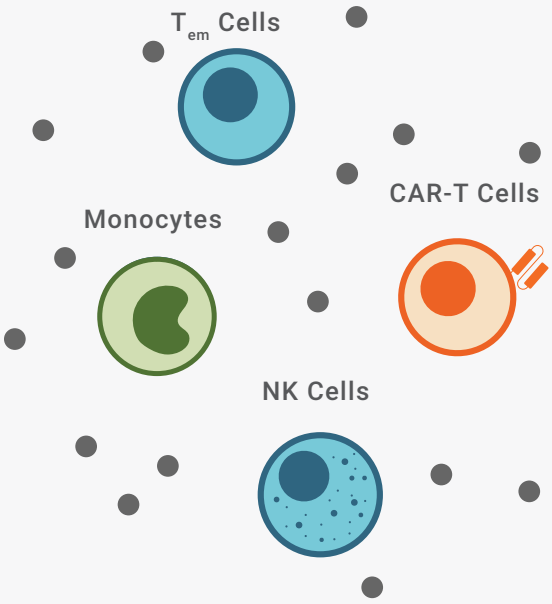
Cytokines are generally pro- or anti-inflammatory, and the balance between these determines the final outcome of an inflammatory response. For example, IL-1β, IL-8, and IFN-γ are pro-inflammatory cytokines involved in early responses and the amplification of inflammatory reactions.

The identification of heterogeneous functional immune cell subsets, made possible with functional phenotyping, is key to understanding the biomarkers of autoimmune response and disease progression.

Challenge 1: Requires Highly-Multiplexed CodePlex Secretome Solution

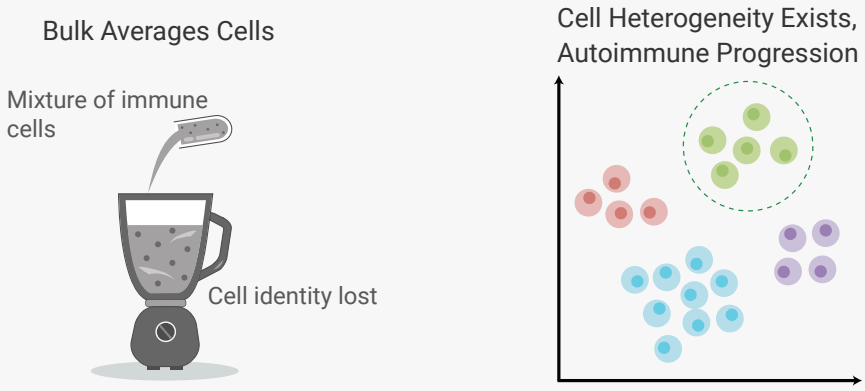
Challenges 4-6: Require Single-Cell Secretome Solution

Cell Types and Cytokines Implicated



Functionally defining each cell type involved in autoimmune response

Why Cell Subsets for Multiplexing Cytokines Matter in Autoimmune Inflammation



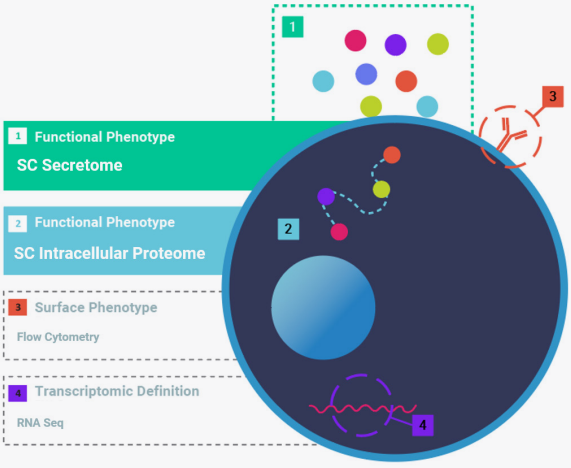
Traditional technologies average serum protein information from all cells. Bruker's cellullar functional phenotyping uncovers cellular differences to identify functional mechanisms in the autoimmune response.

Understanding Cellular Immune Function is Critical for Understanding Autoimmune Inflammation

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 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 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 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 Autoimmune Inflammation

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 and pathways in autoimmune response and progression.

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, sCD40L, sCD137, TNF-b, Granzyme B, IL-15

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

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

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

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)

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).

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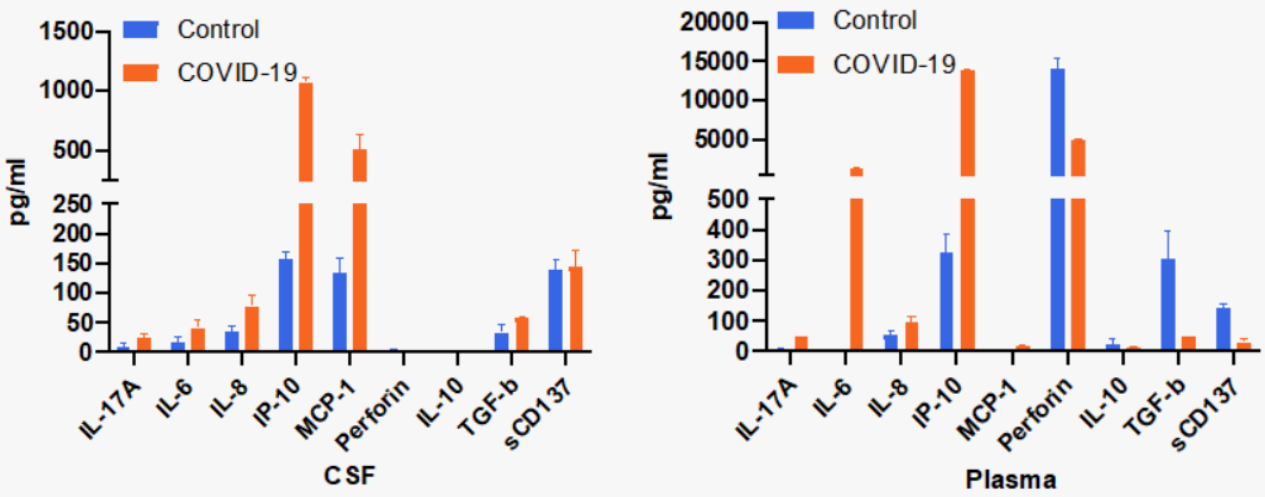
Application 1 – Cytokine Storm and Neurological Manifestations of COVID-19 in Patients

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 [1].

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

Assessing Dysfunctional Innate Immune Cell Subsets in MS with Functional Phenotyping

Products Used

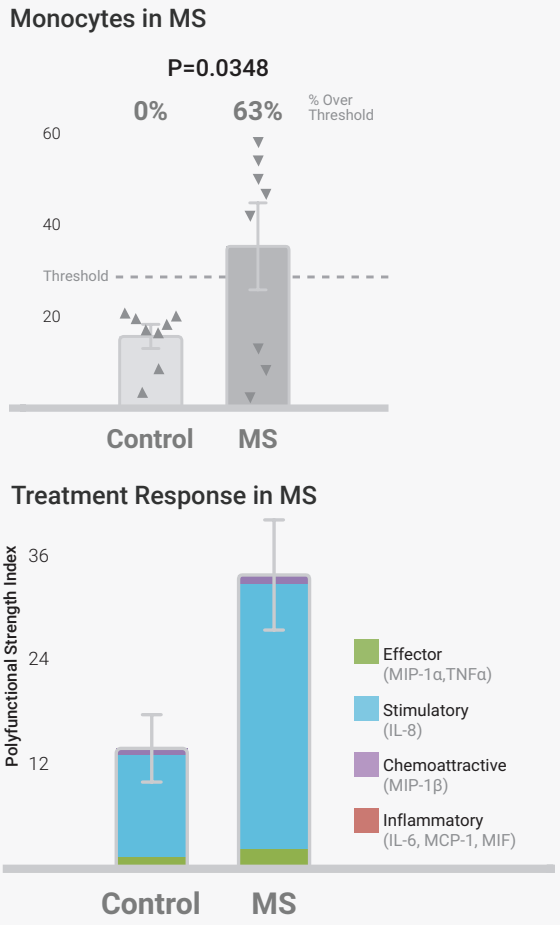
Single-Cell Secretome

Highlights of Identifying Inflammation of Subsets of Monocytes in MS

- Single-Cell Secretome identified a pathologically and therapeutically relevant Toll-like receptor (TLR) 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 different forms of the disease.

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

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



On-treatment MS patients showed deep downregulation of the monocyte polyfunctionality. PSI correlated as an indicator of on treatment/off treatment response in MS [2].

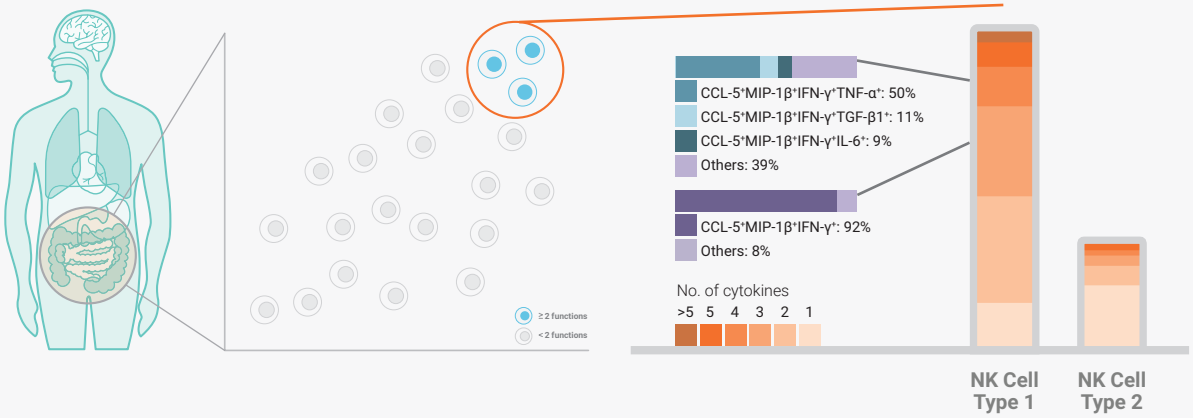
Application 3 – Altered Cytokine Production and Functional Mechanisms of IBD

Single-Cell Secretome Reveals Highly Pro-inflammatory Subsets of NK Cells and Drivers of Crohn's Disease

Products Used

Single-Cell Secretome

Identification of NK Cell Subsets Which are Pro-inflammatory Drivers in Crohn's Disease



NK cell subsets responsible for proinflammatory cytokines in IBD patients. Polyfunctional metrics reveal key differences in NK cell subtypes, which are polarized to proinflammatory cytokine production [3].

Highlights of Uncovering the Precursors of Crohn's Disease with Single-Cell Secretome

- Multiplexed single-cell proteomic analysis reveals that the presence of KIR2DL3 and its interaction with homozygous HLA-C1 results in NK cell cytokine reprogramming, which permits them to promote CD4+ T cell activation and Th17 differentiation *ex vivo*.
- Single-Cell Secretome reveals that in subsets of innate cells, certain highly-polyfunctional NK cell subtypes in Crohn's patients are more polarized to proinflammatory cytokine production.
- The cytokines upregulated in these NK cells further indicate that innate mechanisms drive progression, as far as immune system contribution, and a combination of pro-inflammatory cytokines from these cells may be downregulated through targeting of the NK cell mechanism in KIR2DL3+ NK cells.

Lin ,L et al. Human NK Cells Licensed by Killer Ig Receptor Genes Have an Altered Cytokine Program That Modifies CD4+ T Cell Function. The Journal of Immunology, 2014.

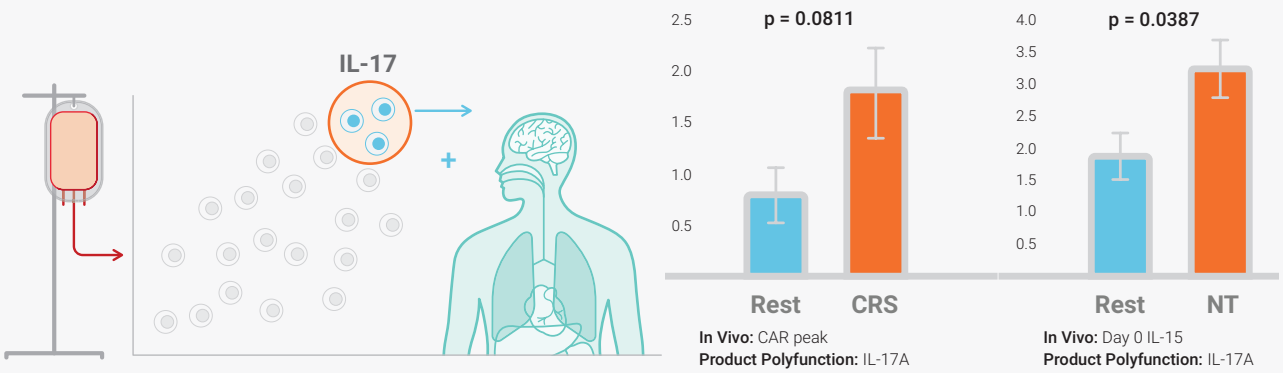
Application 4 – Biomarkers of Neurotoxicity and Immune Related Adverse Events (IRAEs)

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 [4].

Highlights of Bruker's Functional Proteomics and Correlation to CRS

- Single-Cell Secretome reveals 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

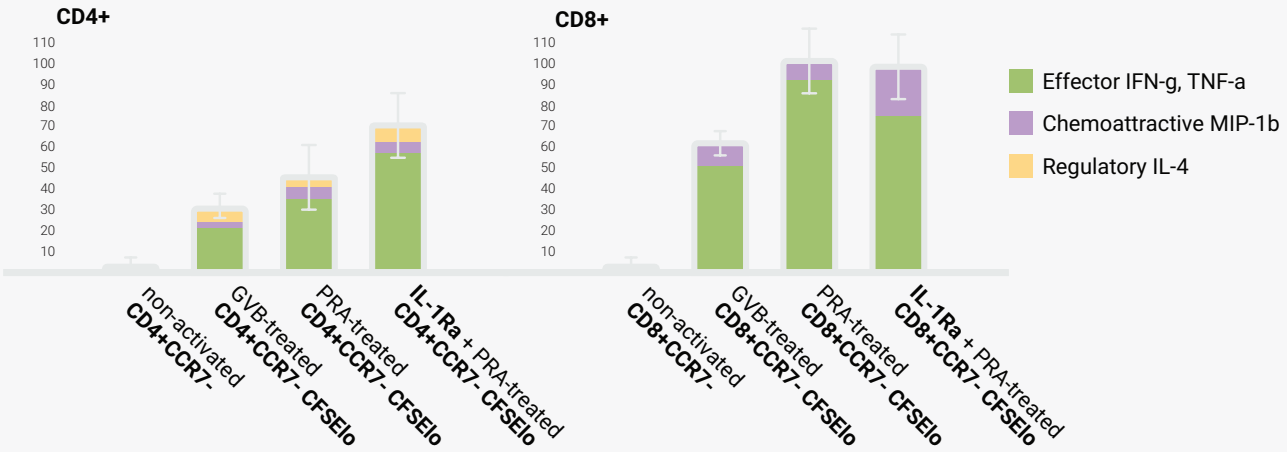
Application 5 – Polyfunctional Cytokine Drivers of Transplant Rejection

Single-Cell Proteomics Reveals Enhanced Polyfunctionality of Alloreactive T_{em} cells That Play an Important Role in Chronic Allograft Rejection

Products Used

Single-Cell Secretome

Identification of Mechanisms of Inflammation in Chronic Allograft Rejection



Functional phenotyping reveals the impact of highly polyfunctional T_{em} on allograft rejection and their potential for patient monitoring before and after organ transplantation [5].

Highlights of Applying Single-Cell Secretome to Discover Drivers of Transplant Rejection

- Single-Cell Secretome identified enhanced polyfunctionality of alloreactive CD4+ and CD8+ effector memory T cells (T_{em}) that play an important role in chronic allograft rejection.
- Data generated via single-cell functional phenotyping revealed the correlation of high polyfunctionality of T_{em} to alloreactivity induced by allogeneic human endothelial cells (ECs).
- This demonstrated that panel reactive antibody (PRA) -treated ECs can promote the formation of highly polyfunctional T_{em} that may accelerate chronic allograft rejection.

Xie, C, et al, Human endothelial cells activated by complement membrane attack complexes differentially affect allogeneic CD4 and CD8 responses. Presented at Human and Translational Immunology, 2019

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Challenges & Applications

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Solutions

- CodePlex Secretome reveals secretomic signatures in CSF and plasma
- Single-Cell Secretome reveals dysfunctional innate immune cell subsets in MS with functional phenotyping
- Single-Cell Secretome reveals highly pro-inflammatory subsets of NK cells and drivers of Crohn's Disease
- Single-Cell Secretome correlates preinfusion CAR-T cell product metrics with grade 3+ Cytokine Release Syndrome (CRS)
- Single-Cell Proteomics reveals enhanced polyfunctionality of alloreactive T_{em} cells that play an important role in chronic allograft rejection

References

1. Farhadian, S et al. Acute encephalopathy with elevated CSF inflammatory markers as the initial presentation of COVID-19. BMC Neurology, 2020.
2. Fujiwara M, et al. Enhanced TLR2 Responses in Multiple Sclerosis. Clinical and Experimental Immunology, 2018
3. Lin ,L et al. Human NK Cells Licensed by Killer Ig Receptor Genes Have an Altered Cytokine Program That Modifies CD4+ T Cell Function. The Journal of Immunology,
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