APPLICATION NOTE

Bruker Proteomic Product Suite for Cancer Immunology

Bruker's functional proteomics reveals unique secretomic signatures and insights into cancer immunology

In this Application Note we outline:

- Challenges in cancer immunology
- Predictive durability biomarkers with a combination PEG-IL2 and checkpoint therapy
- Clinical immune monitoring with immune checkpoint inhibitor therapies
- Early indicators of patient response and relapse with immune checkpoint inhibitor therapies
- Mechanisms of immune persistence in solid tumor indications
- Early indicators of potency in a combination immune checkpoint and TIL Therapy
- Inhibitory mechanisms of monocytes toward immune suppression in Follicular Lymphoma (FL)
- Indicators of immune function and mechanisms of patient relapse

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High Level Challenges and Applications

Application 1: Predictive Durability Biomarkers with a Combination PEG-1L2 and Checkpoint Therapy

Application 2: Clinical Immune Monitoring with Immune Checkpoint Inhibitor Therapies

Application 3: Early Indicators of Patient Response and Relapse with Immune Checkpoint Therapies

Application 4: Mechanisms of Immune Persistence in Solid Tumor Indications

Application 5: Early Indicators of Potency in a Combination Immune Checkpoint and TIL Therapy

Application 6: Inhibitory Mechanisms of Monocytes Toward Immune Suppression in Follicular Lymphoma (FL)

Application 7: Indicators of Immune Function and Mechanisms of Patient Relapse

Bruker Product Types that Address These Challenges:

- Single-Cell Secretome (Human)
- Single-Cell Secretome (Mouse)

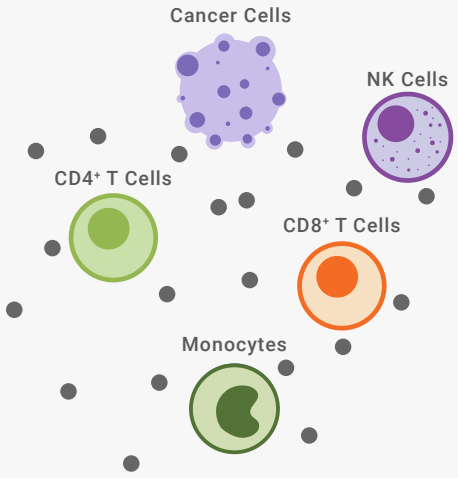
Overcoming Challenges in Cancer Immunology

Cancer cells in the tumor microenvironment secrete cytokines that interact with other cells, which facilitates intracellular communications and jointly moderates pathophysiological processes like cancer-induced angiogenesis and metastasis.

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

- **Challenges 1-7 Require Single-Cell Secretome Solution**

Cell Types and Cytokines Implicated



Functionally defining each cell type involved in the immune response in cancer immunology.

Why Cell Subsets for Multiplexing Cytokines Matter in Cancer Immunology

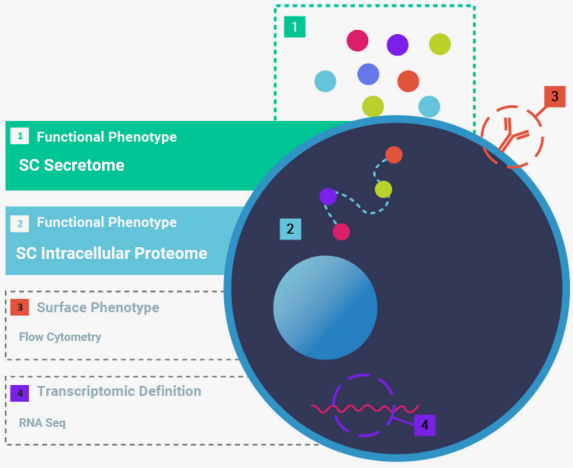
Traditional technologies average serum protein information from all cells. Bruker's cellullar functional phenotyping uncovers cellular differences to identify functional mechanisms in cancer immunology.

Understanding Cellular Immune Function is Critical for Therapy Development in Cancer Immunology

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 the specific cytokines that are produced by each heterogenous immune cell matter, and Bruker's cellullar functional phenotyping uncovers these cellular differences.

Through analysis of cellular RNA or surface phenotypes alone, researchers may miss 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.

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Superpowered Functional Proteomics is Critical for Accelerating Immune Medicines

Bruker's Proteomic solutions are the only way to measure the true function of each cell and identify the rare subsets of superpowered cells driving response. Using Bruker's platform, both single-cell and multiplexed bulk proteomic experiments are fully automated, with data sent directly to IsoSpeak software for analysis. With the IsoLight or IsoSpark system, a process that would traditionally require multiple instruments and steps is accomplished in one instrument with a variety of chip options to suit a wide range of research needs.

Single-Cell Secretome Panels

Human Adaptive Immune
Granzyme B, IFN-γ, MIP-1α, Perforin, TNF-α, TNF-β, GM-CSF, IL-2, IL-5, IL-7, IL-8, IL-9, IL-12, IL-15, IL-21*, CCL11, IP-10, MIP-1β, RANTES, IL-4, IL-10, IL-13, IL-22, TGFβ1, sCD137, sCD40L, IL-1β, IL-6, IL-17A, IL-17F, MCP-1, MCP-4

Non-Human Primate Adaptive Immune
TNF-α, MCP-1, IL-2, IL-4, MIP-1β, IL-6, IL-8, IL-1β, RANTES, IFN-γ, IP-10, MIP-1α, MIF, GM-CSF

Mouse Adaptive Immune
Granzyme B, IFN-γ, MIP-1α, TNF-α, GM-CSF, IL-2, IL-5, IL-7, IL-12p70, IL-15, IL-21, sCD137, CCL11, CXCL1, CXCL13, IP-10, RANTES, Fas, IL-4, IL-10, IL-13, IL-27, TGFβ1, IL-6, IL-17A, MCP-1, IL-1β

Human Innate Immune
IFN-γ, MIP-1α, TNF-α, TNF-β, GM-CSF, IL-8, IL-9, IL-15, IL-18, TGF-α, IL-5, CCL11, IP-10, MIP-1β, RANTES, BCA-1, IL-10, IL-13, IL-22, sCD40L, IL-1β, IL-6, IL-12-p40, IL-12, IL-17A, IL-17F, MCP-1, MCP-4, MIF, EGF, PDGF-BB, VEGF

Human Inflammation
GM-CSF, IFN-γ, IL-2, IL-12, TNF-α, TNF-β, IL-4, IL-5, IL-7, IL-9, IL-13, CCL11, IL-8, IP-10, MCP-1, MCP-4, MIP-1α, MIP-1β, RANTES, IL-10, IL-15, IL-22, TGF-β1, IL-1β, IL-6, IL-17A, IL-17F, IL-21*, Granzyme B, Perforin, sCD40L, sCD137

Mouse Innate Immune
IFN-γ, TNF-α, MIP-1α, IL-15, GM-CSF, IL-5, IL-10, IL-13, IL-6, IL-17A, MCP-1, IP-10, MIP-1β, EGF, PDGF-BB, MIF

Human Natural Killer
Granzyme B, IFN-γ, MIP-1α, Perforin, TNF-α, TNF-β, GM-CSF, IL-2, IL-5, IL-7, IL-8, IL-9, IL-12, IL-15, IL-21*, CCL11, IP-10, MIP-1β, RANTES, IL-4, IL-10, IL-13, IL-22, TGFβ1, sCD137, sCD40L, IL-1β, IL-6, IL-17A, IL-17F, MCP-1, MCP-4

*inquire about availability

The Single-Cell Secretome solution enables the discovery of better biomarkers and accelerated development through functional immune landscaping of each immune cell, allowing for complete single-cell functional characterization. Detect rare subsets of "super cells" to reveal functional biological drivers of persistence, potency, durability and more.

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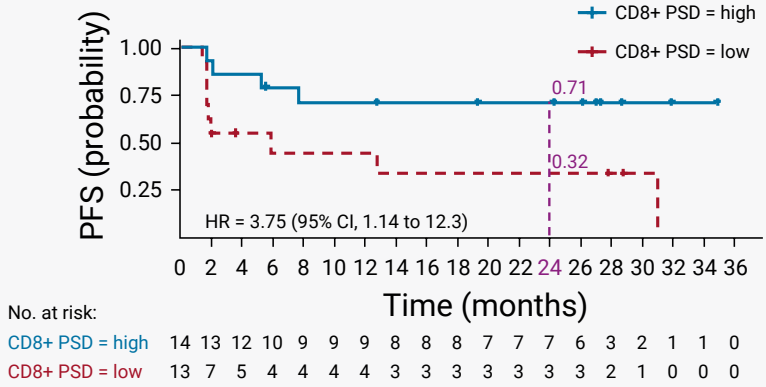
Application 1 – Predictive Durability Biomarkers with a Combination PEG-1L2 and Checkpoint Therapy

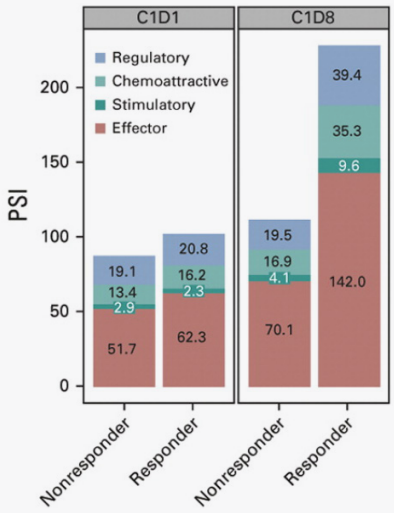
Single-Cell Functional Proteomics Predicts Progression-Free Survival in a Metastatic Melanoma Study

Products Used

Single-Cell Secretome (Human)

Identification of a Blood-Based Biomarker





In this study, 38 patients with stage III/IV melanoma received bempegaldesleukin (BEMPEG) plus nivolumab (NIVO) combination therapy and were evaluated for both clinical response and exploratory biomarkers.

Highlights of Bruker's Functional Proteomics in Predicting Patient Response

- High CD8⁺ Polyfunctional Strength Difference (PSD) between day 8 posttreatment and pretreatment was associated with a higher ORR and a longer Progression-Free Survival (PFS), as compared to low CD8⁺ PSD, over the course of the study (3 years).
- Treatment resulted in a 2.2-fold increase in the Polyfunctional Strength Index (PSI) of CD8⁺ T cells, driven by the production of cytokines with effector functions.
- These results suggest that the combination treatment of BEMPEG + NIVO enhances both T cell fitness and functional capacity and that CD8⁺ PSD is associated with ORR and longer PFS in this patient population, highlighting the potential of polyfunctional strength as a predictive biomarker that positively correlates with patient response.

Diab et al. Bempegaldesleukin Plus Nivolumab in First-Line Metastatic Melanoma. Journal of Clinical Oncology. 2021.

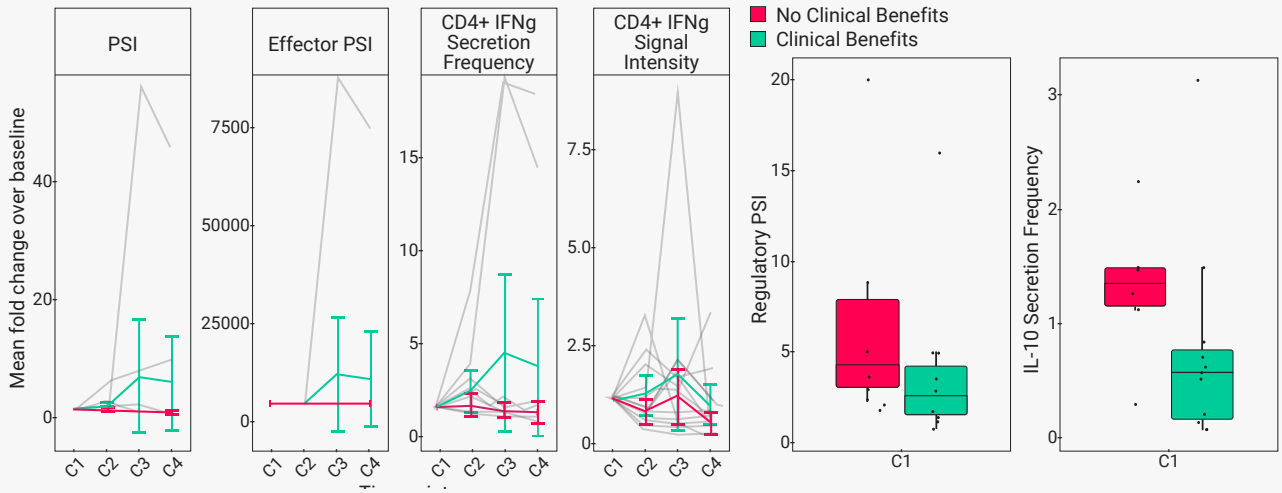
Application 2 – Clinical Immune Monitoring with Immune Checkpoint Inhibitor Therapies

Bruker's Single-Cell Functional Biology Reveals Indications of Immune Health Status in a Phase II Clinical Trial

Products Used

Single-Cell Secretome (Human)

Single-Cell Functional Biomarkers Predict Patient Response to ICIs



In this study, patients with advanced melanoma received either nivolumab plus ipilimumab or ipilimumab alone and were evaluated for both clinical response and translational biomarkers.

Highlights of Bruker's Functional Proteomics as an Indicator of Immune Health

- Patients with clinical benefit (CB) demonstrated increased PSI (Polyfunctional Strength Index) in CD4⁺ T cells, especially among effector cytokines.
- IFNγ secretion was higher in CB patients, as compared to those who did not show benefit.
- Both the expression of the immunosuppressive cytokine IL-10 and the PSI of regulatory cytokines overall was higher at baseline for patients that did not show benefit.
- These results demonstrate the power of single-cell peripheral functional biomarkers in predicting patient response to checkpoint inhibitor immunotherapy and the use of Bruker's unique metric, PSI, in assessing therapeutic efficacy in Phase II clinical trials.

Friedman CF et al, Ipilimumab alone or in combination with nivolumab in patients with advanced melanoma who have progressed or relapsed on PD-1 blockade: clinical outcomes and translational biomarker analyses. *Journal for ImmunoTherapy of Cancer* 2022.

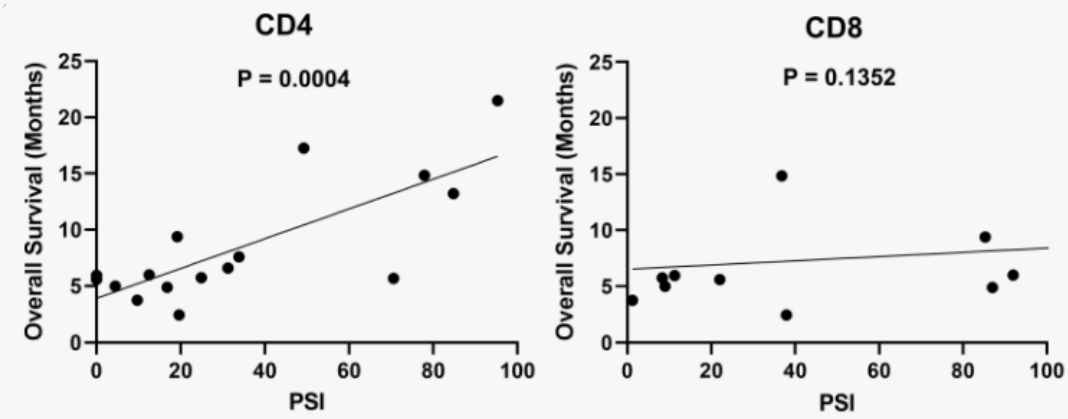
Application 3 – Early Indicators of Patient Response and Relapse with Immune Checkpoint Therapies

Biomarker Predictive of Improved Outcomes in Acute Myeloid Leukemia (AML) Revealed with Bruker's Single-Cell Profiling

Products Used

Single-Cell Secretome (Human)

Predictive Biomarkers of Overall Survival



In this study, 16 patients with relapsed/refractory AML received azacytidine in combination with the PD-1 checkpoint inhibitor nivolumab. Patients were evaluated for clinical response and immune cell subsets from pretreatment bone marrow were analyzed using Bruker's single-cell functional proteomics.

Highlights of Bruker's Functional Proteomics as an Early Indicator of Response and Relapse

- Single-cell functional proteomics was used to identify which cell subsets of bone marrow were associated with response and the specific cytokines that were driving higher polyfunctionality within this subset.
- Bruker technology revealed that pretreatment bone marrow $CD4^+$ T cells, not $CD8^+$ T cells, were inducing patient response to therapy, confirming the significance of this immune cell population in favorable responses to checkpoint inhibitor therapies.
- In addition, the use of single-cell functional phenotyping revealed that AML patients who responded to this combination therapy had distinct and highly polyfunctional subsets of $CD4^+$ T cells prior to therapy, revealing a potential biomarker that may predict how a patient responds to the treatment.

Abbas et al. Single-cell Polyfunctional Proteomics of CD4 Cells from Patients with AML Predicts Responses to Anti-PD-1-based Therapy. Blood Advances. 2021.

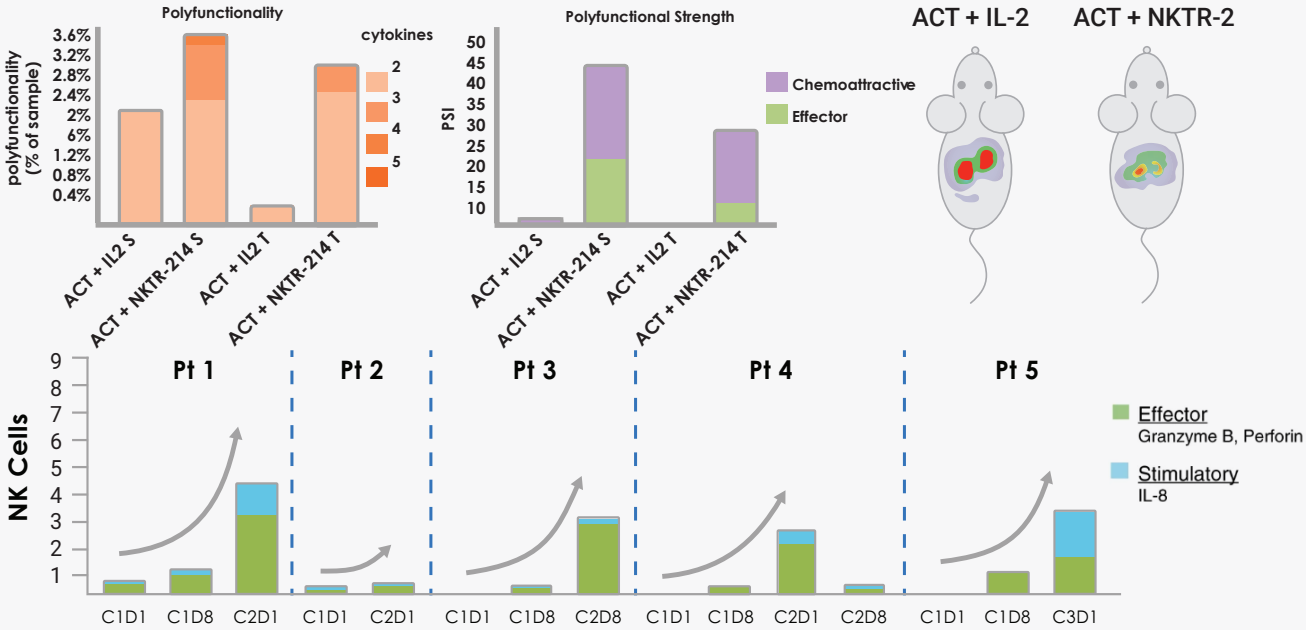
Application 4 – Mechanisms of Immune Persistence in Solid Tumor Indications

Functional Phenotyping Identified Biomarkers of Tumor Killing, Expansion and Persistence of Adoptively Transferred T Cells

Products Used

- Single-Cell Secretome (Mouse)
- Single-Cell Secretome (Human)

Single-Cell Functional Mouse Secretome Provides Pre-Clinical Insights into PEG-IL2 Impact & Superior Anti-Tumor Mechanism



In solid tumor patients undergoing PEG-IL2 therapy, Bruker's platform revealed upregulation of function in circulating CD4⁺ and CD8⁺ T cells and NK cells in the clinical setting in response to this innovative therapy .

Applying Single-Cell Secretome to Cellular Novel Combination Therapies

- Single-cell functional phenotyping was used to profile translational insights between early stage development of combination PEG-IL2 with Adoptive Cell Transfer therapy, and clinical impact on various immune cell types of patient PBMCs.
- Systemic polyfunctional mouse CD8+ T cell response after adoptive transfer was seen in response to PEG-IL2, which correlated to the increases of proliferation, homing, and persistence of anti-tumor T cells *in vivo*.
- The mechanism resulted in superior anti-tumor activity in B16-F10 murine melanoma model as well as the promotion of polyfunctional T and NK cells in peripheral blood of patients with melanoma in a phase 1 clinical trial.

Parisi G, et al. Persistence of adoptively transferred T cells with a kinetically engineered IL-2 receptor agonist. Nature Communications, 2020

Application 5 – Early Indicators of Potency in a Combination Immune Checkpoint and Tumor Infiltrating Lymphocyte (TIL) Therapy

Bruker's Single-Cell Secretome Provides Dynamic Immune Monitoring in a Phase 1 Trial for Metastatic Lung Cancer

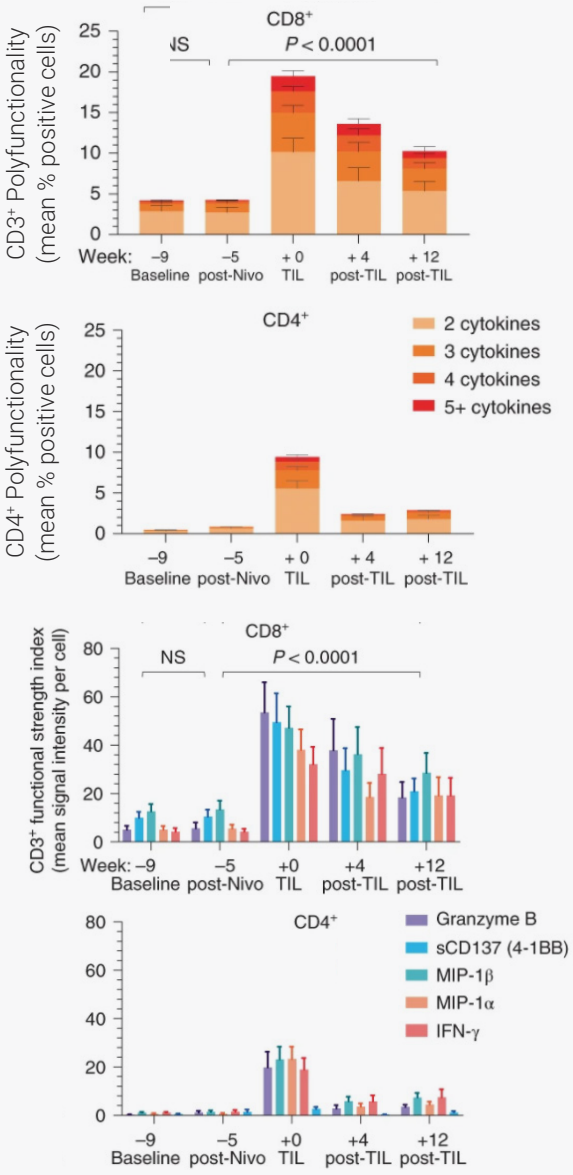
Products Used

Single-Cell Secretome (Human)

Highlights of Bruker's Functional Proteomics as Providing Dynamic Immune Monitoring

- Bruker's polyfunctionality was used to measure the ability of single T cells in blood to secrete multiple different cytokines after stimulation.
- Functional proteomics revealed that polyfunctionality was increased in $CD8^+$ and $CD4^+$ T cells after TIL treatment.
- Additionally, $CD8^+$ T cells had significantly increased secretion of Granzyme B, sCD137, MIP-1β, MIP-1α, and IFNγ after TIL treatment.
- These results highlight the power of Bruker's unique biology, as it helps researchers identify immunotherapy potency and advance TIL therapies by providing dynamic immune monitoring after treatment.

Predictive Product Quality Metrics



In this study, 20 patients with advanced non-small cell lung cancer (NSCLC) received a combination therapy of nivolumab (NIVO) and TILs. Patients were evaluated for clinical response and after treatment using Bruker's single-cell functional proteomics.

Creelan, B. et al. Tumor-infiltrating lymphocyte treatment for anti-PD-1-resistant metastatic lung cancer: a phase 1 trial. Nature Medicine. 2021.

Application 6 – Inhibitory Mechanism of Monocytes Toward Immune Suppression in Follicular Lymphoma (FL)

Identification of the Mechanistic Importance of Monocyte Function in Immune Suppression with Single-Cell Profiling

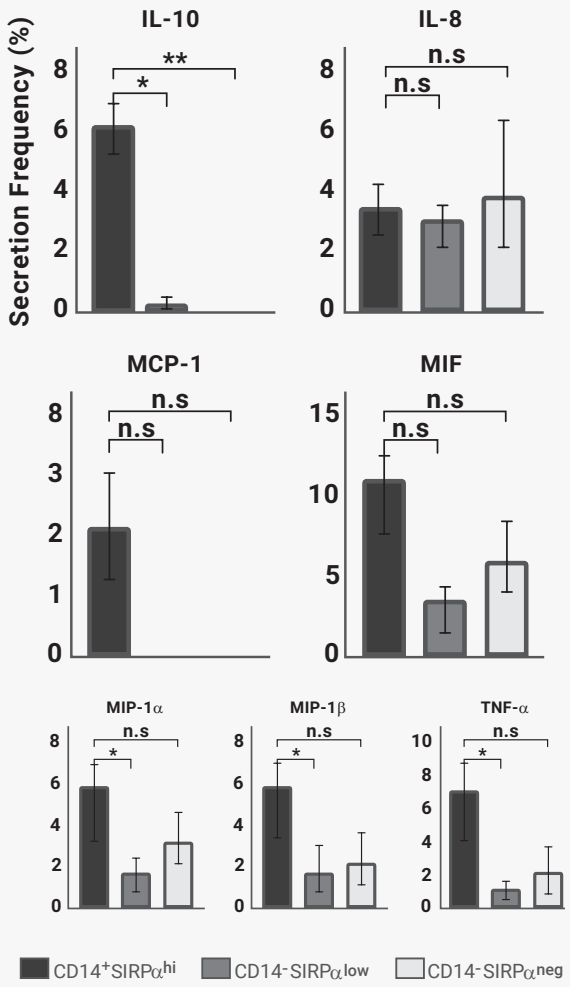
Products Used

Single-Cell Secretome (Human)

Highlights of Prognostic Response with Single Monocyte Function

- CD14+SIRPα^{hi} (signal regulatory protein-α) monocytes/macrophages were associated with an inferior survival in FL, but increased numbers of the CD14−SIRPα^{low} subset appeared to correlate with a better survival.
- 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.
- Single-Cell functional proteomics revealed an inhibitory mechanism of enhanced IL-10 in single-cell polyfunctional CD14+ SIRPα^{hi} subsets reducing T cell proliferation, which provides the functional insights of Mo/MΦs subset for FL patients, which were implicated in inferior survival of patients.

Cytokine/Chemokine Production by CD14+SIRPα^{hi}, CD14−SIRPα^{low}, or CD14−SIRPα^{neg} cells



Cytokine/chemokine production by CD14+SIRPα^{hi}, CD14−SIRPα^{low}, or CD14−SIRPα^{neg} cells determined by the the Single-Cell Secretome 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.

Chen Y-P, et al. SIRPα expression delineates subsets of intratumoral monocyte/macrophages with different functional and prognostic impact in follicular lymphoma. Blood Cancer Journal, 2019

Application 7 – Indicators of Immune Function and Mechanisms of Patient Relapse

Insight into the Mechanism of Action of Venetoclax and Azacytidine in AML Patients with Single-Cell Functional Phenotyping

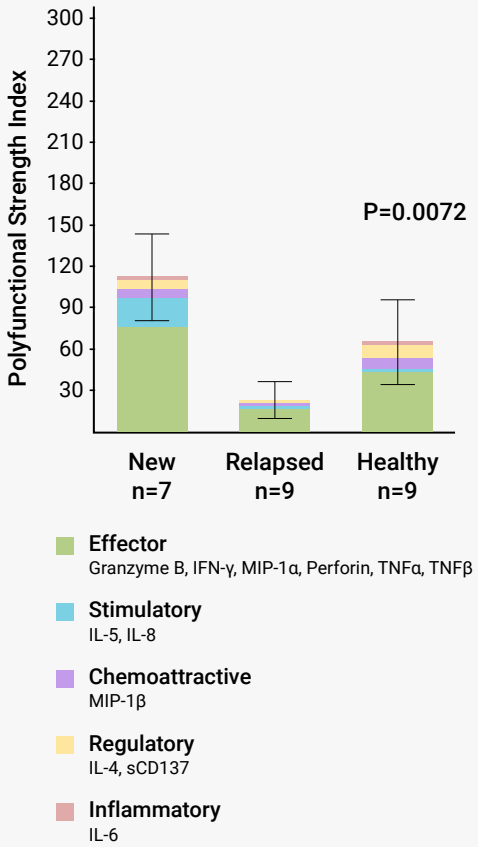
Products Used

Single-Cell Secretome (Human)

Highlights of Bruker's Functional Proteomics in Providing Mechanistic Insights

- Bruker's single-cell proteomics was used to identify the relationship between T cell fitness and polyfunctionality, post-chemotherapy relapse, and response to venetoclax/azacytidine treatment in this study.
- $CD8^+$ T cells of newly diagnosed AML had higher PSI compared to those of AML patients who received chemotherapy and relapsed, suggesting that chemotherapy reduced T cell functionality and thus decreased the efficacy of the combined venetoclax/azacytidine treatment.
- Single-cell functional profiling also provides unique insights into how T cell fitness and functionality contribute to the efficacy of the adoptive T cell therapy in combination with venetoclax/azacytidine, providing mechanistic insights for the optimization of curative medicines.

Insight into Therapeutic Mechanism of Action



This study compared the efficacy of a combination venetoclax/azacytidine therapy in AML patients who were treatment-naïve and patients who had relapsed. This study highlighted the therapeutic potential of combining an adoptive T cell therapy with venetoclax/azacytidine in patients who relapsed post-chemotherapy.

Bok Lee et al., Venetoclax enhances T cell–mediated antileukemic activity by increasing ROS production. Blood. 2021.

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High Level Challenges & Applications

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Application 5 Early Indicators of Potency in a Combination Immune Checkpoint and TIL Therapy

Application 6: Inhibitory Mechanisms of Monocytes Toward Immune Suppression in Follicular Lymphoma

Application 7: Indicators of Immune Function and Mechanisms of Patient Relapse

Solutions

- Single-Cell Functional Proteomics Predicts Progression-Free Survival in a Melanoma Study
- Bruker's IsoCode Technology Reveals Indications of Immune Health Status in a Phase II Clinical Trial of Advanced Melanoma
- Biomarker Predictive of Improved Outcomes in AML Revealed with Bruker's Single-Cell Profiling
- Functional Phenotyping Identified Biomarkers of Tumor Killing, Expansion and Persistence of Adoptively Transferred T Cells
- Bruker's Single-Cell Secretome Provides Dynamic Immune Monitoring in a Phase 1 Trial for Metastatic Lung Cancer
- Identification of the Mechanistic Importance of Immune Cell Subsets and an Association of Monocyte PSI with Disease Severity Function in Immune Suppression with Single-Cell Profiling
- Insight into the Mechanism of Action of Venetoclax and Azacytidine in AML Patients with Single-Cell Functional Phenotyping